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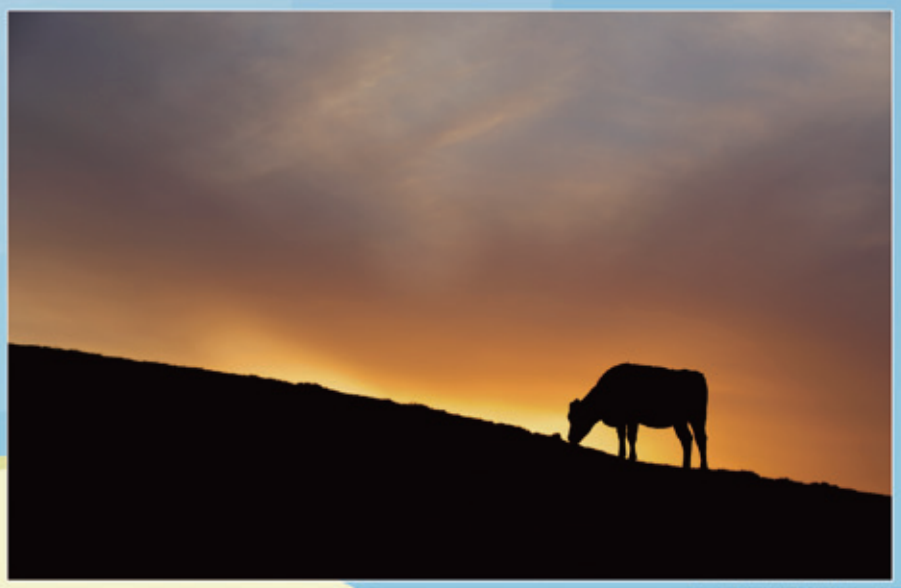

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Oral Session

Proceedings

O-01-1

Improvement the accurate genetic estimation of milk yield trait by addition of wind speed to THI in crossbred Thai Holstein

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Introduction

Climate change in the meaning of high temperature and high humidity caused directly on the decline of milk yield of dairy cows. Dairy cows respond to heat stress in several ways, including reduced feed intake (Beede and Collier, 1986), lower milk yield and milk quality (Bohmanova et al., 2007), and compromised fertility (Haile-Mariam et al., 2008). In Thailand, dairy breeding research trend has been used temperature-humidity index (THI) to solve heat stress problem due to Thailand was located in Tropical zone. Therefore, THI function was used for select heat tolerance dairy cattle. Nowadays, in Thailand, THI function was popular to genetic improvement for many traits in dairy cattle (Boonkum et al, 2011; Charern-osot, 2016; Reebrangrum et al., 2016). THI function base on temperature and relative humidity (NRC, 1971). However, almost factor effect on heat stress in dairy cattle were temperature (T), relative humidity (RH), wind speed (WS) and radiation (RA) leading to new THI function concept as base on T, RH and WS (Baeta et al., 1987). The objective of this study was to compare THI function for finding out THI function has goodness of fit to improve crossbred Thai Holstein cattle in Thailand.

Materials and methods

Data of analysis

Data comprised of 116,828 test-day milk yield records for first lactation from 14,485 cows between 1990 and 2012 and data were obtained from department of livestock development. Daily temperature, humidity and wind speed data were obtained from meteorological department. Data analysis is divided into two types depending on THI function such as 1) THI1 (conventional) and THI2 (+wind speed).

Function temperature-humidity index (THI)

Almost factors effect on heat stress in dairy cattle were air temperature (T), relative humidity (RH), and wind speed (WS). THI1 was calculated by T and RH which, this formula easily to use. However, heat stress have another factor leading to THI2 (+wind speed) by Baeta et al. (1987). THI1 = (1.8T+32) - (0.55-0.0055RH) x (1.8T-26), THI2 = 27.88-(0.45xT^2)-(0.4905xRH)+(0.00088xRH^2)+(1.1507xWS)-(0.12644xWS^2)+ [0.019876 x (TxRH)]–[0.04631 x (TxWS)]

Repeatability Test Day Model

Variance components analysis by restricted maximum likelihood (REML) from REMLF90 and used repeatability test day model where

$$Y_{ijklm} = HMY_i + DIM_k BG_j + AGE_l + \alpha_m + v_m[f(THI)] + p_m + q_m[f(THI)] + e_{ijklm}$$

Where Y_{ijklm} is observation of test-day milk yield from cow m in herd-test, month-test and year-test class i ($HMYi$), DIM_kKBG_j are effect of breed group (BG_j) class j and day in milk class k, AGE_l is age of calving class l, α_m is additive genetic effect, V_Mis additive gene effect include heat stress, P_m is permanent environment, Q_Mis permanent environment include heat stress. e_{ijklm} is residual effect and F(THI) is function of THI

$$f(THI) = \begin{cases} 0; & \text{THI} \leq \text{THI}_{\text{threshold}} \text{ (no heat stress)} \\ \text{THI} - \text{THI}_{\text{threshold}}; & \text{THI} > \text{THI}_{\text{threshold}} \text{ (heat stress)} \end{cases}$$

Genetic parameters

Heritability (h2) of milk yield on heat stress (Ravagnolo and Misztal., 2000)

$$h^2_{f(i)} = \frac{\sigma_a^2 + f(i)^2\sigma_v^2 + 2f(i)\sigma_{av}}{\sigma_a^2 + f(i)^2\sigma_v^2 + 2f(i)\sigma_{av} + \sigma_p^2 + f(i)^2\sigma_q^2 + 2f(i)\sigma_{pq} + \sigma_e^2}$$

Genetic correlation between milk yield and heat stress (Ravagnolo and Misztal., 2000)

$$corr[a, f(i)v] = \frac{f(i)\sigma_{av}}{\sqrt{\sigma_a^2 * f(i)^2\sigma_v^2}}$$

Where $h_{f(i)}^2$ is heritability of milk yield under level of heat stress, *corria*[α ,*f(i)v*] is genetic correlation between milk yield and heat stress, σ_a^2 is additive genetic variance, σ_v^2 is additive genetic variance under heat stress, σ_{av} is covariance between additive genetic and heat stress variance, σ_p^2 is permanent environment variance, σ_q^2 is permanent environment variance under heat stress, σ_{pq} is covariance between permanent environment and heat stress variance, σ_e^2 is error variance and *f(I)* is level of heat stress.

Results and discussions

Average test-day milk yield of crossbred Thai Holstein cattle (first lactation) 12.5 kg and average 305 day milk yield 3,765 kg according Sanpote et al. (2010) found 12.8 kg and 3,654 kg. Average relative humidity, temperature and wind speed were 73 %, 28 oc and 1 m/s respectively. While THI1 and THI2 were 78 and 32 (Table 1.). Threshold point of heat stress on milk yield trait, THI1 and THI2 were 75 and 28.

Genetic parameters

Heritability (h2) of milk yield was downward trend (Figure 1.) show level of heat stress increase effected to h2 were decrease. Average heritability of milk yield, THI1 and THI2 were 0.124 and 0.187 (Table 2), which heritability was low contrast with Sanpote et al. (2010); Boonkum and Duangjinda (2014) found heritability was medium due to used random regression model might be effected to heritability in this study used repeatability model. Genetic correlation (rg) between milk yield and heat stress found weak negative genetic correlation, which THI1 and THI2 were -0.207 and -0.112 (Table 2) show that milk yield decrease was effected of heat stress according Boonkum (2011) found rg was -0.21 in crossbred Thai Holstein cattle first lactation.

Comparison THI function

This study found function THI which THI has goodness of fit for Thailand. Consider accuracy (R^2), THI1 and THI2 were 0.979 and 0.986 found that THI2 was high accuracy than THI1 due to THI2 adjust wind speed which is factor of heat stress there for THI2 can use to improve crossbred Thai Holstein cattle.

Conclusion and Suggestion

The objective of this study was comparison THI function for finding out THI function has goodness of fit to improve crossbred Thai Holstein cattle in Thailand. In summary, even the conventional THI is commonly useful, adjustment wind speed to THI could improve more accurately estimation of milk yield of Cross-bred Thai Holstein cattle in Thailand. However, wind speed data collecting as a result to cost increase, including THI1 had high accuracy, which close to THI2. There for THI1 appropriate to improve crossbred Thai Holstein cattle.

Acknowledgement

This paper was supported from Thermo-tolerance Dairy Cattle Research Group and data was supported by department of livestock development and meteorological department

KEYWORD : Heat stress, Temperature-humidity index, Milk yield, Wind speed

Table 1 Data structure.

Categories	THI1	THI2
Test-day record, n	116,828	116,828
Animal with records, n	14,485	14,485
Animal with pedigrees, n	23,718	23,718
Average 305-d milk yield, kg	3,765	3,765
Average Test-day milk yield, kg	12.5	12.5
Average Relative humidity, %	73	73
Average Temperature, °c	28	28
Average Wind speed, m/s	-	1
Average THI	78	32
Maximum / Minimum THI	86 / 56	40 / 13
Threshold point	75	28

THI = temperature-humidity index, THI1 (conventional), THI2 (+wind speed).

Table 2. Genetic parameters.

THI	average of h^2	r_g	R^2
THI1	0.124	-0.207	0.979
THI2	0.187	-0.112	0.986

THI = temperature-humidity index, THI1 (conventional), THI2 (+wind speed), h^2 = heritability, r_g = genetic correlation between milk yield and heat stress effect, R^2 = coefficient of determination.

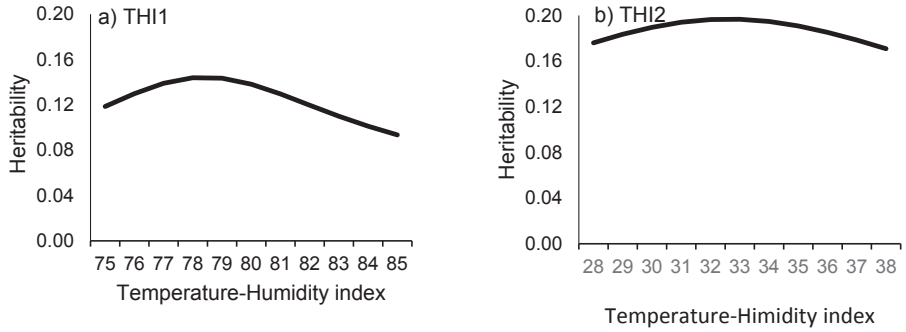

Figure 1. Heritability of THI1 and THI2 under heat stress

REFERENCES

Baeta, F. C., N. F. Meador, M. D. Shanklin, and H. D. Johnson. 1987. Equivalent temperature index at temperatures above the thermoneutral for lactating dairy cows. ASAE Paper No. 874015. American Society of Agricultural Engineers (ASAE), St. Joseph, MI.

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543-554.

Bohmanova, J., I. Misztal, and J. B. Cole. 2007. Temperature-humidity indices as indicators of milk production losses due to heat stress. J. Dairy Sci. 90:1947-1956.

Boonkum, W. 2011. Studies on Genetics of Heat Stress Effect in Thai Crossbred Holsteins. Ph. D. Thesis. Khon Kaen University, Khon Kaen.

Boonkum, W. and M. Duangjinda. 2014. Genetic evaluation for milk production trait in crossbred Holstein dairy cattle separated by region of Thailand using Random regression test-day model. KHON KAEN AGR. J. 42 SUPPL 1:260-266.

Boonkum, W., I. Misztal, M. Duangjinda, V. Pattarajinda, S. Tumwasorn, and S. Buaban. 2011. Short communication: Genetic effects of heat stresson days open for Thai Holstein crossbreds. J. Dairy Sci. 94:1592–1596.

Charern-osot, W. 2016. Association study of candidate gene marker and geneti evaluation of fat to protein

ratio of milk with heat stress in Thai-Holstein crossbred. Ms. Thesis. Khon Kaen University, Khon Kaen.

Haile-Mariam, M., M. J. Carrick, and M. E. Goddard. 2008. Genotype by environment interaction for fertility, survival, and milk production traits in Australian dairy cattle. J. Dairy Sci. 91:4840-4853.

NRC. 1971. A Guide to Environmental Research on Animals. Natl.Acad. Sci., Washington, DC.Reebrangrum, K. M. Duangjinda, S. Buaban, J. Sanpote and W. Boonkum. 2016. Effect of heat stress on genetic for milk protein and milk fat yield traits in Thai x Holstein crossbreds. KHON KAEN AGR. J. 43(1):151-160.

Sanpote, J., S. Buaban, M. Duangjinda and W. Boonkum. 2010. Genetic evaluation for milk production trait in crossbred Holstein dairy cattle separated by region of Thailand using Random regression test-day model. KHON KAEN AGR. J. 38:56-64.

O-01-3

Analysis of realized heritability and other genetic parameters in a long-term selection experiment for non-destructive deformation in White Leghorns

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Abstract

Objective: Although many advances have been made in the past decades to improve eggshell strength, it is still estimated that the yearly economic losses incurred by the poultry industry due to poor eggshell quality amount to hundreds of millions of dollars worldwide. Against this backdrop, our research aims to improve the understanding of the long-term effects of selection for non-destructive deformation on other eggshell traits in egg-laying chickens. To this end, emphasis was placed on the analysis of the realized heritability and of the other essential genetic parameters.

Methodology: In this study, we examined a long-term divergent selection experiment for eggshell strength performed on seventeen generations of White Leghorns. The selection process was based on low and high non-destructive deformation values, creating a strong line and a weak line, respectively. Variance components and other genetic parameters were estimated by using the REML approach, and the breeding values were predicted under a three-trait animal model through BLUP methodology. The three traits considered were non-destructive deformation, eggshell breaking strength, and eggshell thickness.

Results: The realized heritability for non-destructive deformation was 0.255 in the strong line, and 0.164 in the weak line. In the strong line, the heritability estimates were 0.478 for non-destructive deformation, 0.369 for eggshell breaking strength, and 0.451 for eggshell thickness. In the weak line, these values were 0.418, 0.504, and 0.548, respectively. The genetic correlation between all traits was large, with absolute values estimated to lie between 0.748 and 0.912.

Conclusion: The effectiveness of the selection process for non-destructive deformation, the moderately high heritability estimates, and the high genetic correlations are results which not only indicate the potential for long-term genetic improvement through selection for non-destructive deformation, but also suggest that non-destructive deformation is a useful tool to assess eggshell strength that can replace the destructive methods currently being used.

Introduction

Although many advances have been made in the past decades to improve eggshell strength, it is still estimated that the yearly economic losses incurred by the poultry industry because of poor eggshell quality amount to hundreds of millions of dollars globally (Seydim and Dawson, 1999). For this reason, scholars regularly emphasize the fact that eggshell quality is and will remain an essential aspect of research for the poultry industry in the near future (Bain *et al.*, 2006).

Against this backdrop, this study aims to investigate the long-term effects of selection for non-destructive deformation on other eggshell traits in egg-laying chickens. Indeed, one of the advantages of using non-destructive deformation as a criterion to assess eggshell strength in comparison with mainstream methods, such as the measurement of the breaking strength of the eggshell or of the thickness of the eggshell, lies in the fact that it does not require the destruction of the egg to be applied. Even though the study of the deformation of the eggshell is not a recent phenomenon (Voisey and MacDonald, 1978), more knowledge is needed to better understand the long-term effects of selection for non-destructive deformation. To this end, this paper lays stress on the analysis of breeding values and genetic parameters, e.g. the heritabilities, realized heritabilities, and genetic correlations between the traits measured.

Materials and methods

The experiment reviewed in this study is a two-way selection experiment conducted in seventeen generations of White Leghorn, based on low and high eggshell deformation values, referred to as the strong (shell) line and the

weak (shell) line, respectively. All generations were selected using the non-destructive deformation value only, except for generation 2, which was selected based on eggshell breaking strength due to technical difficulties. Selection was based on individual performance from generation 1 to generation 13, however a within-family selection procedure was implemented from generation 14 onward in order to prevent the inbreeding coefficient from increasing.

In total, the number of records measured in the strong and weak lines were 4,255 and 4,094, respectively. Each record corresponds to the average of three measurements on three different eggs for a given individual. Given that males do not have their own records, male selection was performed by using the full-sib mean. This value was also used for the calculation of the selection differential.

With respect to statistical analysis, ASReml-R was used to estimate the variance components (Patterson and Thompson, 1971; Butler, 2009), and BLUP methodology was implemented to predict the breeding values (Henderson, 1975) under a three-trait animal model. Three random effects, corresponding to the three traits measured, i.e. non-destructive deformation, eggshell breaking strength, and eggshell thickness, were included in the model, as well as one fixed effect, i.e. the generation effect. The relationship between individuals was accounted for by using full pedigree information.

Results and discussion

Genetic parameters

The genetic parameters were estimated by using REML methodology under the three-trait animal model described in the Methods section (Table 1). The genetic correlation between non-destructive deformation and eggshell breaking strength was -0.821 ($\pm$ 0.032) in the strong line and -0.811 ($\pm$ 0.030) in the weak line. Between non-destructive deformation and eggshell breaking strength, the genetic correlation was -0.850 ($\pm$ 0.024) in the strong line and -0.912 ($\pm$ 0.016) in the weak line. The genetic correlations were negative given that low non-destructive deformation values are associated with stronger eggshells, and the stronger the eggshell, the higher the strength required to break the shell and the thicker the eggshell. The large correlations estimated in this experiment are similar to those found by Grunder *et al.* (1989). Moreover, large correlations were also found between eggshell breaking strength and eggshell thickness, in both lines.

In the strong line, the heritability estimates were 0.478 ($\pm$ 0.044) for non-destructive deformation, 0.369 ($\pm$ 0.037) for eggshell breaking strength, and 0.451 ($\pm$ 0.038) for eggshell thickness. In the weak line, these values were 0.418 ($\pm$ 0.038), 0.504 ($\pm$ 0.040), and 0.548 ($\pm$ 0.038), respectively. The moderately high heritability values estimated for non-destructive deformation are somewhat higher than those of Van Tijen and Kuit (1970), and indicate the potential for genetic improvement of eggshell quality through selection for non-destructive deformation.

Realized heritability

We determined the realized heritability over the course of the experiment based on the cumulative selection differential and selection response calculated for all seventeen generations of White Leghorns (Table 2 and Table 3). In the strong line, the value of the realized heritability for non-destructive deformation was 0.255, whereas it was 0.164 in the weak line.

In addition to the realized heritability computed using the selection differential and response for non-destructive deformation, we also calculated the realized heritability for eggshell breaking strength and eggshell thickness, given that the genetic correlations between the three eggshell traits examined were very large in both lines. For eggshell breaking strength, the realized heritability values were 0.295 and 0.163, respectively, and for eggshell thickness, they were 0.220 and 0.280.

Although these values are smaller than the heritability estimates calculated using REML methodology, they confirm the potential for long-term eggshell strength improvement through selection by showing the effectiveness of the selection process for non-destructive deformation over the generations.

Conclusion

The present study aimed to investigate the long-term effect of selection for non-destructive deformation on two other eggshell traits, namely eggshell breaking strength and eggshell thickness. To this end, a population of White

Leghorns was divided into two lines, a strong line and a weak line, based on low and high deformation values, respectively.

This experiment, which was conducted for seventeen generations, was analyzed by using REML methodology to estimate variance-covariance components and other genetic parameters, and by using BLUP methodology for the prediction of the breeding values. The selection differential, the selection response, and realized heritabilities were also examined.

The results obtained from the analysis of this experiment showed the effectiveness of the selection process for non-destructive deformation, and the moderately high heritability estimates and the high genetic correlations are findings which suggest the potential for long-term genetic improvement through selection for non-destructive deformation. It was therefore concluded that non-destructive deformation is a useful way to assess eggshell strength that may be able to replace the destructive methods currently being used.

KEYWORD : Eggshell strength, Genetic parameters, Long-term selection, Non-destructive deformation, White Leghorn

Table 1. Heritability, genetic correlation (above diagonal), and phenotypic correlation (below diagonal) estin standard error).

	Strong line		
	NDD[1]	**BS**	**ST**
NDD	0.478 ± 0.044	-0.821 ± 0.032	-0.850 ± 0.024
BS	-0.690 ± 0.011	0.369 ± 0.037	0.748 ± 0.038
ST	-0.799 ± 0.008	0.677 ± 0.011	0.451 ± 0.038
	Weak line		
	NDD	**BS**	**ST**
NDD	0.418 ± 0.038	-0.811 ± 0.030	-0.912 ± 0.016
BS	-0.727 ± 0.011	0.504 ± 0.040	0.808 ± 0.028
ST	-0.829 ± 0.007	0.715 ± 0.011	0.548 ± 0.038

[1] NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm).

Table 2. Cumulative selection differential and response (strong line).

Gen [1]	NDD		BS		ST	
	SD	SR	SD	SR	SD	SR
1-2	N/A	N/A	0.28	-0.03	15.5	-7.0
2-3	9.8	5.0	0.68	-0.01	36.7	-6.0
3-4	18.7	-1.3	1.05	0.15	54.8	3.6
4-5	26.9	4.8	1.35	0.47	69.0	18.0
5-6	33.5	5.5	1.73	0.54	85.8	9.5
6-7	40.7	5.7	2.09	0.62	105.1	18.3
7-8	45.9	10.0	2.32	0.67	117.3	27.7
8-9	51.9	6.5	2.64	0.63	136.1	12.6
9-10	59.1	10.3	2.99	0.70	156.2	26.5
10-11	65.1	8.0	3.36	0.78	174.1	30.9
11-12	71.0	12.8	3.67	0.86	189.7	32.9
12-13	74.5	16.4	4.12	1.02	199.3	36.9
13-14	78.4	17.9	4.38	0.97	211.1	38.4
14-15	80.2	18.5	4.49	0.97	215.9	39.5
15-16	83.1	18.5	4.65	1.02	224.0	37.0
16-17	84.9	18.2	4.76	1.27	229.5	42.8
17-18	87.0	22.2	4.90	1.45	237.2	52.3
h^2	0.255		0.295		0.220	

[1] Gen, Generation; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SD, Cumulative selection differential; SR, Cumulative selection response; h^2, Realized heritability; N/A, Not available.

Table 3. Cumulative selection differential and response (weak line).

Gen [1]	NDD		BS		ST	
	SD	SR	SD	SR	SD	SR
1-2	N/A	N/A	0.18	0.21	8.7	18.6
2-3	9.8	-4.0	0.58	0.23	30.2	19.7
3-4	21.7	4.0	0.93	0.17	49.2	12.7
4-5	32.6	4.4	1.23	0.15	68.3	14.2
5-6	43.8	7.8	1.60	0.30	90.2	31.5
6-7	55.6	11.8	1.93	0.36	109.3	31.0
7-8	65.4	10.5	2.17	0.48	123.2	33.2
8-9	78.1	17.2	2.51	0.52	144.6	46.5
9-10	91.4	25.4	2.86	0.76	164.9	62.2
10-11	105.2	35.9	3.13	0.81	182.0	68.7
11-12	119.8	32.9	3.40	0.95	197.7	74.7
12-13	126.9	36.3	3.71	1.00	209.1	78.1
13-14	137.7	33.9	3.87	1.01	219.5	78.2
14-15	142.5	40.7	3.94	1.15	224.0	85.7
15-16	147.5	42.9	4.02	1.17	229.3	88.0
16-17	148.1	33.8	4.04	0.83	229.2	77.1
17-18	147.8	24.2	4.06	0.66	229.6	64.3
h^2	0.164		0.163		0.280	

[1] Gen, Generation; NDD, Non-destructive deformation (μm/kg); BS, Eggshell breaking strength (kg); ST, Eggshell thickness (μm); SD, Cumulative selection differential; SR, Cumulative selection response; h^2, Realized heritability; N/A, Not available.

REFERENCES

Bain, M. M., Dunn, I. C., Wilson, P. W., Joseph, N., De Ketelaere, B., De Baerdemaeker, J., & Waddington, D. (2006). Probability of an egg cracking during packing can be predicted using a simple non-destructive acoustic test. *British poultry science, 47*(4), 462-469.

Butler, D. (2009). ASReml-R. R package (Version3.0). VSN International Ltd. Hemel Hempstead, UK.
Available from: http://www.vsni.co.uk/ (Accessed July 1, 2016)

Grunder, A. A., Hamilton, R. M. G., Fairfull, R. W., & Thompson, B. K. (1989). Genetic parameters of egg shell quality traits and percentage of eggs remaining intact between oviposition and grading. *Poultry Science, 68*(1), 46-54.

Henderson, C. R. (1975). Best linear unbiased estimation and prediction under a selection model. *Biometrics*, 423-447.

Patterson, H. D., & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika*, 58(3), 545-554.

Seydim, A. C., & Dawson, P. L. (1999). Packaging effects on shell egg breakage rates during simulated transportation. *Poultry science, 78*(1), 148-151.

Van Tijen, W. F., & Kuit, A. R. (1970). The heritability of characteristics of egg quality, their mutual correlation and the relationship with productivity. *Instituut Voor De Pluimveeteelt 'Het Spelderholt' Mededeling* (172), 201-210.

Voisey, P. W., & MacDonald, D. C. (1978). Laboratory measurements of eggshell strength. 1. An instrument for measuring shell strength by quasi-static compression, puncture, and non-destructive deformation. *Poultry Science, 57*(4), 860-869.

O-01-4

Accuracy of Genomic-Polygenic Prediction for Milk Yield and Fat Percentage in the Thai Dairy Cattle Population

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INTRODUCTION

Advanced genotyping technologies provide valuable information on thousands of genotypes across the genome for dairy genetic evaluations. Genomic evaluation utilizes a combination of genotypes with pedigree and phenotypes to increase accuracy of prediction (Thomasen et al., 2012) and rate of genetic progress in dairy cattle (Buch et al., 2012). As a result, dairy genomic evaluations are routinely conducted in several countries (VanRaden et al., 2013; Bauer et al., 2015; Koivula et al., 2015). To speed up genetic improvement for economically important traits in dairy cattle raised under tropical environments, a national genomic-polygenic evaluation was implemented in Thailand in 2015 (Jattawa et al., 2015), and genomic breeding values for individual animals were made available to dairy producers in the 2016 sire and dam summary (Koonawootrittriron et al., 2016). A comparison between genomic-polygenic and polygenic evaluations would help explain dairy producers, stakeholders, researchers, and other interested people understand the advantages and disadvantages of implementing a genomic-polygenic evaluation in Thai multibreed dairy population. Thus, the objectives of this study were to compare prediction accuracies and animal rankings for 305-day milk yield (MY) and 305-day fat percentage (FP) using genomic-polygenic and polygenic models, and to evaluate genomic-polygenic EBV (GPEBV) trends for each trait as Holstein (H) fraction increased from 0% to 100%.

MATERIALS AND METHODS

phenotypic and pedigree records of 9,339 first-lactation cows from 1,002 farms located in Northern, Northeastern, Western, Central, and Southern Thailand were used in this research. These cows calved between 1989 and 2015 and were the progeny of 1,346 sires and 7,875 dams. Traits were MY and FP.

DNA samples were extracted from blood or semen samples of 2,661 animals (89 sires and 2,572 cows) in the population. These DNA samples were genotyped with four GeneSeek Genomic Profiler (GGP) chips (GeneSeek Inc., Lincoln, NE, USA), i.e., GGP9K (n = 1,412), GGP20K (n = 570), GGP26K (n = 540), and GGP80K (n = 139) chips. Animals genotyped with GGP9K (8,590 SNP), GGP20K (19,161 SNP), and GGP26K (25,979 SNP) were imputed to GGP80K (76,694 SNP) using FImpute 2.2 (Sargolzaei et al., 2014). The actual and imputed SNP genotypes with minor allele frequencies lower than 0.04 (n = 2,375) and with call rates lower than 0.9 (n = 175) were removed. The resulting genotype file contained 74,144 actual and imputed SNP markers available for the genomic-polygenic evaluation.

Variance and covariance components for MY and FP were estimated using a bivariate single-step genomic-polygenic model (GPM; Aguilar et al., 2010) and a bivariate polygenic model (PM). The GPM utilized pedigree, phenotypic, and genomic information, whereas the PM used only pedigree and phenotypic information. Fixed effects for GPM and PM included contemporary group (herd-year-season), calving age, and heterosis. Random effects were animal additive genetic and residual. Random effect means were assumed to be zero for both models. The PM variance-covariance matrix among additive genetic effects was equal to $A * \sigma_a^2$, where A = additive relationship matrix among all animals, "*" = Kronecker product, and σ_a^2 = additive genetic variance. The GPM variance-covariance matrix among additive genetic effects was equal to:

$$\begin{bmatrix} A_{11} + A_{12}A_{22}^{-1}(G_{22} - A_{22})A_{22}^{-1}G_{21} & A_{12}A_{22}^{-1}G_{22} \\ G_{22}A_{22}^{-1}A_{21} & G_{22} \end{bmatrix} * \sigma_a^2,$$

where A_{11}= additive relationship submatrix among non-genotyped animals, A_{12}= additive relationship submatrix between non-genotyped and genotyped animals, A_{22}^{-1} = inverse of the additive relationship submatrix for genotyped animals, G_{22}= matrix of genomic relationships for genotyped animals (VanRaden, 2008; Aguilar et al., 2010). The AIREMLF90 program (Tsuruta, 2014) was utilized to estimate variance and covariance components using an average information restricted maximum likelihood algorithm. Subsequently, estimates of variances and covariances were used to compute GPM and PM estimated breeding values (EBV) for all animals.

Prediction accuracies for MY and FP animal EBV were obtained as $\sqrt{1-\frac{PEV}{\sigma_a^2}} * 100$, where PEV = prediction error variance, and σ_a^2 = additive genetic variance. Prediction accuracies from GPM and PM were computed for all animals in the population, only genotyped animals, only genotyped cows, and only genotyped sires (young sires and proven sires). Proven sires in this research were sires that had ten daughters or more, whereas young sires were sires with less than ten daughters. Rankings of animal EBV from GPM and PM were compared using Spearman's rank correlations for all animals in the population. The GPEBV for each trait were plotted against Holstein fraction of animals from 0% to 100%. Regressions of GPEBV on Holstein fraction were computed for each trait to assess GPEBV trends as Holstein percentage increased.

RESULTS AND DISCUSSION

The MY and FP prediction accuracies computed for animal EBV from GPM and PM are shown in Figure 1. Accuracy distributions from GPM and PM were similar. However, GPM had higher numbers of animals with high prediction accuracies (50% and above) for MY and FP, and lower numbers of animals with low prediction accuracies (below 50%) than PM. The mean prediction accuracies for MY and FP from GPM were, on the average, 4.54% higher than those from PM. Thus, inclusion of genomic information in addition to pedigree and phenotypes increased EBV prediction accuracies in the Thai dairy population above those achieved using only pedigree and phenotypes.

Noticeably, inclusion of genomic information in GPM had a higher impact on increasing prediction accuracies for non-genotyped animals (4.76%) than for genotyped animals (3.49%; Table 1). This likely occurred because most genotyped animals here were sires and dams that had high number of progeny and other relatives in the population (on the average 29 ± 42 daughters per animal), whereas most non-genotyped animals were cows with few relatives (on the average 5 ± 8 daughters per animal) or had only their own phenotypes. Thus, EBV for genotyped animals were predicted with high accuracy even using only pedigree and phenotypes due to the large amount of information from their daughters and other relatives. Further, gains in prediction accuracy were substantially higher for genotyped young sires (14.34%) than for genotyped proven sires (0.18%). This was in agreement with previous studies reporting that inclusion of genomic information in dairy genetic evaluations had a high impact on increasing the accuracy of prediction for young animals (Pollott et al., 2014; Bauer et al., 2015). This outcome showed the advantage of using a genomic-polygenic evaluation to shorten generation interval and speed up genetic progress in the Thai dairy population.

The Spearman rank correlations between rankings of all animals, all sires, and all cows using EBV from GPM and PM are shown in Table 2. Rank correlations between GMP and PM EBV were high for all animals (0.88 for MY, and 0.83 for FP), all sires (0.91 for MY, and 0.87 for FP) and all cows (0.88 for MY, and 0.82 for FP). These high correlations indicated that EBV rankings from GPM were in close agreement with EBV rankings from PM. However, selection of replacement animals based GPEBV rankings would be expected to achieve faster selection responses than from PEBV rankings due to their higher accuracy.

Figure 2 shows that linear regression coefficients of GPEBV on Holstein fractions of all animals were positive for MY (0.36; $P < 0.0001$), but close to zero for FP (- 0.0002; $P < 0.001$). These regression coefficients indicated that animals with higher Holstein fraction tended to have higher GPEBV for MY, but lower GPEBV for FP than animals with lower Holstein fractions. However, regression coefficient values for both traits were low indicating that animals in this population with high, medium, or low Holstein fraction exhibited a wide range of GPEBV. This degree of variation indicated that using GPEBV to select replacement animals would be effective to increase MY and FY in the Thai dairy cattle population. Thus, producers should consider selecting animals based on individual GPEBV instead of simply choosing groups of animals with high Holstein fraction as replacements in their dairy operations.

CONCLUSSION

The GPM yielded higher prediction accuracies for MY and FP than the PM. Rankings between GPEBV and PEBV for MY and FP were similar. However, selecting animals with GPEBV would be expected to yield higher rates of genetic progress in the Thai dairy population than with PEBV due to their higher accuracy. Lastly, animals with higher Holstein fraction tended to have higher GPEBV for MY, but lower GPEBV for FP than animals with lower Holstein fractions.

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KEYWORD : Dairy, Multibreed, Breeding, Genomic, Prediction accuracy

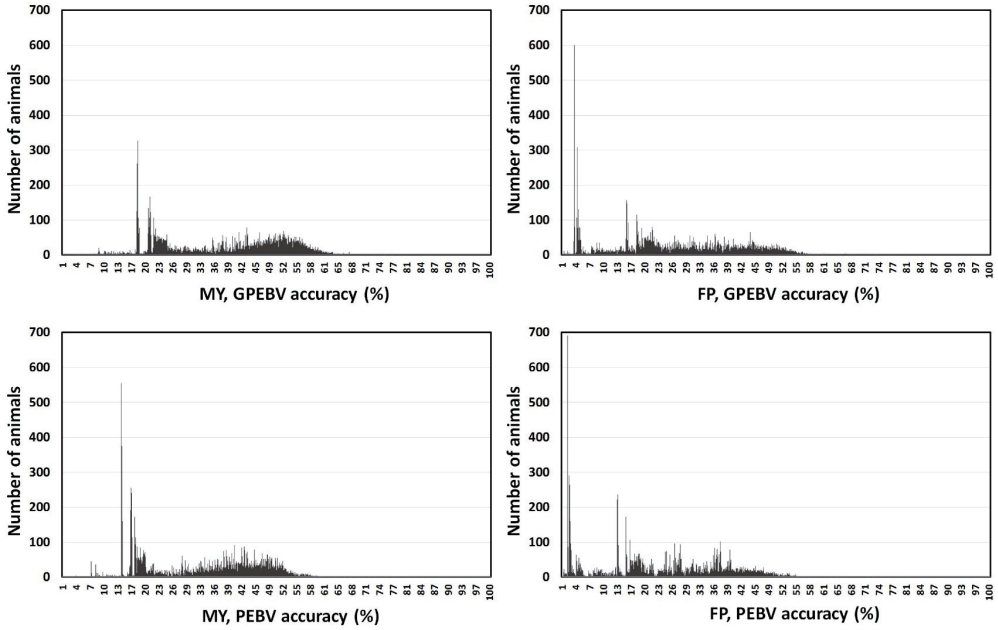

Figure 1. Number of animals by accuracies of EBV from genomic-polygenic model (GPEBV) and polygenic model (PEBV) for 305-d milk yield (MY) and 305-d fat percentage (FP)

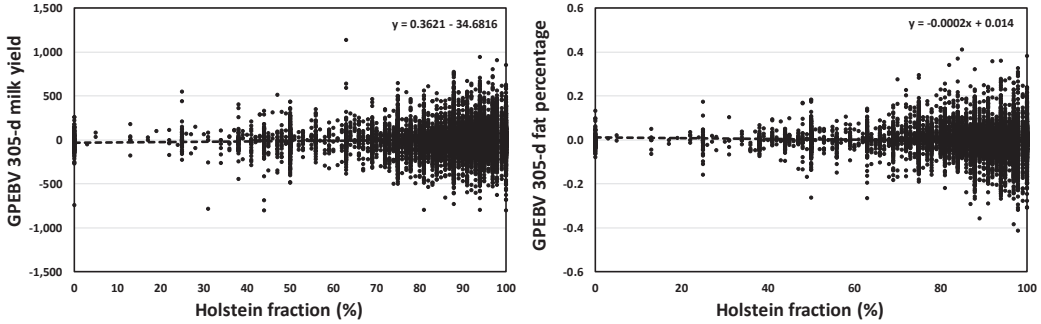

Figure 2. Genomic-polygenic EBV (GPEBV) as Holstein fraction increases from 0% to 100% for 305-d milk yield and 305-d fat percentage

Table 1. Mean prediction accuracies for 305-d milk yield (MY) and 305-d fat percentage (FP) from genomic-polygenic (GPM) and polygenic (PM) models

Type of animal[a]	Number of animals	GPM		PM		Accuracy Increase
		MY	FP	MY	FP	
All animals	17,363	38.37 (14.84)[b]	27.46 (15.59)	33.11 (13.76)	23.63 (14.95)	4.54
Non-genotyped animals	14,702	36.72 (15.14)	26.38 (15.95)	31.12 (13.47)	22.45 (14.72)	4.76
Genotyped animals	2,661	46.17 (10.20)	32.47 (12.67)	42.55 (10.94)	29.12 (14.82)	3.49
Genotyped cows	2,572	34.50 (17.03)	26.24 (18.58)	26.86 (13.60)	18.95 (14.61)	7.46
Genotyped sires	89	64.50 (16.26)	43.81 (17.22)	59.96 (19.74)	43.17 (20.38)	2.59
Genotyped proven sires	58	69.48 (11.31)	47.10 (15.54)	67.23 (12.79)	49.00 (15.94)	0.18
Genotyped young sires	31	42.29 (16.72)	26.47 (15.71)	27.51 (10.03)	12.47 (11.88)	14.39

[a] Genotyped young sires = sires that had less than ten daughters; genotyped proven sires = sires that had ten daughters or more

[b] Numbers in brackets are SD of mean prediction accuracies

Table 2. Rank correlations between genomic-polygenic EBV and polygenic EBV for 305-d milk yield and 305-d fat percentage

Trait	Rank correlation	P-value
All animals		
305-d milk yield	0.88	<.0001
305-d fat percentage	0.83	<.0001
Sires		
305-d milk yield	0.91	<.0001
305-d fat percentage	0.87	<.0001
Cows		
305-d milk yield	0.88	<.0001
305-d fat percentage	0.82	<.0001

REFERENCES

Bauer, J., J. Přibyl, and L. Vostrý. 2015. Short communication: Reliability of single-step genomic BLUP breeding values by multi-trait test-day model analysis. J. Dairy Sci. 98: 4999-5003.

Buch, L.H., M.K. Sørensen, P. Berg, L.D. Pedersen, and A.C. Sørensen. 2012. Genomic selection strategies in dairy cattle: Strong positive interaction between use of genotypic information and intensive use of young bulls on genetic gain. J. Anim. Breed. Genet. 129: 138-151.

Jattawa, D., M.A. Elzo, S. Koonawootrittriron, and T. Suwanasopee. 2015. Comparison of genetic evaluations for milk yield and fat yield using a polygenic model and three genomic-polygenic models with different sets of SNP genotypes in Thai multibreed dairy cattle. Livest. Sci. 181: 58-64.

Koivula, M., I. Strandén, J. Pösö, G.P. Aamand, and E.A. Mäntysaari. 2015. Single-step genomic evaluation using multitrait random regression model and test-day data. J. Dairy Sci. 98: 2775-2784.

Koonawootrittriron, S., M.A. Elzo, T. Suwanasopee, W. Chunpet, and T. Tongprapi. 2016. D.P.O. Sire & Dam Summary 2016. Dairy Farming Promotion Organization, Ministry of Agriculture and Cooperatives of Thailand, Bangkok.

Pollott, G.E., A. Charlesworth, and D.C. Wathes. 2014. Possibilities to improve the genetic evaluation of a rare breed using limited genomic information and multivariate BLUP. Animal 8: 685-694.

Thomasen, J.R., B. Guldbrandtsen, G. Su, R.F. Brøndum, and M.S. Lund. 2012. Reliabilities of genomic estimated breeding values in Danish Jersey. Animal 6: 789-796.

Tsuruta, S., 2014. Average Information REML with several options including EM-REML and heterogeneous residual variances. Available at: http://nce.ads.uga.edu/wiki/doku.php?id=application_programs.

VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414-4423.

VanRaden, P.M., D.J. Null, M. Sargolzaei, G.R. Wiggans, M.E. Tooker, J.B. Cole, T.S. Sonstegard, E.E. Connor, M. Winters, J.B. van Kaam, A. Valentini, B.J. Van Doormaal, M.A. Faust, and G.A. Doak. 2013. Genomic imputation and evaluation using high-density Holstein genotypes. J. Dairy Sci. 96: 668-678.

O-01-6

Genetic fluctuation study with microsatellite markers in germplasm-preserved Wujie Black Muscovy

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INTRODUCTION

Black Muscovy duck, derived from rainforest of South America and mainly lived in swamp, were brought in Taiwan at the mid 17th century by the Spanish and Portuguese trading ships and have been raised for more than 300 years (Hung et al., 1996). The black Muscovy ducks have two characteristics of drought enduring and heat resistant, which are more appropriate to live in countryside in Taiwan. In early years, the farmers produced mule ducks by crossbreeding with black Muscovy drakes and brown Tsaiya ducks. This was the major application of black Muscovy ducks in Taiwan (Chou and Huang, 1970). However, there were two problems in this production system of mule ducks, the first one was the black Muscovy duck are seasonal reproductive avian and has broodiness so the reproductive season is shorter than other breeds of duck; and the second one was the mule ducks produced from black Muscovy ducks had smaller body size and the there were deep-colored pin feathers on their skin. That caused negative influence on preference of carcass appearance. To elevate the body weight and to improve the plumage of mule ducks, the white Muscovy duck was introduced from Australia, America and Netherlands, successively. As a consequence, the population size of black Muscovy duck has dramatically declined, and many of them have deviated from their breed features because of hybridization with white Muscovy duck (Kang et al., 1992; Chou and Huang, 1970).

Considering the economic and cultural values, to preserve the diversity of genetic resource of black Muscovy, a germplasm-preserved population had been established from 1987 and nomenclatured as Wujie Black Muscovy at 2013 by the ILan Branch, Livestock Research Institute. Each new generation of this population had been reproduced following random mating from 1st to 7th generation, and then the rotational mating system had been applied to further avoid severe inbreeding depression and maintain the biodiversity (Livestock research institute, 2013).

Previously, except for monitoring of vital traits, such as growth, reproduction, and egg traits generation by generation, to perceive the genetic change without the interference of environment effects, we conducted a cross-generation genetic analysis in Wujie Black Muscovy using 11 microsatellite markers derived from Tsaiya duck in germplasm preservation work and confirmed the capability of those markers in genetic monitoring of Wujie Black Muscovy (Chang et al., 2015). However, a comparison of genetic analysis among different generations should be conducted to figure out the exact effect of the rotational mating system on maintenance of biodiversity and genetic structure. Therefore, here we conducted a cross-generation genetic analysis before (the 6th generation (G6)) and after (the 9th (G9) and the 13th (G13) generation) rotational mating system applied and tried to investigate the genetic fluctuation of Wujie Black Muscovy population.

MATERIALS AND METHODS

A total of 92 Wujie Black Muscovy, each 15 drakes and 15 ducks of G6 and G13 generation and 16 drakes and 16 ducks of G9, were used as experimental animals. Genotype data of G9 and G13 were obtained in the previous study (Chang et al., 2015), and the one of G6 was conducted by following processes with only the PCR recipe slightly modified. The genomic DNA was extracted from fresh blood sample treated by EDTA using EasyPure Genomic DNA mini kit (Bioman, Taiwan). Eleven microsatellite markers, which specially developed from Brown Tsaiya ducks, were used in this study, including APT001, APT004, APT008, APT010, APT012, APT017, APT020, APT025, APT026, APT032 and APT033 (Hsiao et al., 2008). The PCR reaction was performed in a final volume of 15 μ L, containing 100 ng genomic DNA, 1.5 mM MgCl, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM dNTP, 0.4 μ M of forward and reverse primer and 0.01 U Taq DNA polymerase (Takara, Japan). The PCR conditions were 95 for 10 min, 30 cycles of 95 for 20 s, 50 (APT025 and APT026) or 60 for 30 s, 72 for 30 s and a final extension step of 10 min at 72 . After capillary electrophoresis conducted by National Center for Genome Medicine of Academia Sinica in Taiwan, ABI 3730 sequencer and Peak Scanner Software v1.0 (Applied Biosystems, USA) were employed

in genotyping of these markers.

The population genetic parameters such as number of alleles (Na), number of effective alleles (Ne), observed (H_O) and expected (H_E) heterozygosities, polymorphism information content (PIC), and F_{IS} in Wright's fixation index (Wright, 1978) were evaluated using POPGENE software package v1.32 (Yeh et al., 1999), Cervus program v3.0 (Kalinowski et al., 2007), and Hardy-Weinberg equilibrium was tested by GENEPOP v3.4b (Raymond and Rousset, 1995). Furthermore, the differentiation between populations was estimated with FSTAT 2.9.3 (Goudet, 2002). Additionally, we ran STRUCTURE v2.3.3 (Pritchard et al., 2000) from K = 1-10, 20 independent runs for each K value with $1x10^7$ Markov chain Monte Carlo iterations after a burnin period of $5x10^3$ repetitions. The best K was evaluated following the Evanno method (Evanno et al., 2005) using the STRUCTURE HARVESTER v0.6.91 application (Earl and vonHoldt, 2012). And adegenet package of R software (Jombart and Ahmed, 2011) were performed to infer the population structure by conducting principal component analysis (PCA).

RESULT AND DISCUSSION

The genetic variation of these microsatellite markers in G6 of Wujie Black Muscovy were shown in Table 1. A total of 38 alleles (ranged from 2 to 6) with an average of 3.5 alleles per microsatellite locus was observed and an average 2.4 effective alleles per microsatellite locus. The observed and expected heterozygosity of those polymorphic markers ranged from 0.100 to 0.724 with an average number of 0.362 and 0.097 to 0.767 with an average number of 0.486, respectively. In Wujie Black Muscovy population, there were eight reasonably (PIC > 0.25) to highly (PIC > 0.5) informative markers. It showed that the set of eleven microsatellite markers used in this study should have enough polymorphisms to investigate the genetic structures. In terms of population genetics, all of eleven microsatellite markers tested fitted Hardy-Weinberg equilibrium. Although, the mean of F_{IS} value was 0.208, it had no significant difference from 0. It demonstrated that G6 of Wujie Black Muscovy was not faced with inbreeding depression problems.

Compare the genetic parameter of G6 with G9 and G13 shown in Table 2 (Chang et al., 2015), the results showed that although the number of alleles in G6 was slightly more than the other generations did, there were almost no differences in heterozygosities among the three generations. In addition, in all of the three generations, the estimated F_{IS} value had no significant differences from 0, which indicated that the three generations of Wujie Black Muscovy population were not endangered by inbreeding depression. However, the difference of observed and expected heterozygosity and the F_{IS} value of G6 were slightly larger than the other generations, it suggested that rotational mating system may has the effect for minimizing inbreeding and elevating observed heterozygosity. When taking a view of F_{ST}, there was almost no differentiation between each two of the generations, except for low differentiation between G6 and G13 (F_{ST} = 0.0580), while the F_{ST} was larger between G6 and G9 (F_{ST} = 0.0247) rather than that between G9 and G13 (F_{ST} = 0.0145), which may resulted from the change of mating system. Furthermore, the STRUCTURE result showed a trend that the populations, before (G6) and after (G9 and G13) rotational mating system been applied, had different genetic structures. According to Nomura and Yonezawa (1996), in a close population, it is impossible to avoid inbreeding completely, but if it proceeds gradually, it may not cause serious inbreeding depression, because deleterious alleles can be eliminated gradually through nature selection. Our experiment results suggested that rotational mating system may be an effective way to maintain heterozygosity, reduce population differentiation, and slow down the inbreeding progress.

In the future, the rotational mating system would be carried on, and we will apply more highly polymorphism microsatellite markers for continuous genetic monitoring, accompanied with phenotype monitoring, to maintain the Wujie Black Muscovy population sustainably.

KEYWORD : Wujie Black Muscovy, Genetic Diversity, Genetic Fluctuation, Microsatellite Markers

Figure 1. Genetic cluster analysis for the G6, G9 and G13 generation of Wujie Black Muscovy population using STRUCTURE software. K: possible number of subpopulation (in this figure K = 2); Q: proportional membership of Wujie Black Muscovy to genetic clusters. Colors correspond to different genetic clusters. Each vertical bar represents a single individual.

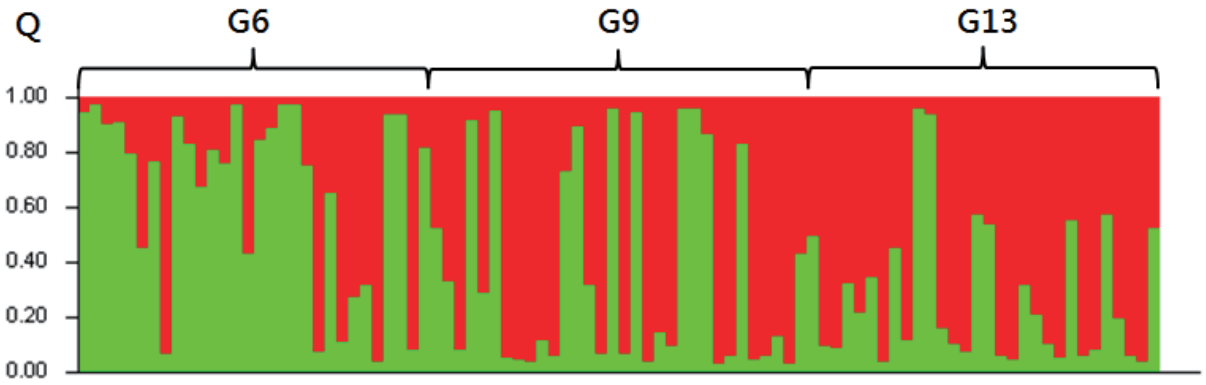

Table 1. List of primer sequences of 11 Tsaiya microsatellite markers in this study

Locus[a]	Primer sequences (5'→3')	T_a (℃)[b]	Duck genome scaffold no.[c]
APT001	F: GTCCCACTGGTTTGCTGTCC R: ACTACGCATGGCAGTGAGGTT	60	1509
APT004	F: GGGCAGGAAAATCTCCTGAAT R: TCTCAGTGGCTGAGCGGTC	60	192
APT008	F: CAAAGAAATCCTAGAACATCATTCAAAT R: TCTTCTGGCTTTTCACCTTAGTTTAGTA	60	358
APT010	F: CACTCAGGCTTTTAGGTCCATTAATA R: CATCTGAGAATGCACTTACTGTCAAA	60	1199
APT012	F: TTGAGCCTCAGGTTCTAAACTCCTA R: TCATAACATTTCAGACCAGTTTTCAGA	60	5
APT017	F: TGGATGGACAGACGGGTGA R: TGGAAGTTTTGATTTCTAGTGCTTACA	60	481
APT020	F: TTCCAAGTTTGTCATGCCAATAGA R: CTGACCATGTTAGGGCGTTTTAG	60	197
APT025	F: TCCTAAGAAACGTTGCTTCATAGACC R: GAGTTAAGCTTCATCACTCTGTGACTG	50	121
APT026	F: CCCTGAAAGGCTGTTTTATATATCCA R: ATGTAAATAAAGTAGCCTTGCACGGT	50	477
APT032	F: TCACTTTCTTGACTCTCCTTGGTTT R: TGACTTGAATTCTGTTCAGGATAAATG	60	45
APT033	F: CTTCACCCTACCTCATAAGGAACTG R: ATTCCAAATCTGCAAGGTGAGTATTA	60	14

[a] Hsiao *et al.* (2008). Developed from Tsaiya duck.

[b] Annealing Temperature

[c] The orthologous microsatellites in the duck genome scaffold

Table 2. Genetic variation of the G6 of Wujie Black Muscovy analyzed by 11 Tsaiya microsatellite markers

Locus	Fragment size (bp)	N_a[a]	N_e[b]	H_O[c]	H_E[d]	PIC[e]	F_{IS}[f]
APT001	228-260	2	1.6	0.167	0.381	0.305	0.562
APT004	289-297	2	1.1	0.100	0.097	0.090	-0.031
APT008	164-180	4	2.3	0.500	0.589	0.497	0.151
APT010	184-192	2	1.2	0.167	0.155	0.141	-0.077
APT012	157-177	3	3	0.167	0.684	0.591	0.756
APT017	169-177	2	1.1	0.133	0.127	0.117	-0.047
APT020	169-201	6	3.9	0.724	0.759	0.706	0.046
APT025	120-136	5	4.1	0.567	0.767	0.713	0.261
APT026	134-142	3	2.7	0.600	0.635	0.554	0.055
APT032	201-237	6	3	0.655	0.678	0.621	0.034
APT033	217-229	3	1.9	0.200	0.474	0.403	0.578
Average		3.5	2.4	0.362	0.486	0.431	0.208
SD		1.6	1.1	0.244	0.258		0.292

[a] Number of alleles

[b] Expective number of alleles

[c] Observed heterozygosity

[d] Expected heterozygosity

[e] Polymorphic information content

[f] Wright's fixation index, within population inbreeding estimate

Table 3. Genetic variation of G9 and G13 of Wujie Black Muscovy analyzed by 11 Tsaiya microsatellite markers (Chang et al., 2015)

Locus	G9							G13						
	Fragment (bp)	N_a[a]	N_e[b]	H_O[c]	H_E[d]	PIC[e]	F_{IS}[f]	Fragment (bp)	N_a[a]	N_e[b]	H_O[c]	H_E[d]	PIC[e]	F_{IS}[f]
APT001	228-260	2	1.7	0.233	0.413	0.324	0.436	228-260	2	1.9	0.200	0.472	0.357	0.576
APT004	289-297	2	1.6	0.367	0.381	0.305	0.037	289-297	2	1.5	0.300	0.345	0.282	0.130
APT008	164-188	4	2.8	0.690	0.658	0.575	-0.049	164-176	3	2.0	0.600	0.505	0.402	-0.188
APT010	184-192	2	1.0	0.033	0.033	0.032	0.000	184-192	2	1.1	0.067	0.066	0.062	-0.015
APT012	157-177	3	1.6	0.167	0.382	0.333	0.563	157-177	3	1.2	0.040	0.189	0.176	0.788
APT017	169-177	2	1.1	0.133	0.127	0.117	-0.047	169-177	2	1.1	0.133	0.127	0.117	-0.047
APT020	173-201	5	3.0	0.767	0.681	0.604	-0.126	169-201	6	4.2	0.700	0.773	0.725	0.094
APT025	120-136	5	3.1	0.567	0.685	0.621	0.172	120-136	5	3.9	0.793	0.754	0.697	-0.052
APT026	134-142	3	2.5	0.600	0.608	0.526	0.013	134-142	3	1.6	0.367	0.382	0.333	0.039
APT032	201-237	5	3.2	0.724	0.699	0.623	-0.036	201-237	4	3.1	0.700	0.685	0.604	-0.022
APT033	217-225	2	1.8	0.259	0.440	0.338	0.411	217-229	3	1.5	0.233	0.358	0.309	0.349
Average		3.2	2.1	0.413	0.464	0.400	0.125		3.2	2.1	0.376	0.423	0.369	0.150
SD		1.3	0.8	0.264	0.229		0.236		1.3	1.1	0.275	0.243		0.299

[a] Number of alleles

[b] Expective number of alleles

[c] Observed heterozygosity

[d] Expected heterozygosity

[e] Polymorphic information content

[f] Wright's fixation index, within population inbreeding estimate

REFERENCE

Chang, Y. Y., C. F. Huang, L. Y. wei, M. C. Hsiao, and H. C. Liu. 2015. Germplasm preservation and prior study of genetic monitoring by Tsaiya microsatellite markers in Wujie Black Muscovy (*Cairina moschata*). J. Chin. Soc. Anim. Sci. 44:131-146.

Chou, K. Y., and H. H. Huang. 1970. Chinese-American Joint Commission on Rural Reconstruction. The duck industry in Taiwan. (Animal Industry Series no. 8).

Earl, D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611-2620.

Goudet, J. 2002. FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3. Department of ecology and evolution, Lausanne University, Lausanne, Switzerland.

Hsiao, M. C., H. C. Liu, Y. C. Hsu, Y. H. Hu, and S. R. Lee. 2006. Isolation and characterization of microsatellite markers in Tsaiya duck. Asian-Aust. J. Anim. 21:624-627.

Hung, F. L., C. D. Tien, and M. C. Chen. 1996. The history of raising Muscovy duck (6). Chin. Livest. Magazine 28:97-101. (in Chinese)

Jombart, T., and I. Ahmed. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 21:3070-3071.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099-1106.

Kang, C. L., S. R. Lee, J. F. Huang, and C. C. Huang. 1992. The preservation and utilization of Tsaiya duck and Black Muscovy duck germplasm. Annual experimental report, Taiwan Livestock Research Institute.

Livestock research institute. 2013. Verification letter of Wujie Black Muscovy. Retrieved July 11, 2016, From http://www.angrin.tlri.gov.tw/duck/BM-268_Application.pdf.

Nomura, T., and Yonezawa, K. 1996. A comparison of four systems of group mating for avoiding inbreeding. Genet. Sel. Evol. 28:141-159.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M., and F. Rousset. 1995. Genepop (version 3.4): Population genetics software for exact tests and ecumenicism. J. Hered. 896: 248-249.

Wright, S. 1978. Variability within and among natural populations, in: Evolution and the Genetics of Populations, Vol. 4. University of Chicago, Chicago. pp. 53-56.

Yeh, F. C., T. Boyle, Y. Rongcai, Z. Ye, and J. M. Xin. 1999. POPGENE, Version 1.31. A Microsoft Window based free ware for population genetic analysis. University of Alberta, Edmonton.

O-02-3 A study on the correction factors between temperature-humidity index and body surface temperature for Hanwoo heifer (Bos taurus coreanae)

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Objective

Body temperature is one of the most common indicators used for the diagnosis of illness and has been used in the measurement of the physiological status of animals. Infrared thermography (IRT) is a simple, rapid and non-invasive method of measuring the body surface temperature (BST) of animals (Colak et al, 2008). The present study aims to make correction factors for a correlation analysis between body surface temperature (BST) of Hanwoo heifer and temperature-humidity index with seasonal environmental factors.

Methology

The experiment was performed at a beef cattle farm in Gyeonggi-do, South Korea, for 1 year. 8 Hanwoo heifers were used in this exprements. Each heifers were housed in a pen during all times, with a space allowance of $10m^2$ per head. The heifer house was covered with a 4.5m roof that induced ventilation and included a winch curtain, which prevented the entry of cold wind in the winter. The curtain was mostly open, except when there was strong cold wind in winter. Feed was mixed in the form of total mixed ration (TMR) which was based on the feeding standard of Hanwoo. The chemical compositions of experimental diets are presented in Table 1. The animals were allowed free access to feed and water. Sawdust was spread on the floor to a thickness of 15cm, and a dry condition was maintained by regular sawdust replacement.

An infrared thermal imaging camera CX-320U (COX, Taejeon, Korea) was used to measure BST. Infrared thermography was collected seven times a day at 07, 09, 11, 13, 15, 17, 19 hours, with three replications per animal each season. Samples of BST collected from a distance greater than 3 m from the camera were excluded due to the sensitivity of the camera. BST of animals exposed to direct sunlight may cause very inaccurate results and, therefore, IRTs were collected out of direct sunlight.

Ambient temperature (AT) and relative humidity (RH) were recorded seven times a day, according to the infrared thermal imaging sampling time. The temperature-humidity index (THI) was calculated using the formula of Ravagnolo et al. (2000).

Five distinct areas were measured to evaluate BST, the eyes, nose, horns, ears and rear. The eyes region includes conjunctiva and the skin that surrounds the conjunctiva; nose pertains to the nasal cavity; horns pertain to the stratum comeum (epikaras) of both horns; ears include all of the external ear that is exposed to the outside and is visible; and rear area refers to the vicinity of the anus that was hidden by the tail. The average and maximum surface temperature of each area measured was determined using Thermal Imaging Analyzer software (ver. A.8, COX, Taejeon, Korea).

Statistical analysis was conducted using ANOVA of the general linear modelling procedure of the SAS Program (ver. 9.1, SAS Institute, Cary, NC, USA). Comparison among averages of the treatment groups was conducted using Duncan's test at a significance level of 5%.

Result

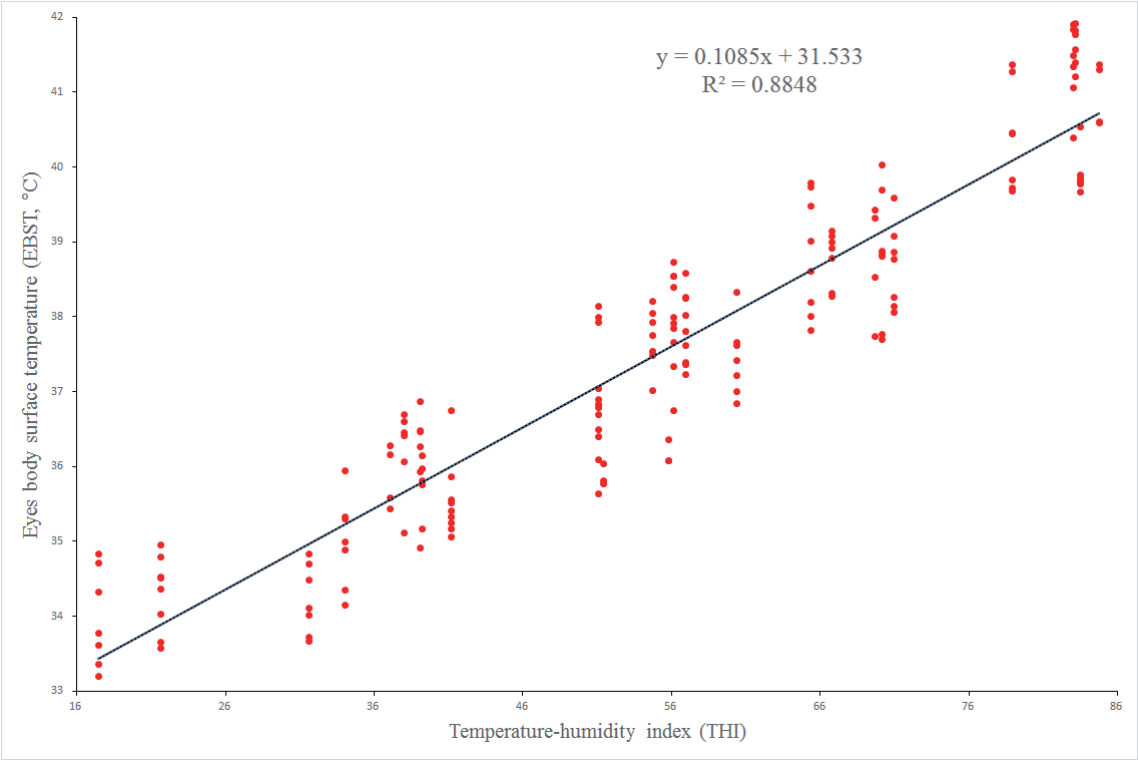
The changes in BST of the five regions according to season and time are presented in Table 2. A wide range of BST was observed throughout the experimental period, which suggests that BST is directly affected by ambient temperature. Core temperature maintain about 37~38°C for seasonal changes. The THI was calculated using the formula of Ravagnolo et al (2000). THI were 34.0~56.9 in spring (AT: -1.0~13.4°C), 75.1~84.7 in summer (AT: 24.9~33.6°C), 55.8~70.9 in autumn(AT: 13.0~26.0°C) and 17.5~39.2 in winter (AT: -10.4~1.0°C) (Table 2). The BST of cattle showed a varied result according to each body region. The BSTs of nose, horns and ears were significantly ($P<0.05$) lower than those of the eyes and rear area.ANOVA using the general linear models procedure and a regression analysis was performed using SPSS 23 software (ver, 2013). In the regression analysis, a coefficient of determination were between THI and BST [eyes (R^2=0.88), rear (R^2=0.72), nose (R^2=0.83), horns

(R^2=0.86) and ears (R^2=0.85)].

Conclusion

These basically means that body surface temperature have a strong correlation with environmental factors. However, nose, horns and ears has a wide gap for BST each measuring times. It is indicated that eye and rear are taken as significant factors.

KEYWORD : body surfact temperature, temperature-humidity index, Hanwoo heifer, seasonal variation



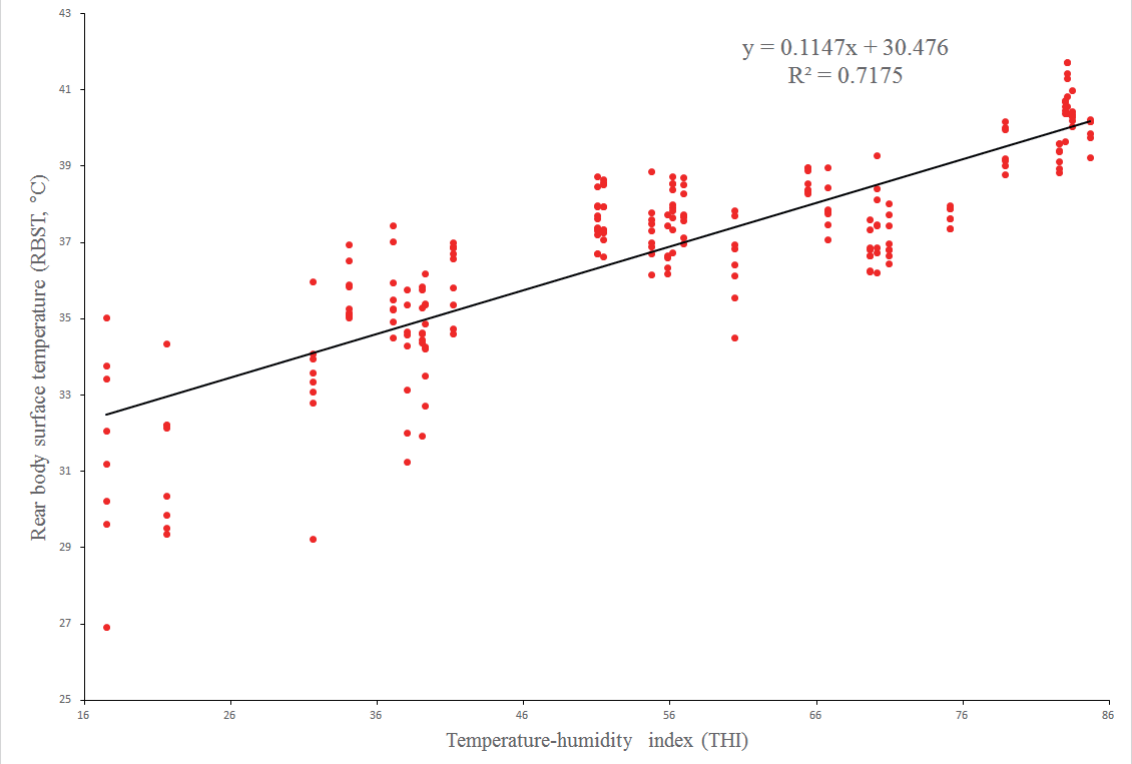


Table 1. The chemical compositions of experimental diets.

Item	TMR*
Dry matter (%)	63.12
	----------% DM----------
CP[1)]	14.60
CF[2)]	14.52
NDF[3)]	37.60
ADF[4)]	19.61
EE[5)]	4.97
Ash	6.85
Ca	0.58
P	0.56
TDN**	74.26

[1)] Crude protein, [2)] Crude fiber, [3)] Neutral detergent fiber, [4)] Acid detergent fiber, [5)] Ether Extract

* Total mixed ration ** Total digestible nutrition.

Table 2. Seasonal changes in body surface temperature in different body regions of the Hanwoo heifer (n=8)

Collection time	Body region	Spring			Summer			Autumn			Winter		
		Mean	s.d.	AT, RH (THI)	Mean	s.d.	AT, RH (THI)	Mean	s.d.	AT, RH (THI)	Mean	s.d.	AT, RH (THI)
0700 hours	Eyes	35.07	1.02	-1°C	37.72	0.18	24.9°C	35.57	0.64	13.0°C	33.65	1.11	-10.4°C
	Nose	29.03	1.42	75% (34.0)	36.09	0.67	84% (75.1)	28.57	2.03	73% (55.8)	22.88	2.10	83% (17.5)
	Horns	23.03	2.13		35.06	0.67		29.46	2.49		10.32	1.56	
	Ears	21.10	2.52		35.43	1.03		27.26	1.81		10.68	2.91	
	Rear	35.66	0.70		37.68	0.25		36.83	0.62		31.54	2.62	
0900 hours	Eyes	35.47	0.55	2°C	40.40	0.71	27.9°C	37.12	1.03	16.0°C	34.31	0.51	-8.0°C
	Nose	27.61	1.71	55% (41.1)	38.62	1.41	75% (78.9)	32.77	1.14	72% (60.4)	24.02	2.13	82% (21.6)
	Horns	27.25	1.75		38.39	0.61		33.47	1.19		19.37	2.15	
	Ears	22.27	1.66		38.60	1.19		31.83	1.19		14.31	2.96	
	Rear	36.15	0.94		39.48	0.56		36.50	1.11		31.26	1.75	
1100 hours	Eyes	35.15	0.53	8.8°C	39.67	1.54	31.2°C	37.91	1.06	24.0°C	34.09	0.57	-3.5°C
	Nose	28.52	1.46	36% (51.4)	39.05	1.18	66% (82.6)	35.95	1.28	41% (69.6)	22.33	2.65	67% (31.6)
	Horns	27.63	0.71		39.58	1.02		35.39	0.45		24.61	1.71	
	Ears	22.32	1.16		38.87	1.19		34.52	0.74		18.80	3.75	
	Rear	37.73	0.70		39.28	0.30		36.81	0.47		33.26	1.89	
1300 hours	Eyes	37.98	0.62	12.7°C	41.73	0.38	31.5°C	38.51	0.72	26.0°C	35.73	1.06	-2.0°C
	Nose	33.25	1.45	27% (56.1)	40.52	0.67	67% (83.1)	36.26	1.35	31% (70.9)	26.53	2.62	41% (38.0)
	Horns	32.49	0.74		40.68	0.55		36.11	1.18		26.14	1.86	
	Ears	32.81	2.57		40.27	0.81		35.53	0.67		20.47	4.99	
	Rear	38.13	0.71		41.15	0.54		37.12	0.56		33.89	1.60	
1500 hours	Eyes	37.84	0.47	13.4°C	41.57	0.99	33.6°C	38.64	0.95	25.0°C	35.06	1.04	-1.0°C
	Nose	34.15	1.60	25% (56.9)	41.10	0.71	59% (84.7)	36.74	1.21	34% (70.1)	27.70	2.28	41% (38.0)
	Horns	32.75	0.93		41.46	0.82		36.56	0.51		25.95	1.47	
	Ears	32.81	2.22		40.41	0.75		35.97	1.27		23.76	3.20	
	Rear	37.81	0.59		39.91	0.38		37.58	1.00		34.58	1.12	
1700 hours	Eyes	37.82	0.52	11.3°C	41.45	0.58	31.8°C	38.95	0.55	22.0°C	35.47	1.40	-1.4°C
	Nose	33.31	1.90	24% (54.7)	40.07	0.56	64% (83.0)	36.78	1.08	35% (66.7)	27.44	3.11	39% (39.0)
	Horns	32.44	1.23		39.93	0.69		36.18	1.36		28.35	1.31	
	Ears	33.27	1.69		39.90	0.59		35.57	1.69		22.85	5.56	
	Rear	37.32	0.77		40.46	0.38		37.91	0.57		34.62	1.22	
1900 hours	Eyes	36.91	0.77	7.7°C	39.91	0.29	30.4°C	38.84	0.79	20.0°C	36.28	1.31	-2.0°C
	Nose	32.24	2.48	23% (51.0)	38.78	0.39	80% (83.5)	37.01	0.98	52% (65.4)	27.48	2.71	47% (37.0)
	Horns	30.08	1.44		38.40	0.26		36.62	1.86		30.02	2.84	
	Ears	32.19	2.83		39.06	0.67		36.28	1.53		23.56	2.06	
	Rear	37.60	0.62		40.38	0.30		38.60	0.30		35.74	1.02	

AT, ambient temperature (°C); RH, relative humidity (%); THI, temperature-humidity index (Ravagnolo et al. 2000)

Mean followed by the same letter are not significantly different (P=0.05) among the five regions of the body (columns)

REFERENCES

Bergsten, C. 1993. A photometric method for recording hoof diseases in cattle, with special reference to hemorrhages of the sole. Acta Vet. Scand. 34:281 286.

Berry, R. J. 1999. A systems study of lameness in dairy cattle: Effectsof behavior and production. Ph.D. Diss. University of Edinburgh,UK.

Berry, R. J., A. D. Kennedy, S. L. Scott, B. L. Kyle, and A. L. Schaefer. 2003. Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis detection. Can. J. Anim. Sci. 83:687 693.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. E. D. Olfert, B. M. Cross, and A. A. McWilliam, ed. CCAC, Ottawa, ON, Canada.

Donovan, G. A., C. A. Risco, G. M. DeChant-Temple, T. Q. Tran, and H. H. van Horn. 2004. Influence of transition diets on occurrence of subclinical laminitis in Holstein dairy cows. J. Dairy Sci.87:73 84.

Eddy, A. L., L. M. van Hoogmoed, and J. R. Snyder. 2001. Review: The role of thermography in the management of equine lameness. Vet. J. 162:172 181.

Enevoldsen, C., Y. T. Gro ¨ hn, and I. Thysen. 1991. Sole ulcers in dairy cattle: Associations with season, cow characteristics, disease, and production. J. Dairy Sci. 74:1284 1298.

Green, L. E., V. J. Hedges, Y. H. Schukken, R. W. Blowey, and A.J.Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85:2250 2256.

Greenough, P. R., F. J. MacCallum, and A. D. Weaver. 1981. Lamenessin cattle. Pages 124 150 (Chapter 8) in Anatomy of the Digital Region. 2nd ed. A. D. Weaver, ed. Lippincott Co., Philadelphia, PA.

Greenough, P. R., and J. J. Vermunt. 1990. Evaluation of subclinical laminitis and associated lesions in dairy cattle. Update in cattle lameness. Pages 45 54 in Proc. VIth Int. Symp. Dis. Ruminant Digit, Liverpool, UK. Br. Cattle Vet. Assoc., Frampton-on-Severn,UK.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005 1028.

Schaefer, A. L., N. Cook, S. V. Tessaro, D. Deregt, G. Desroches, P.L. Dubeski, A. K. W. Tong, and D. L. Godson. 2004. Early detection and prediction of infection using infrared thermography. Can. J.Anim. Sci. 84:73 80.

Schwartzkopf-Genswein, K. S., and J. M. Stookey. 1997. The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle. Can. J. Anim. Sci. 77:577 583.

Stone, W. C. 2004. Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E. Suppl.):E13 E26.

van Amstel, S. R., J. K. Shearer, and F. L. Palin. 2004. Moisture content, thickness, and lesions of sole horn associated with thin soles in dairy cattle. J. Dairy Sci. 87:757 763.

Vermunt, J. J., and P. R. Greenough. 1995. Structural characteristics of the bovine claw: Horn growth and wear, horn hardness and claw conformation. Br. Vet. J. 151:157 180.

O-02-6 THE CHARACTERIZATION OF ADIPOSE TISSUE VIA A METABOLIC SYNDROME MINIPIG MODEL

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Introduction

The observed increase in the incidence of metabolic syndrome (MetS) is a worldwide phenomenon (Aguilar-Salinas et al., 2003). This syndrome is a major risk factor for developing type 2 diabetes mellitus (T2D) and cardiovascular disease (CVD), which are the main causes of death in the world (Aguilar-Salinas et al., 2004). Diagnosis of MetS requires three of the following five criteria being satisfied: central obesity, dyslipidemia, high cholesterol, low concentration of high density cholesterol (HDL), and hyperglycemia (Grundy, 2005). MetS represents a cluster of cardiovascular risk factors, including insulin resistance, lipid abnormalities, and obesity that are associated with increased risk of CVD (Wang et al., 2010). In addition, MetS is a growing public health issue that is becoming hyper-endemic around the world, with related increases in health care use and cost (Boudreau et al., 2009).

The main storage compartment for lipid in the human is adipose tissue (Trayhurn and Beattie, 2001). It appears that in addition to fat amount, the fat composition of adipose tissue modulates several metabolic processes that take place in adipocytes (Westcott et al., 2005). Moreover, metabolic activity, such as releasing inflammatory factors of subcutaneous, visceral, and pericardial adipose tissue seem relative to metabolic diseases. Inflammation receives increased attention for its potential role in the pathogenesis of disorders ranging from insulin resistance and type 2 diabetes to fatty liver and CVD (Badimon et al., 2011).

Mice, rats, and swine are known to recapitulate MetS; however, none of these models fully reproduce the combined symptoms of MetS (Neeb et al., 2010). Ossabaw, Yucatan and domestic swine are commonly used large animal models for studying MetS (Touchard and Schwartz, 2006). Although these porcine models are already used, they still have some shortcomings.

Objective

The aim of this study is to setup a metabolic syndrome mininpig model, and the characteristics of adipose tissues (subcutaneous, visceral, and pericardial adipose tissue) were analyzed to elucidate their role in metabolic regulation.

Methodology

Animals and experimental diets

All animal care procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National Taiwan University. Four-month-old Lee-Sung minipigs which was from National Taiwan University were used in this study (initial weight 24.91 ± 1.73 kg). They were randomly assigned to two groups: control diet (C) and Western diet (W), for a 5-month experimental period. Feed composition, intake and feed method were designed according to the previous study (Li et al., 2015).

Blood parameters

Commercial kits (Fortress Diagnostics, Northern Ireland, UK) was used to measure plasma glucose, triglyceride, total cholesterol and high density lipoproteins (HDL). The values of low density lipoproteins (LDL) were calculated by total cholesterol minus HDL according to the manufacturer's instruction.

Cytokines detections

The concentration of each cytokine (TNF- α , IL-6) in plasma and adipose tissue was quantified by commercial ELISA kit (R & D, USA) following the instructions which provide by manufacturer. Absorbance was measured at 450 nm and the amounts of cytokines were linearized by plotting the log of the O.D. and the best fit line can be determining by regression analysis.

HE staining

The adipose tissues (1 cm^3) were harvested after pigs were sacrificed. Samples were fixed in 10% neutral formalin solution for a week, embedded in paraffin and sectioned at 5 mm. The sections were stained with hematoxylin and eosin.

Measurements of oxidative stress

Thiobarbituric acid reactive substances (TBARS), a method for screening and monitoring lipid peroxidation, was applied to evaluate the oxidative stress. Adipose tissue was homogenized with TBARS assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). The malondialdehyde (MDA) adduct was measured colorimetrically at 530-540 nm (Nabeshima et al., 2013). Results are expressed as nM MDA/mg tissue.

Determination of fatty acid composition

To determine the fatty acid composition, lipids were extracted from the subcutaneous, visceral, and pericardial fat. The extracts were suspended in 200 ml of hexane for gas chromatography analysis on a capillary column (HP-88 60 m × 0.25 mm × 0.20 mm, Agilent J&W, USA). The gas chromatograph was an Agilent 7890 series GC equipped with a flame ionization detector (FID/EPC G3440A) and an autoinjector module. The oven temperature was start at 125℃ , then was increased at 8℃ per min to 145℃ and held at 145℃ for 26 min, then was increased by 2℃ per min to 220℃ and held at 220℃ for 1 min. Components were identified by comparison of the retention time with those of authentic standards (Supelco 37 Comp. Fame Mix™, Supelco Inc. Belletonte PA) (Hsu et al., 2014).

Statistics

Data were performed as mean ± standard error (SE). The results were analyzed by one-way ANOVA followed by Student's t-test.

Results

Comparing with C group, W pigs had significantly higher body weight from the third month to the end of experimental period (Figure 1a). In W pigs, the body weight is also significantly heavier than C pigs in the end of experiment (Figure 1b). The body composition showed that the fat ratio is significantly higher in W pigs (Figure 1c). Besides, W pigs exhibited more back fat (Figure 1d). W pigs had higher plasma levels of glucose, triglycerides and total cholesterol (Figure 1e). In addition, glucose intolerance was also observed in W pigs (Figure 1f). Comparing with C group, greater blood concentration of TNF- α was observed (Figure 1g). The results of blood parameters in the W pigs indicate that the metabolic syndrome was induced in W pigs successfully.

The HE stain showed that W pigs had bigger lipid droplets in the visceral fat and pericardial fat than C pigs did (Figure 2a-d). Comparing with C group, W pigs exhibited more TBARS in pericardial and visceral fat (Figure 3). Moreover, more TBARS were observed in pericardial fat than in visceral fat of W pigs. In W pigs, greater expression (mRNA and protein) of TNF- α and IL-6 were observed in pericardial fat than in visceral fat (Figure 4 a-d). These results indicated that western diet induced more inflammation and oxidative stress in the pericardial fat.

The fatty acid composition was measured by gas chromatography. In subcutaneous fat (Table 1), W pigs had higher percentage of C17:1 and, C20:3n6, and lower percentage of C10:0 and C20:0. W pigs had higher percentage of C17:1 and C20:3n6 in visceral fat. On the contrary, the percentage of C10:0, C20:0, C16:1, C18:2n6, and C18:3n6 was higher in C pigs. In pericardial fat, W pigs had higher percentage of C11:0, C17:1, C20:1n9, C20:3n6 and lower C20:0, C18:2n6. Western diet did not change the fatty acid composition in the subcutaneous fat. In visceral and pericardial fat, western diet did not change the content of saturated fatty acids; however, it increased more monounsaturated fatty acids and decreased polyunsaturated fatty acids, suggesting a potential role of unsaturated fatty acids in the link with metabolic-related diseases.

Discussion

The current study setup a MetS minipigs with server pericardial adipose tissue. The characteristics of pericardial fat were identified as well.

Ossabaw swine have great elevations in four metabolic features of MetS (Neeb et al., 2010). In fact, some literature thought that the Ossabaw pig is perhaps the best MetS model because of its "thrifty genotype" (Trask et al., 1989). Similar with Ossabaw pig, Lee-Sung minipigs fed with western diet were obese, hyperglycemia, dyslipidemia, and glucose intolerance. However, this Lee-Sung minipig model had some advantages than Ossabaw pig model. There are 2% cholesterol needed in the western diet for Ossabaw pigs, while no cholesterol supplement in the diet for Lee-Sung pigs. To induce the MetS, a period of 8-10 months are needed for Ossabaw pig (Pedersen et al., 2013), but only 6 months are needed for Lee-Sung minipigs.

Recently, some research find that pericardial fat is associated with the prevalence of CVD and myocardial infarction (Mahabadi et al., 2009). As a result, to establish animals model which have pericardial fat become

important. Unfortunately, there are no commercial animal model for PF study. In this study, PF is observed in western diet induced Lee-Sung mini pigs, suggesting Lee-Sung minipigs as an appropriate experiment platform for further PF investigation.

Conclusion

In summary, the Lee-Sung minipigs were observed metabolic syndrome which induced by western diet. Pericardial fat expressed more reactive oxygen species and inflammatory cytokines. The role of adipose tissue distribution in metabolic regulation could provide more information to characterize the differences among three adipose tissues.

KEYWORD：adipose tissue, Lee-Sung minipig, metabolic syndrome, inflammation, fatty acid profiles

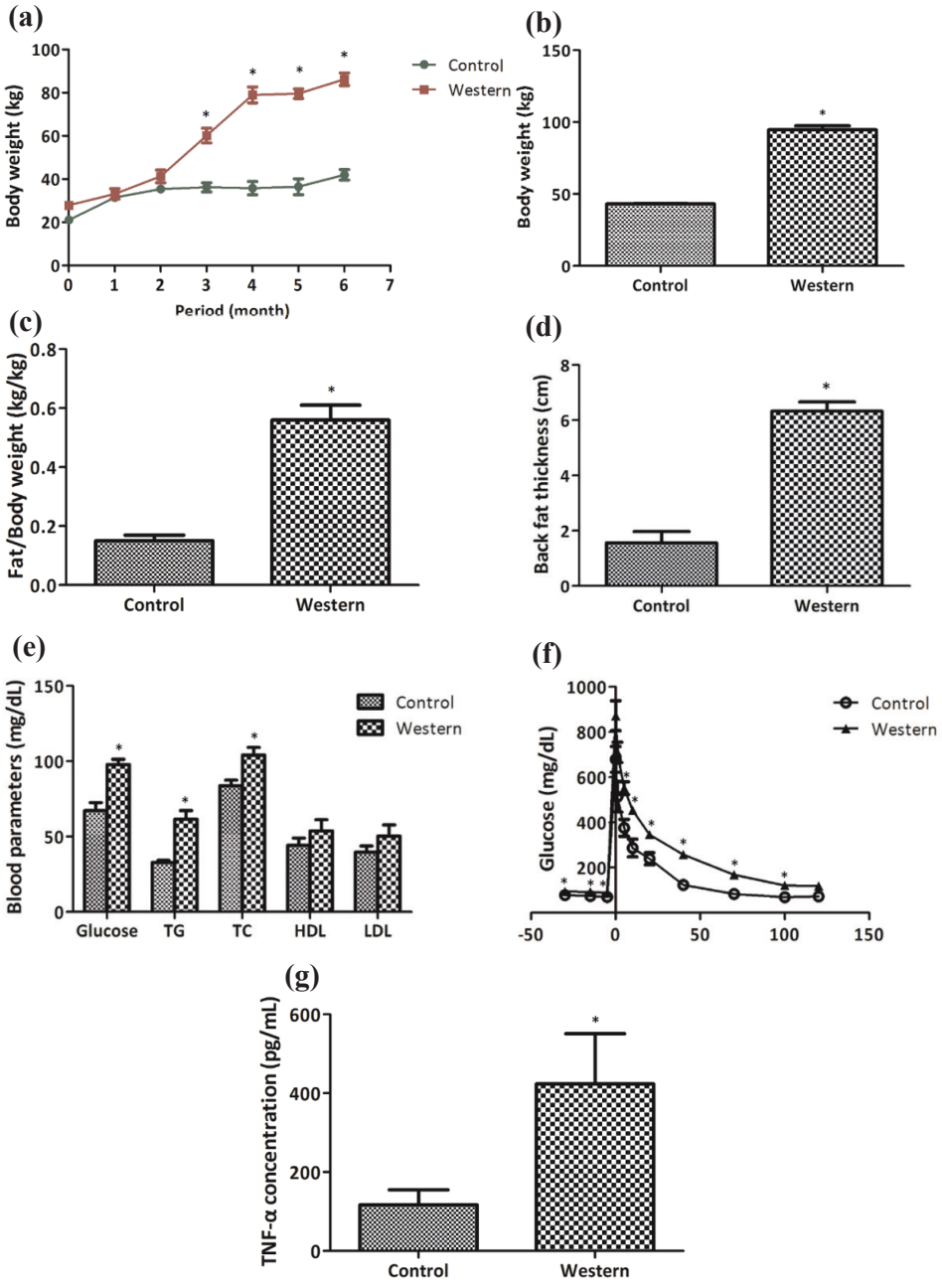

Figure 1 (a) Body weight increase after 6 months; (b) final body weight; (c) fat percentage; (d) backfat thickness; (e) blood parameters; (f) intravenous glucose tolerance test, IVGTT; (g) blood TNF-α concentration. n=4. All results are expressed as mean ± SEM. *$p < 0.05$ vs. control group.

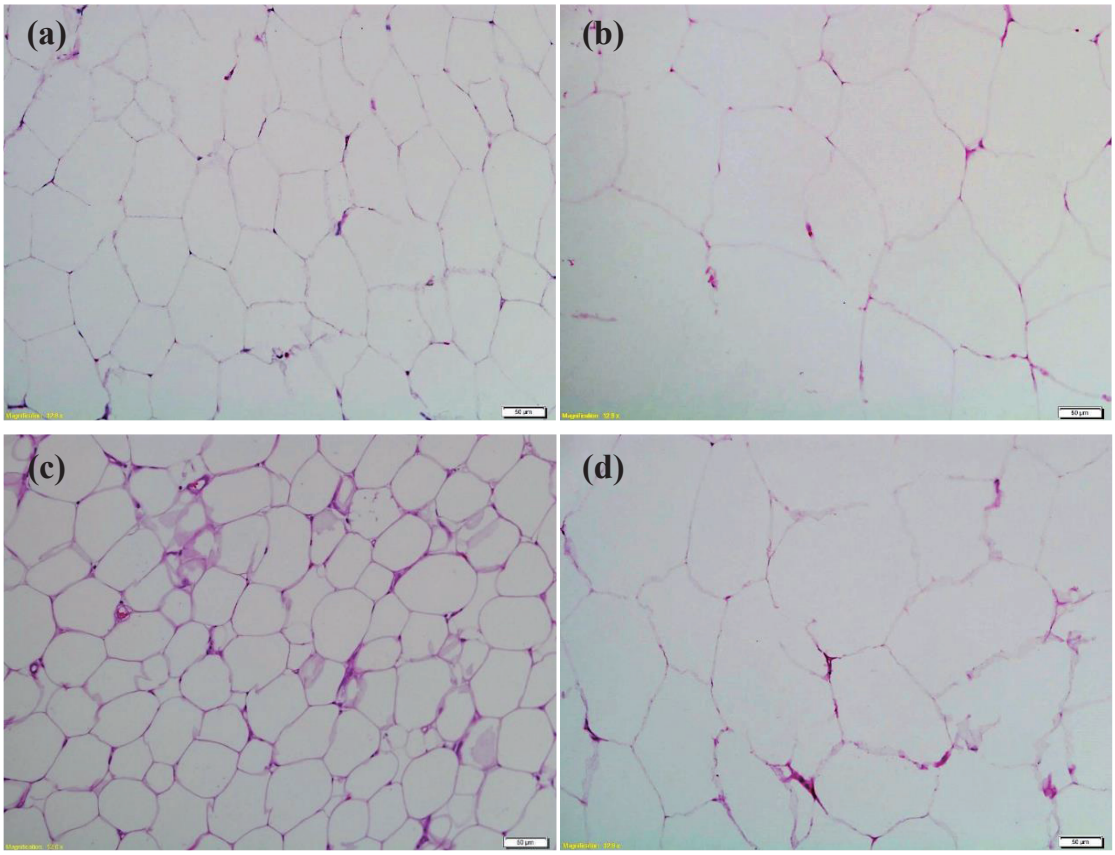

Figure 2 HE staining of visceral adipose tissues: (a) control and (b) western group; pericardial adipose tissue: (c) control and (d) western group. Bar scale: 50 μm.

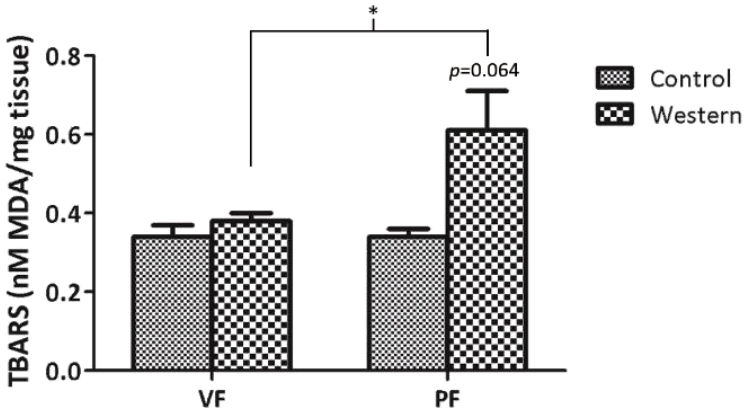

Figure 3 Oxidative stress of visceral fat and pericardial fat. n=4. All results are expressed as mean ± SEM. *$p < 0.05$ vs. visceral fat.

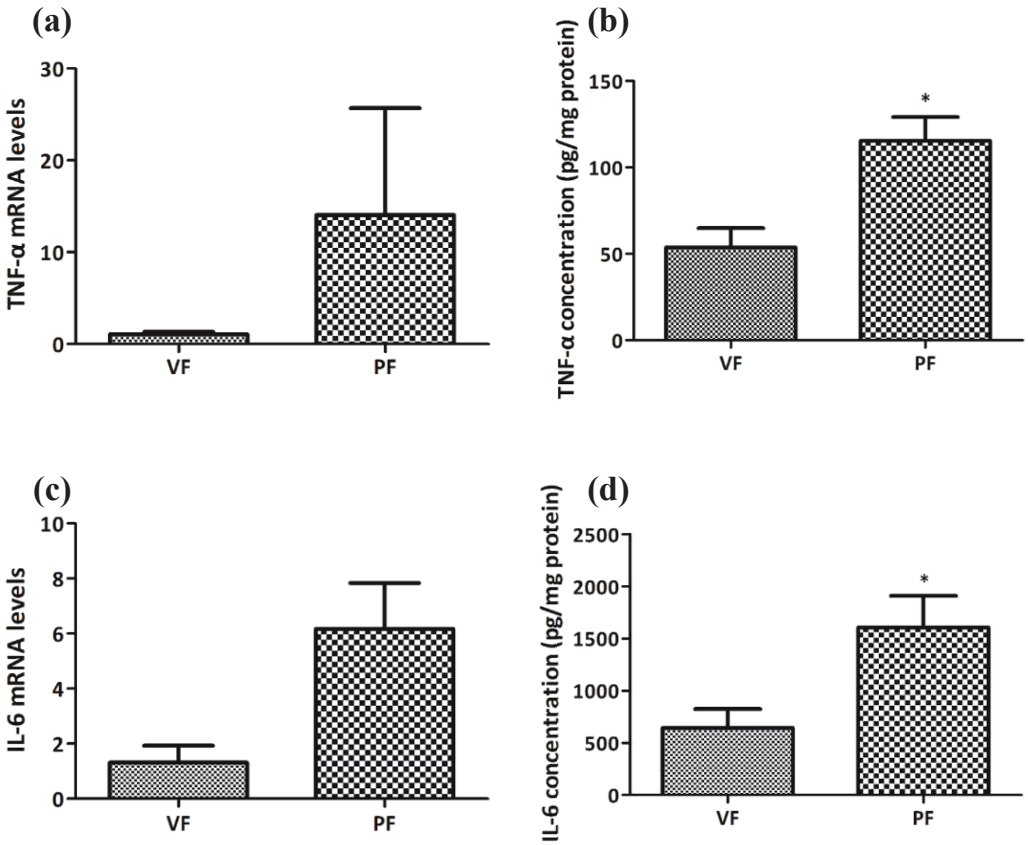

Figure 4 TNF-α and IL-6 mRNA (a, c) and protein expression (b, d) of visceral fat and pericardial fat in western pigs. n=4. All results are expressed as mean ± SEM. *$p < 0.05$ vs. VF.

Table 1 The composition of subcutaneous fat, visceral fat and pericardial fat. All results are expressed as mean ± SEM. *$p < 0.05$ vs. control group.

Subcutaneous fat	Area percent fatty acid content (%)	
	Control	Western
C16:0	22.9 ± 0.8	24.0 ± 2.1
C18:0	10.2 ± 1.0	12.6 ± 2.1
C18:1n9c	45.2 ± 3.2	40.9 ± 4.9
C18:2n6c	18.6 ± 1.3	15.0 ± 0.5
SFA	36.0 ± 1.7	38.8 ± 4.2
MUFA	48.9 ± 2.9	44.2 ± 4.8
PUFA	15.1 ± 4.5	17.0 ± 0.6
n6	13.8 ± 4.7	15.7 ± 0.5
Visceral fat (VF)	Area percent fatty acid content (%)	
	Control	Western
C16:0	25.9 ± 0.6	22.7 ± 1.4
C18:0	16.9 ± 0.7	15.8 ± 1.2
C18:1n9c	32.7 ± 1.1	42.2 ± 2.9
C18:2n6c	16.9 ± 0.8	12.6 ± 0.4*
SFA	45.7 ± 0.3	40.4 ± 2.7
MUFA	34.2 ± 1.1	44.4 ± 2.9
PUFA	20.1 ± 1.0	15.2 ± 0.6*
n6	17.9 ± 0.9	13.7 ± 0.6*
Pericardial fat (PF)	Area percent fatty acid content (%)	
	Control	Western
C16:0	25.7 ± 1.3	24.1 ± 1.2
C18:0	14.1 ± 2.3	16.0 ± 0.8
C18:1n9c	36.2 ± 1.9	38.4 ± 2.5
C18:2n6c	20.4 ± 1.2	14.3 ± 1.0*
SFA	38.6 ± 3.1	42.1 ± 2.2
MUFA	38.9 ± 2.0	43.4 ± 2.4
PUFA	22.5 ± 1.2	16.7 ± 1.2*
n6	21.6 ± 1.1	15.3 ± 1.1*

REFERENCES

Aguilar-Salinas CA, J. Gó mez-Pérez FJ, Valles V, Ríos-Torres JM, Franco A. Analysis of the agreement between the World Health Organization criteria and the national cholesterol education program III: definition of the metabolic syndrome: results from a population based survey. 2003. Diabetes Care. 26: 1635.

Aguilar-Salinas CA, Rojas R, Gomez-Perez FJ, Valles V, Rios-Torres JM, Franco A, Olaiz G, Rull JA, Sepulveda J. High prevalence of metabolic syndrome in Mexico. 2004. Arch Med Res. 35: 76–81.

Badimon L, Storey RF, and Vilahur G. Update on lipids, inflammation and atherothrombosis. 2011. Thrombosis and Haemostasis. vol. 105, supplement 1, pp. S34–S42.

Boudreau DM, Malone DC, Raebel MA, Fishman PA, Nichols GA, Feldstein AC, et al. Health care utilization and costs by metabolic syndrome risk factors. 2009. Metab Syndr Relat Disord. 7(4): 305–314.

Grundy SM. The issue of statin safety: where do we stand? 2005. Circulation. 111: 3016–3019.

Wang J, Sarnola K, Ruotsalainen S, Moilanen L, Lepistö P, Laakso M, Kuusisto J. The metabolic syndrome predicts incident congestive heart failure: a 20-year follow-up study of elderly Finns. 2010. Atherosclerosis. 210: 237–242.

Hsu HC, Chen CY and Chen MF. N-3 polyunsaturated fatty acids decrease levels of doxorubicin-induced reactive oxygen species in cardiomyocytes -- involvement of uncoupling protein UCP2. 2014. J Biomed Sci. 21: 101.

Li SJ, Liu CH, Chang CW, Chu HP, Chen KJ, Mersmann H J, Ding ST, Chu CH and Chen CY. Development of a dietary-induced metabolic syndrome model using miniature pigs involvement of AMPK and SIRT1. 2015. Eur J Clin Invest. 45(1): 70–80.

Mahabadi AA, Massaro JM, Rosito GA, et al. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. 2009. European Heart Journal. 30(7): 850–856.

Nabeshima A, Yamada S, Guo X, Tanimoto A, Wang KY, Shimajiri S et al. Peroxiredoxin 4 protects against nonalcoholic steatohepatitis and type 2 diabetes in a nongenetic mouse model. 2013. Antioxid Redox Signal. 19: 1983–1998.

Neeb ZP, Edwards JM, Alloosh M, Long X, Mokelke EA, Sturek M. Metabolic Syndrome and Coronary Artery Disease in Ossabaw Compared with Yucatan Swine. 2010. Comparative Medicine. 60(4):300–315.

Pedersen R, Ingerslev HC, Sturek M, Alloosh M, Cirera S, Christoffersen BO et al. Characterisation of gut microbiota in Ossabaw and Gottingen minipigs as models of obesity and metabolic syndrome. 2013. PLoS ONE. 8: e56612.

Touchard AG, Schwartz RS. Preclinical restenosis models: challenges and successes. 2006. Toxicol Pathol. 34: 11–18.

Trask AJ, Katz PS, Kelly AP, Galantowicz ML, Cismowski MJ, West TA et al. Dynamic micro- and macrovascular remodeling in coronary circulation of obese Ossabaw pigs with metabolic syndrome. 2012. J Appl Physiol. 113: 1128–1140.

Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. 2001. Proc Nutr Soc. 60: 329–339.

Westcott E, Windsor A, Mattacks C, Pond C, Knight S. Fatty acid compositions of lipids in mesenteric adipose tissue and lymphoid cells in patients with and without Crohn's disease and their therapeutic implications. 2005. Infl amm Bowel Dis. 11: 820–827.

O-03-2

Is eleutherine (Eleutherine americana) potential as feed additive for poultry?

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Introduction

Feed additive in livestock production system is subjected to improve intake, liveweight gain, feed efficiency and health status. Feed additive includes antibiotics, flavour, ionophore and growth hormone. The use of antibiotics and growth hormone synthetics produce negative effect on animal and environment by enhancing microbiota resistance in the digestive tract (Windisch *et al.*, 2008). Furthermore, some antibiotics have serious undesirable side effect which limit their application. Therefore, there is serious need to develop new antibacterial agents that are very effective with minimal undesirable side effects. Plants are representation of potential source for feed additive such as antibiotics.

Eleutherine has long been recognized as spicy food and herbal medicine (Ifesan *et al.*, 2010). Bulbs of eleutherine have been reported to have antibacterial activity on gram-positive and gram-negative bacteria (Ifesan *et al.,* 2009; 2010), as well as fungi (Ifesan *et al.*, 2010). Recently, Phoem and Voravuthikunchai (2013) proposed that bulbs of eleutherine extracts could be used as growth media for nonpathogen bacteria. These indicated that the bulbs extract could be used as phytobiotics and prebiotics sources. Therefore, the objectives of present preliminary research are to explore the potential of bulbs extract to be used as phytobiotic and/or prebiotic in poultry through *in vitro* evaluation.

Material and Methods

Research materials: The bulbs of eleutherine were washed and cleaned with tap water and then chopped. Chopped bulbs were dried to about 10% of water content and then ground to powder. The powders were individually extracted using methanol, ethylacetate, diethylether and hexane, respectively. Extraction process was done for 7 days. Crude extracts were rotary evaporated until the extract became completely dry as pellet. The pellet was then subjected to the chemical analysis for its bioactive compunds. The pellet were also individually disolved in distilled water for further analysis and evaluation.

Antioxidant evaluation: Antioxidant activity was conducted using DPPH (diphenylpicrylhydrazyl) method and the concentration of tested extract was 0.25, 0.50, 0.75 and 1 mg pellet in the solution. Krings and Berger (2001) suggested that scavenge of free radical is assessed on the absorbance at the wave length of 517 nm and the unit is expressed as ppm of AEAC (*ascorbic acid equivalent antioxidant capacity*).

Antibacterial evaluation: Agar diffusion based on Ayad *et al*. (2000) was applied to evaluate antibacterial activity of the bulbs extract. Suspension of the tested bacteria (*Escherichia coli* and *Staphylococcus aureus*) were prepared to contain approximately 10^8cfu/mL and the disc containing solid agar were innoculted by spreading up 1 ose of bacteria suspension. A 100 µL of crude extract from individual solvent which was preparing at the level of 0, 0.25, 0.50, 0.75 and 1mg extract/mL were placed in the hole (4 mm depth and 8mm diameter). Inhibition zone diameter was measured four times after allowing 24 h at 37°C in the incubation equipment. A control positive was synthetic antibiotic of tetracycline

Growth promoting assay: Four types of eleutherine extract and two controls were subjected to the growth-promoting assay based on Phoem and Voravuthikunchai (2013), and tested bacteria was *Lactobacillus acidophilus*. Bacteria growth was assessed using turbidimeter (NTU; nephelometric turbidity unit). Test tubes were containing 9 ml of liquid growth media and 1 mL of extracts (1 mg/mL) and sterile distilled water, respectively and positive control was 10 mL of growth media. The test tubes were anaerobically incubated for 2, 6, 10, 14, 20, 24, 48 and 72 hour.

Experimental design and statistical analysis: Experiment was designed as Block Design, in which blocks were types of solvent and four level of pellet conectration in the solution as treatments within 3 or 4 replicates. Treatment levels were 0.25, 0.5, 0.75 and 1 mg. Parameters were antioxidant activity, antibacterial activity and growth of bacteria. Data were analyzed using analysis variance and least significant different (LSD) for comparison means analysis (Steel and Torrie, 1990).

Results and Discussion

Bioactive compounds:

The bulbs of eleutherine have been extracted using four different solvents: methanol, ethylacetate, diethylether and hexane. Extracted materials produce bioactive compounds as shown in Table 1. The absence of bioactive compounds in the extract is likely related to the lack of sensivity of equipment and method used, and also low concentration of extract in the solution. Bioactive compounds could therefore be detected when concentration of the eleutherine extract is elevated in the solution. Nonetheless, all extracts have tannin compounds.

Table 1. Bioactive composition of 1 mg pellet/ml solution

Solvents	Fenol (mg/kg)	Flavonoid (%)	Tannin (%)
Methanol	nd	1.29	0.09
Ethylacetate	nd	63.48	0.20
Diethylether	nd	nd	0.10
Hexane	nd	nd	0.04

nd=not detected

Antioxidant activity

Mean values of antioxidant activity of extract are presented in Table 2. All extracts produced antioxidant activity and tended to be different within types of organic solvent, in which methanol produced the highest value of antioxidant activity. This study revealed that antioxidant activity is elevated as concentration of extracts increased in the solution. This pattern agrees with the results of Rusdi et al. (2009 and 2014). Natural antioxidant has been reported improving nutrients digestibility, feed efficiency, egg production and egg quality (Radwan *et al*., 2008). Furthermore, inclusion of natural antioxidant during laying period significantly reduced melonaldehyde-egg yolk and had postive effect on oxidation stability of egg-shell and improved fertility as well as egg hatchability. Meanwhile, Abd El-Hakim *et al.* (2009) reported that antioxidant generated from plant materials significantly improved a daily liveweight gain of broiler for the first 3-week old.

Table 2. Antioxidant activity of exctracted eleutherine from different solvents at level of 0.25, 0.50, 0.75 and 1 mg of pellet in the solution (n=3)

	ppm of AEAC				SEM
Solvents	0.25	0.50	0.75	1.0	
Methanol	18.57^{a}	25.03^{b}	35.98^{c}	55.36^{d}	4.21
Ethylacetate	4.79^{a}	13.67^{b}	24.85^{c}	38.16^{d}	3.80
Diethylether	612.08^{a}	13.43^{b}	14.71^{c}	20.33^{d}	0.95
Hexane	0^{a}	0^{a}	0^{a}	7.92^{b}	1.07

SEM, standard error of the mean. Means in the same row with different superscript differ significantly ($P<0.01$)

Antibacterial activity

Antibacterial activity result is summarized in Table 3. The activity was firstly recorded when exctract is 0.5 mg in the solution and it increases as concentration increases. Similar pattern has been reported by Akiyama et al. (2001); Pereira et al. (2007); Sakunpak dan Panichayupakaranant (2012). They found antibacterial activity on polyphenol compounds. Previous studies reported that polyphenol, phenol, flavonoid and essential oil generated from plants reduce the growth of pathogen bacteria of *E. coli*, *S.aureus, L. monocutogenes* and *Salmonella spp* (Friedman *et al.,* 2004; Oussalah *et al*., 2006). The presence of antibacterial activity in both gram positive and gram negative bacteria in the current study proved that these extracts could be categorized as a broad spectrum antibiotic to replace synthetic antibiotics.

Table 3. Bacteria growth inhibition of extracted eleutheurine from different solvents on *Escherichia coli* and *Staphylococcus aureus* at the level of 0.25, 0.50, 0.75 and 1 mg of pellet in the solution (n=4)

Solvent	Inbitition (mm)									
	Escherichia coli					*Staphylococcus aureus*				
	0.25	0.50	0.75	1.00	SEM	0.25	0.50	0.75	1.00	SEM
Methanol	nd	3.00^{a}	7.50^{b}	13.25^{c}	1.30	nd	4.00^{a}	7.25^{b}	10.25^{c}	0.99
Ethylacetate	nd	3.00^{a}	7.75^{b}	8.25^{c}	0.89	nd	7.25^{a}	8.75^{b}	10.00^{c}	1.01
Diethyether	nd	3.75^{a}	6.00^{b}	8.50^{c}	0.82	nd	4.75^{a}	6.00^{b}	10.75^{c}	1.01
Hexane	nd	4.25^{a}	5.75^{b}	7.75^{c}	0.75	nd	5.50^{a}	8.50^{b}	9.25^{c}	0.95
Tetracycline			26.00					25.50		

nd = not detected. SEM, standard error of the mean. Means in the same row within bacteria with different superscript differ significantly (P<0.01)

Bioactive compounds in the particular media generally produce antioxidant and antibacterial activity on bacteria, fungi and even more it may reduce the growth of mosquito's larvae (Ferreira *et al*., 2008). The rate of 0.5 mg in the present study is not high enough to produce an antibacterial activity on all type of extracts. This is supported by the results of Banso and Adeyemo (2007). They found that inhibition growth is achieved when tannin concentration in the media is 4.0 to 5.5 mg/mL. Furthermore, Sakunpak and Panichayupakaranant (2012) reported the value of concentration of 10 mg/mL in the media to produce antibacterial activity.

Table 4. The growth of bacteria *Lactobacillus acidophilus* (NTU) on media added with 1 mg of bulbs extract from methanol, ethylacetate, diethylether and hexane

	Incubation time (h)							
Solvent	2	6	10	14	20	24	48	72
Methanol	0	2.18^{b}	3.62^{b}	4.74^{c}	9.10^{ab}	12.25^{a}	39.63^{ac}	77.43^{a}
Ethylacetate	0	2.44^{b}	2.68^{c}	3.59^{d}	8.54^{bc}	11.87^{a}	76.53^{b}	94.35^{b}
Diethyether	0	3.07^{a}	6.92^{a}	8.09^{a}	9.57^{a}	11.84^{a}	60.20^{ab}	97.45^{b}
Hexane	0	2.18^{b}	2.42^{c}	3.50^{d}	9.42^{a}	11.83^{a}	26.25^{cd}	81.55^{a}
Control (+)	0	2.19^{b}	3.22^{b}	5.75^{b}	8.14^{c}	9.66^{b}	12.15^{d}	58.65^{c}
Control (-)	0	1.19^{c}	1.63^{d}	1.66^{e}	2.42^{d}	2.63^{c}	3.38^{d}	32.60^{d}
SEM		0.13	0.23	0.31	0.34	0.53	10.96	2.32

SEM, standard error of the mean. Means in the same colon with different superscript differ significantly (P<0.01)

Table 4. The growth of bacteria *Lactobacillus acidophilus* (NTU) on media added with 1 mg of bulbs extract from methanol, ethylacetate, diethylether and hexane

Solvent
Incubation time (h)
2
6
10
14
20
24
48
72

Methanol
0
2.18[b]
3.62[b]
4.74[c]
9.10[ab]
12.25[a]
39.63[ac]
77.43[a]
Ethylacetate
0
2.44[b]
2.68[c]
3.59[d]
8.54[bc]
11.87[a]
76.53[b]
94.35[b]
Diethyether
0
3.07[a]
6.92[a]
8.09[a]
9.57[a]
11.84[a]
60.20[ab]
97.45[b]
Hexane
0
2.18[b]
2.42[c]
3.50[d]
9.42[a]
11.83[a]
26.25[cd]
81.55[a]
Control (+)
0
2.19[b]
3.22[b]
5.75[b]
8.14[c]
9.66[b]
12.15[d]
58.65[c]
Control (-)
0
1.19[c]
1.63[d]
1.66[e]
2.42[d]
2.63[c]
3.38[d]

32.60[d]
SEM
0.13
0.23
0.31
0.34
0.53
10.96
2.32
SEM, standard error of the mean. Means in the same colon with different superscript differ significantly (P<0.01)

Bacterial growth

Current study clearly revealed the ability of eleutherine extract to stimulate the growth of *Lactobacillus acidophilus* bacteria (see Table 4 and Figure 1). Type of organic solvents produced different growth pattern of bacteria, in which methanol solvent, in general, tended to performing better growth than the other solvents. The growth was linearly improved as incubation time increased. This trend agrees with the previous studies of Maligan *et al*. (2006) and Usmiati *et al*. (2011). Moreover, Maligan *et al*. (2006) reported alogaritmics phase growth was achieved in 35 h incubation time and continuously increased until 70 h incubation time. In addition, *Lactobacillus* bacteria was growing and improving within 21 days on yoghurt milk (Usmiati *et al.,* 2011). Present study indicated that the growth rate of bacteria with eleutherine extract was significantly higher than those without eleutherine extract (P<0.01).

Bacterial lactate acid producer of *Lactobacillus* and *Bifidobacterium* have been reported to have benefit effect on the health (Bernet e*t al*., 1993), and other effects are following: nutrition, physiology and antibacterial (Naidu and Clemens, 2000). In fact that all non digested carbohydrate that categorized as prebiotics may stimulate the growth of those bacteria and therefore enhance animal productivity. For example, isomalto-oligosakarida (IMOS), trans-galakto-oligosakarida (TGOS), mannan-oligosakarida (MOS) and pectin- oligosakarida are categorized as prebiotics. These prebiotics produce different mechanisms in stimulating the growth improvement of livestocks and IMOS was selectively fermented *Bifidobacteria* and *Lactobacilli* but not for *Salmonella* or *E.coli* (Chung and Day, 2004). The manno-oligosaccharida enhanced the population of *Lactobacilli* in the ileum (Yang *et al*., 2008). Moreover, the growth improvement of livestocks is ralated to the improvement of energy used (Yang *et al*., 2008).

The current *in vitro* result agrees with the results of Gibson *et al*. (2004) stating that the non digested carbohydrate of eleutherine has positive effects on the particular non pathogenic bacteria in the colon and improved health status. Similarly, Phoem and Voravuthikunchai (2013) reported that eleutherine could be used as prebiotics to stimulate the growth of non pathogenic bacteria through enhancement of short-chain acids production. Furthermore, oligosaccharides extract from eleutherine elevated the growth of *Bifidobacteria* from 9.63 to 12.8 log cfu/ml and 5.80 to 8.85 log cfu/ml for mix- and pure culture media respectively. Therefore, they concluded that extracted materials from eleutherine could be used as a functional food for human.

Conclusion

Extracted materials from the bulbs of eleutherine consisted of bioactive compounds and produced antioxidant and antibacterial activity. The extracts also have ability to enhance the growth of bacteria *Lactobacillus acidophilus*. Therefore, it could be concluded that extracted materials from eleutherine bulbs are potentially to be used as feed additive “phytobiotics and prebiotics.

KEYWORD : Eleutherine, Extract, Bioactive, Phytobiotics, Prebiotics

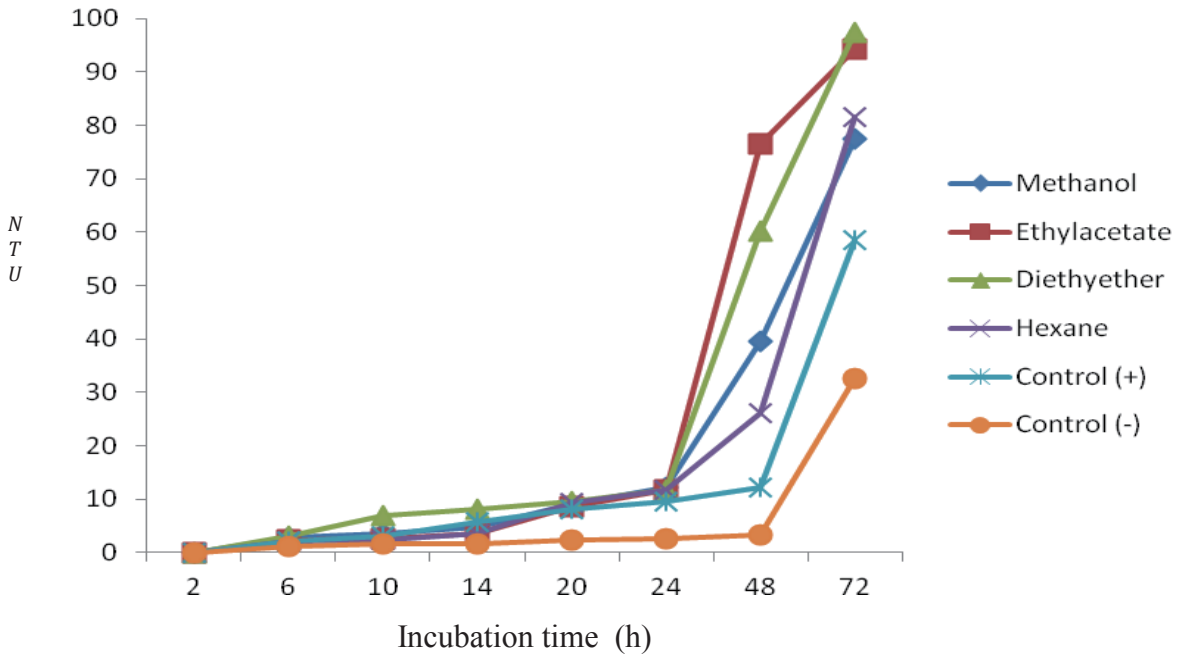

Figure 1.The growth curve of *Lactobacillus* on media with additional with 1mg of bulbs extracted from methanol, ethylacetate, diethylether and hexane

References

Abd El-Hakim, A.S., Cherian, G. And Ali, M.N. 2009. Use of organic acid, herbs, and tehir combination to improve the utilization of commercial low protein broiler diets, International Journal of Poultry Science, 8(1):14-20

Ayad, E.H.E., Verheul, A., Wouterd, J.T.M. and Smit, G. 2000. Application of wild starter cultures for flavour development in pilot plant cheese making. International Dairy Journal, 10:169-179

Banso, A. and Adeyomo, S.O. 2007. Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. African Journal of Biotechnology, 6:1785-1787

Bernet, M.F., Brassart, D., Neeser, J.R. and Servin, A.L. 1993. Adhesion of human bifidobacterial strains to cultured human intenstinal epithelial cells and inhibition of enteropathogen-cell interaction. Applied of Enviroment and Microbiology, 59:4121-4128

Chung, C.H. and Day, D.F. 2004. Efficacy of Leucanostoc mesentroides (ATCC13146) isomaltooligosaccharides as a poultry prebiotic. Poultry Science, 83:1302-1306

Ferreira, P.M.P., Farias, D.V., Oliveira, J.T.de and Carvallo, A de F. Moringa oleifera:bioactive compounds and nutritionl potential. Review Nutrition Campinas, 21(4):431-437

Friedman, M., Henika, P.R., Levin, C.E. and Mandrell, R.E. 2004. Antibacterial activities of plant extract essensial oils and their component against Escherichia coli O157:H7 and Salmonella enterica in apple juice. Journal of Agricultural and Food Chemistry, 52; 6042-6048

Gibson, G.R., Probert, H.M., Van Loo, J., Rastall, R.A. and Roberfroid, M.B. 2004. Dietary modulation of the human clolonic bacteria:apdating the concept of prebiotics. Nutrition Research Review, 17:259-275

Ifesan, B. O. T., Ibrahim, D. and Voravuthikunchai, S. P. 2010. Antimicrobial activity of crude ethanolic extract from Eleutherine Americana.Journal of Food, Agriculture & Environment, 8 (3&4): 1233-1236.

Ifesan, B. O. T., Hamtasin, C., Mahabusarakam, W. and Voravuthikunchai, S. P. 2009. Inhibitory effect of Eleutherine americana Merr. extract on Staphylococcus aureus isolated from food. Journal of Food Science. 74:31-36.

Krings, U and Berger, R.G. 2001. Antioxidant activity of somes roasted foods. Food Chemistry, 72:223-229

Maligan, J.M., Kusnadi, J. dan Murtini, E.S. 2006. Stusdi viabilitas bakteri probiotik Bifidobacerium bifidum, Lactobacillus acidophilus dan Lactobacillus casei terimobilisasi pada sistem emulsi air dalam minyak jagung dan daya tahannya

pada perlakuan lanjutan. Jurnal Teknologi Pertanian, 7(3): 141-149

Naidu, A.S. and Clemens, R.A. 2000. Probiotics. In: Natural Food Antimicrobial System. Naidu, A.S. (ed). CRC Press, LLC pp.431-462

Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M. 2006. Inhibitory effects of selected essential oils on the growth of four pathogenic bacteria E. coli O157:H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food Control, 18: 414-420

Phoem, A.N. and S.P. Voravuthikunchai. 2013. Eleutherine americana as growth promotor for infant intestinal microbiota. Anaerobe, 20:14-19

Radwan, N. L., Hassan, R.A., Qota, E.M. and Fayek, H.M. 2008. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. International Journal of Poultry Science, 7:134-150

Rusdi, Asriani, H. dan Rosmiaty A.. 2009. Antioksidan dan antimikroba biji mangga (Mangifera indica). Laporan Penelitian. Lemlit UNTAD. Palu

Rusdi, Asriani, H and Rosmiaty, A. 2014. Evaluation of Phytogenic Potential of Legume Leaves for Broiler Chicken. Proceedings of the 16th AAAP Animal Science Congress Vol. II.10-14 November 2014, Gadjah Mada University, Yogyakarta, Indonesia.pp.521-524

Sakunpak, A. and P. Panichayupakaranant. 2012. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenon, chamuangone. Food Chemistry, 130:826-831

Steel, R.G.D. and Torrie, J.A. 1990. Principles and Procedures of Statistics. McGraw Hill. New York.

Usmiati, S., Broto, W. dan Setyanto, H. 2011. Karakteristik dadih susu sapi yang menggunakan starter bakteri probiotik. Juranl Ilmu Ternak dan Veteriner (JITV), 16 (2):141-153

Windisch,W.M.,Schedle,K.,Plitzner,C. and Kroismyr, A. 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86:E140-E148

Yang, Y., Iji., P.A., Kocher, A., Thomson, E., Mikkelsen, L.L. and Choct, M. 2008. Effects of manna-oligosaccharida in broiler chicken deits on growth performance, energy utilization, nutrient digestibility and intestinal microflora. Brisith Poultry Science, 49:186-194

O-03-3

UTILIZATION EFFECT OF BREAD WASTE AS CORN SUBSTITUTION ON HYBRID DUCK PERFORMANCES

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ABSTRACT

The research was aimed to evaluate the utilization of bread waste (BrW) as corn substitution on feed to the live performance of hybird duck in term of feed intake, body weight gain, feed conversion ratio, and income over feed cost (IOFC). One hundred of straight run (unsexed) of 14 days old used as research material, reared on similar feeding management since day old duck. The Feed composted by yellow corn, rice brand, broiler concentrate (CP 34%), and bread waste. The feed treatments consisted were P0 = feed without bread waste (control); P1 = feed in with 10% corn substituted with BrW); P2 = feed in which 20% corn substituted with BrW; and P3 = feed in which 30% corn substituted with BrW, with 5 replications for each treatment. The variables measured were feed intake, body weight gain, feed conversion, and value income over feed cost. Collected data were analyzed by Analysis of Variance of the Completely Randomized Design (CRD) and for significant effect variable were analyzed by Duncan's Multiple Range Test (DMRT). The result showed that the used of BrW in the duck feed had highly significant effect ($P<0.01$) on feed intake and income over feed cost, increase body weight gain, but no significant effect to feed conversion ($P>0.05$). Based on the research result, it can be concluded that bread waste can be used up to 20% as corn substitution.

KEYWORDS: Hybrid Duck, Bread Waste, Corn, Live Performance

Introduction

The meat duck farming was grown fast in the local area in East Java. Even though, the rearing cost of duck higher than broiler chicken due to higher feed consumption and slower growth but the duck carcass have better commercial price in local market. The farmer try to looking for the alternative feedstuff to minimize the feed cost. One of the locally available sources was bread waste.

Bread waste is by product bread industry consisted rejected quality bread or unconsumed and expired bread. The bread was made from 90%flour and other ingredient like egg, milk, and yeast containing β -carotene, thiamin (vitamineB1), riboflavine (vitamine B2), niasin, Fe and Ca(Astawan,2007). Bread waste was potential energy sources for animals contain gross energy 4217 kcal/kg or equivalent to metabolizable energy 2952 kcal/kg. The research result of Salamun (2009) showed that corn substitution by bread waste meal until 30% did not significance effect to broiler performance, and increased the feed efficiency and income over feed and chick cost. This present study was try to minimize feed cost by substitution of yellow corn as energy sources for broiler hybrid duck, and evaluate the production performance, carcass characteristic and vital organ due to corn substitution by bread waste meal.

Material and Method

The research and feed analysis was conducted in Research station of Sumber sekar and Feed and Nutrition Laboratory, Faculty of Animal Husbandry, University of Brawijaya, Malang East Java, Indonesia.

Two hundred unsexed of 2 weeks old hybrid ducks (Peking x Khaki Campbell), with average body weight 346 $\pm$ 15.79 g/bird (CV 4.55%), reared for 5 weeks period.2) The duck plotted on 20 litter cages 150x100x75 cm size for 10 birds each, completed with feeder and drinker. The bread waste was collected from local bakery industry in Malang regency. The bread waste grounded in 1mm sieve size. The basal feed was formulated based on the duck nutrients requirement (Table 1), and formulation of the treatment feed was found in Table 2.

Table1. Nutrient content (% DM) of research feedstuff based on analysis and ME = 70% GE

Table2. Formulation and Nutrient content of Treatment Feed

Method

The research method used was feed experimental on completely randomized design with 4 treatments and 5 replications, with 10 birds each. The basal feed consist of yellow corn, Consentrate CP 144, rice bran, and bread waste meal (BWM). The feed amount given according to the duck requirement on different ages for 5 weeks, with 2 times daily feeding frequency(07:00 with 40% and 16:00 with 60% total) and water drinker was available *ad libitum.* The feeding treatment of this research were:

P_0 = Basal feed (control)
P_1 = Basal feed with 20% corn Substitution by BWM
P_2 = Basal feed with 40% corn Substitution by BWM
P_3 = Basal feed with 60% corn Substitution by BWM

Variable measured were :

Feed consumption (g/bird/day) = feed allowed - feed waste Body weight gain (g/week) = BW present -BW last week Feed Conversion = KonsumsiFeed consumption (g)

Body weight gain (g)

Carcass percentage = Carcass weight/ final body weight 5. IOFC = (Final BW x carcass price/kg) - (Σ feed consumption x feed cost/kg) % carcass proportion = weight carcass proportion / final body weight % vital organ = organ weight/ final body weight

The collected data were tabulated using software Microsoft Excel, and analyzed by analysis of variance (ANOVA) in completely randomized design, and followed by Duncan multiple range test (Steel dan Torrie, 1991).

Result and Discussion

The data result of the feeding treatment of corn substitution by bread waste meal (BWM) to the production performance, carcass characteristic and % vital organ were presented on Table 3.

The feed consumption were highly significant effected ($P< 0.01$) by the treatment. The feed consumption tend to decreased by increasing BWM utilization as corn substitution. The highest feed consumption was found at control group and lowest at P3 (3342 vs3336 g/ bird).

This decreasing of feed consumption caused by high of lipid content of the bread waste. The bread waste consist not only plain bread but most of them derived from tasted bread which containing margarine with high energy content. The high energy concentration on the feed caused the bird decrease feed consumption.

The previous result reported by Wahju (2004) about biscuit waste as corn substitution showed the similar result, due to the energy content of the biscuit higher than yellow corn (3370 vs 3038 Kcal/kg). The lipid content also affected the feed consumption. The normal lipid content of the poultry feed about 3.5% (Widodo, 2006), but in this treatment feed was more than 5%. The other problem of the high lipid content according to Perry *et al.* (2003) was rancidity and low feed palatability that caused lowering energy availability and consumption.

Table3. The Effect of Corn substitution by BWM to production performance, and carcass of hybrid duck.

The other important factor affecting to the feed consumption was a feed density. The density of the treatment feed bread waste was 687 g/l. This value was lower compared to the corn density 719 g/l. The feed density have positive correlation to the feed consumption. An increasing of feed density will be increase feed consumption, due to crop will be full filled by smaller particle of the high density feedstuff, and the bird will get more nutrients from similar amount of feed consumption.

The body weight gain, feed conversion and carcass percentage was not affected by the feed treatment. The highest BW gain of the treatment feed similar to control group (935.3 vs931.2;928.1 and 923,4g/bird). The factors affected BW gain were feed consumption and nutrient content of the feed. The increasing amount of bread waste cause lower feed consumption and BW gain. The research ofSalamun (2009) used bread waste as corn substitute until 30% feed level was not effected to broiler life performance.

The average feed conversion ratio (FCR) on Table 3 showed that the lowest FCR of duck treatment was on control group followed by P2, P3 and P1 (3.57 vs 3.58;3.60; and 3.62).The difference carbohydrate content of the corn (mostly consist of amylose) and bread waste (glucose, sucroseand fructose), then combination of both energy source will be have better result. The corn substitution by bread waste until 40% feed level did not significant

effect to FCR of broiler chicken (Salamun, 2009). The FCR value also affected by passage rate, feed form, feed composition and nutrients balance (Anggorodi, 1985).

The Income over feed cost (IOFC)value of treatment feed (Tabel 3) showed a very significant increasing value ($P<0.01$) with utilization of bread waste as corn substitution. The highest IOFC value derived from 60% corn substitution and lowest on control(IDR 4706 vs 3410). The similar result also reported by Widjiastuti (2009) which utilize bread waste until 30% on broiler feed. The feedstuffs prices used in this research was varied. The corn price and CP 144 concentrate was IDR 4000 and 7800/kg, which more expensive compared to bread waste and rice bran (both IDR 2500/kg). And, the selling price of duck carcass in the local market was IDR20.000/kg. The variation of feed consumption and feed price among treatment caused the production cost also difference, but the BW gain and final BW was almost similar. This condition produced the IOFC value tended to increased.

The percentage of carcass retail cut and internal organ of hybrid duck was not affected by the corn substitution treatment. This result explained that there is no negative effect of the utilization of bread waste as corn substitution to the carcass as well as to the internal organ weight.

CONCLUSION

The utilization of bread waste as corn substitution until 60% showed good result of live hybrid duck life performance. There is no negative effect to the carcass and internal organ weight, but increased the IOFC value return.

KEYWORD : Hybrid Duck, Bread waste, Corn, Live performances

Table1. Nutrient content (% DM) of research feedstuff based on analysis and ME = 70% GE

Feedstuff	EM (Kcal/g)	CP	Lipid	Crude Fiber
Yellow corn	3370	8.6	3.9	2
Rice bran	2860	10.2	7	3
Consentrate	2600	37	3	5
Bread waste meal	3038[2]	13.37	11.02	0.48

Table2. Formulation and Nutrient content of Treatment Feed

Feedstuff	Treatment Composition (%)			
	P0	P1	P2	P3
Consentrate CP 144	25	25	25	25
Yellow corn	50	40	30	20
Rice bran	25	25	25	25
Bread waste meal	0	10	20	30
Total	100	100	100	100
Nutrient Content (based on calculation)				
Metabolizable (Kcal/kg)	3050	3014	2979	2943
Crude Protein	16.1	16.59	17.08	17.57
Lipid	4.45	5.55	6.37	6.58
Crude fiber	3	2.84	2.69	2.54

Table3. The Effect of Corn substitution by BWM to production performance, and carcass of hybrid duck.

Variables	Treatment				Sig.
	P0	P1	P2	P3	
Feed Consumption,g/bird	3342±1.55[b]	3340±1.73[b]	3337±1.21[a]	3336±2.30[a]	**
BWG, g/bird	935±15.25	928±15.70	931±14.21	923±30.83	NS
Feed Conversion	3.57±0.06	3.60±0.06	3.58±0.05	3.62±0.12	NS
% Carcass	56.73±4.90	58.21±2.85	59.06±1.63	57.07±3.38	NS
IOFC (IDR/bird	3410±306[a]	3782±316[ab]	4357±280[bc]	4707±619[c]	**
Carcass cutting (% BW)					
- Wing	8.90±0.81	9.07±1.56	8.21±0.84	9.28±0.98	NS
- thigh	16.52±1.04	17.31±0.99	17.65±1.61	17.02±1.02	NS
- Breast	11.62±2.20	11.37±1.44	13.76±1.66	10.94±1.81	NS
- Back	19.88±2.69	20.57±1.29	19.44±2.80	19.83±0.97	NS
Vital Organ (% BW)					
- Heart	0.62±0.08	0.67±0.11	0.70±0.14	0.64±0.15	NS
- Liver	3.41±0.20	3.53±0.38	3.39±0.22	3.25±0.18	NS
- *gizzard*	3.38±0.31	3.31±0.63	3.43±0.52	3.45±0.57	NS

Note:Different superscript on same row means the very significant effect (P<0.01)

REFERENCES

Anggorodi, H.R.1995. Poultry Nutrition. Gramedia Pustaka. Jakarta.

Astawan, M. 2007. The Fibre and Nutrient content of bread, noodle, and rice.. Institut Pertanian Bogor Press. Bogor

NRC. 1994. Nutritien Requirement Of Poultry . Eighth Revised Edition. National Academy Press. Washington DC.

Perry, T, W. A. E. Cullison and R.S. Lowrey. 2003. Feed & Feeding. Sixth Edition. Pearson Education, inc. Upper Saddler River. New Jesrey

Salamun, R. 2009. The effect of rejected bread meal as corn substitution on Broiler feed to the male broiler performance. Universitas Diponegoro. Semarang

Steel, R.G dan J.H. Torrie. 1992. Principle and Procedure Statistic, Geometrical approach. PT. Gramedia. Jakarta..

Wahju, J. 1992. Poultry Nutrition Science. 4th Ed.4. Gajah Mada University Press. Yogjakarta.

Widodo, W. 2006. Contextual Poultry Feed and Nutrition. Faculty of Animal Husbandry Universitas Muhammadiyah Malang

Widjiastuti dan Sujana. E. 2009. Utilization of waste bread meal on broiler feed and its implication to the feed efficiency. National Seminar Forum. Development of production system and Local Resources management for self sufficient of Animal food resources. PadjajaranUniversity. Bandung.

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Effects of Multi-probiotics and Synbiotics on Laying Ducks Performance and Egg Quality

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Introduction

Poultry production use of probiotics, prebiotics and synbiotics that enrich certain bacterial population in the digestive system are considered as alternatives to antibiotic growth promotants (Patterson and Burkholder, 2003). Probiotics are naturally occurring microorganism include bacteria, fungi or yeast. Addition of probiotics to layer diets are claimed to improve performance and some egg quality traits (Li et al., 2006; Ramasamy et al., 2009; Mikulski et al., 2012; Mohammadian et al., 2013). On the other hand, probiotics showed no significant effect on egg production (Mikulski et al., 2012; Zarei et al., 2011; Kalavathy et al., 2009; Ramasamy et al., 2009). Prebiotics are non-digestible feed ingredients, have selective effects on the intestinal microflora such as fructooligosaccharides and inulin, galactooligosaccharide, transgalactooligosaccharide and mannanoligosaccharide. A positive effect of prebiotics on some eggshell quality in laying hens (Swiatkiewicz et al., 2010; Amani et al., 2013). Synbiotic or symbiotic are combination of probiotics and prebiotics and in a form of synergism (Collins and Gibson, 1999). This combination could improve the survival of probiotic organism, because its specific substrate is available for fermentation. Synbiotics improved growth performance of broilers (Awad et al., 2009; Abeer El-Shenway and Mosaad Soltan, 2015). In addition, Amani et al., 2013 and observed improvement in egg production in laying hens. However, the results of Zarei et al., 2011 showed that inclusion of synbiotics had no significant effect on egg production.

There are few studies reported in literature on the effects of multi-probiotic supplementation on the diets of livestock and poultry (Mountzouris et al., 2007; Mountzouris et al., 2009; Wang et al., 2009) because of its many diverse functions. A large number of experiments has been carried out in hens (Zarei et al., 2011; Mikulski et al., 2012; Amani et al., 2013; Tang et al., 2015), but the literature for ducks is very limited (Li et al., 2011). The present study evaluated the effects of multi-bacterial species probiotic and synbiotic product may induce considerable beneficial synergetic effects on performance and egg quality of Khaki Campbell ducks.

Materials and Methods

A total of ninety 25-wk-old Khaki Campbell ducks were randomly assigned to 3 treatments with 3 replicates and 10 birds/cage in a completely randomized design. Hens were allotted to treatments so that the mean BW for each treatment was similar. The birds were placed in 2 x 2.5 x 1 m in an open-housing and concrete slat floor. The three diet treatments were the control (commercial feed with no supplement), diet supplemented with multi-probiotic (Bactosac-P) at 1.0 kg/ton and diets supplemented with synbiotic (Synbac®) at 1.0 kg/ton diet. A commercial probiotic used (Bactosac-P, K.M.R. Biotech Co., LTD.) was a 7-bacteria species product that comprised of 1 x 10^{10} cfu/kg of *Lactobacillus acidophilus*, 1 x 10^{10} *Lactobacillus plantarum*, 1 x 10^{10} *Bacillus subtilis,* 1 x 10^{10} *Bacillus licheniformis,* 1 x 10^{10} *Streptococcus faecium,* 1 x 10^{9} *Pediococcus pentosaceus* and 1 x 10^{9} *Saccharomyces cerevisiae*. A commercial synbiotic used (Synbac®, K.M.R. Biotech Co., LTD.) was combination of probiotic, 4-bacteria species product that comprised of 5 x 10^{9} *Bacillus subtilis,* 5 x 10^{9} *Pediococcus acidilacticii*, 5 x 10^{9} *Enterococcus faecium,* and 1 x 10^{9} *Saccharomyces cerevisiae* and prebiotic, xylo-oligosaccharide. Ducks were allowed *ad libitum* access to feed and water throughout the 16-wk experimental period during which the hens were 25 to 40 wk of age.

Data collection

Performance

Egg production, egg weight and survival rate were recorded daily during experiment (25-40 wk). Feed consumption and feed conversion ratio (kg feed/kg egg) data were recorded every 28 days (period). Egg mass were calculated from egg production and egg weight.

Egg quality

At the end of every 28 day laying cycle for three consecutive days, internal and external egg qualities were analyzed. The specific gravity of a whole egg was measured by Archimedes's method with an instrument designed by the measurement of the egg weight in air (*Wa*) and in water (*Ww*). The specific gravity was calculated by the formula [Specific gravity = *Wa*/(*Wa*-*Ww*)] (Wells, 1968) at the same. Haugh unit was calculated with following formula where the H_A is albumen height and W_E is egg weight (Haugh unit = 100log H_A + 7.57 - 1.7$W_E^{0.37}$) (Card and Nesheim, 1972). The shell thickness were measured from the three different parts of shell in each (equator, top and truncated edge) egg using a micrometer and was averaged and recorded as shell thickness.

Statistical analysis

All data were statistically analyzed as a completely randomized design by analysis of variance (ANOVA) using the SAS (2001) software. Duncan's multiple range test was used to determine treatment differences.

Results and Discussion

Laying performance

Laying performance are summarized in Table 1. No mortalities were recorded over the total feeding period. Patterson and Burkholder (2003) suggested that a daily intake of 108 to 109 microorganisms could exert beneficial effects in animals. Supplementation of probiotics and synbiotic caused higher egg production, egg weight and egg mass than control, but these differences were not significant statistically (P>0.05). Feed intake was not affected by dietary treatments. Feed conversion ratio improved (P>0.05) by probiotics or synbiotic dietary treatments. This result agreed with those reported by Mikulski et al. (2012), Zarei et al. (2011), Kalavathy et al. (2009), Ramasamy et al. (2009), Chen and Chen, (2004) fed commercial multi train probiotic to layers and showed no significant differences in egg production and egg weight compared to the control. Also, other reports disagreed with the results of Li et al., (2011), who also indicated that egg production (P<0.05), egg weight and egg mass were improved in Shaoxing duck fed probiotic-supplemented (*Bacillus subtilis*) diets. Moreover, Mikulski et al. (2012), Abdelqader et al. (2013), Mohammadian et al. (2013) indicated that probiotics increased egg production, egg weight and egg mass. The positive effects of *Bacillus subtilis* supplementation to laying ducks could be due to decrease in the multiplication of harmful bacteria resulting from improvement in gut environment and enhanced nutrient utilization (Miles, 1993). *Bacillus subtilis* may, also, enhance enzymatic activity in the digestive tract resulting in improving nutrient utilization. The main source of inconsistencies may be the breed and age of layers, microbial species, and supplemental dose (Mikulski et al., 2012). Adding synbiotic did not significantly affect egg production which has been reported by Swiatkiewicz et al. (2010) and Amani et al. (2013). Synbiotic affects the host by improving the survival and establishment of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth by activating the metabolism of one or a limited number of health promoting microorganisms and thus improving the host (Nekoubin and Sudagar, 2012). Moreover, synbiotic could increase the digestibility and available of many nutrient elements such as, mineral elements and proteins.

Egg quality

The effects of dietary probiotics and synbiotic on egg quality are shown in Table 2. Dietary treatments did not significantly affect shell weight and shell thickness. However, probiotics and synbiotic tended to increase shell weight and shell thickness. During the third and fourth period of the experiment (33 to 36 and 37 to 40 wk), probiotics and synbiotic had improved (P<0.05) egg specific gravity compared with control treatment. Haugh unit was significantly (P<0.05) improved by both feed additives on the second (29 to 32 wk) and overall periods (25 to 40 wk). The laying ducks fed probiotics containing diets produced the heaviest yolk weight (P<0.05) on the third periods (33 to 36 wk). These same results were obtained by Amani et al. (2013) who reported that supplementations with probiotics (Protexin® and Clostat®) and symbiotic (Diamond®) had no significant effects on the Haugh units of eggs from 27 to 39 wk of age. Li et al. (2011), who also indicated that probiotic (*Bacillus subtilis*) inclusion did not influence significantly on the egg weight, shell thickness, horizontal-vertical, egg yolk color and Haugh units (P>0.05). However, Abdelqader et al. (2013) found significant improvement in eggshell thickness in hens fed *Bacillus subtilis*, inulin and their combination as a symbiotic. Although egg weight is a relatively high heritability trait, the increased egg weight and eggshell thickness in our study were probably due to the improved intestinal microbial balance, which may benefit the utilization of nutrients (Zhang and Kim, 2014). The positive effect of the prebiotics on eggshell quality could be due to connected with the stimulation of mineral availability. On the other hand, the mechanism of the positive effect of prebiotics on mineral utilization can be

attributed to the high solubility of minerals because of the increased production of short-chain fatty acids (Scholz-Ahrens et al., 2007)

Conclusion

The results of the present study demonstrated that probiotics and synbiotics show potential for improving egg production, egg weight, egg mass, specific gravity, Haugh unit, yolk weight, and eggshell quality during the overall period of laying ducks.

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KEYWORD : Laying duck, Probiotic, Synbiotic, Performance, Egg quality

Table 1. Effect of dietary treatments on performance of laying ducks.

Item	Treatment			SEM	P-value
	Control	Probiotic	Synbiotic		
Egg production (%)					
From 25-28 wk	88.93	96.79	92.14	4.898	0.555
From 29-32 wk	86.55	93.45	93.93	2.592	0.159
From 33-36 wk	82.03	87.38	89.05	3.106	0.318
From 37-40 wk	87.50	91.42	94.16	2.831	0.317
From 25-40 wk	86.25	92.26	92.32	2.616	0.248
Egg weight (g/duck/day)					
From 25-28 wk	67.40	67.92	67.49	0.739	0.873
From 29-32 wk	69.77	70.14	70.79	0.807	0.679
From 33-36 wk	72.50	72.96	72.82	0.547	0.836
From 37-40 wk	73.96	74.28	73.44	0.793	0.763
From 25-40 wk	70.91	71.33	71.14	0.629	0.896
Egg mass (g/duck/day)					
From 25-28 wk	59.97	65.74	62.17	3.411	0.521
From 29-32 wk	60.35	65.57	66.47	1.768	0.099
From 33-36 wk	59.47	63.77	64.80	2.181	0.263
From 37-40 wk	64.65	67.92	69.15	1.865	0.286
From 25-40 wk	61.11	65.75	65.65	1.837	0.206
Feed intake (g/duck/day)					
From 25-28 wk	158.89	158.03	158.94	0.975	0.771
From 29-32 wk	144.97	143.63	144.83	3.251	0.905
From 33-36 wk	147.32	146.72	147.03	1.252	0.944
From 37-40 wk	146.36	144.21	146.47	3.153	0.854
From 25-40 wk	149.38	148.15	148.15	0.997	0.639
Feed conversion ratio					
From 25-28 wk	2.358	2.327	2.355	0.019	0.516
From 29-32 wk	2.079	2.048	2.046	0.056	0.900
From 33-36 wk	2.032	2.011	2.019	0.026	0.852
From 37-40 wk	1.980	1.941	1.994	0.053	0.769
From 25-40 wk	2.107	2.077	2.099	0.027	0.737

Table 2. Effect of dietary treatments on egg quality parameters of laying ducks.

Item	Treatment			SEM	P-value
	Control	Probiotic	Synbiotic		
Specific gravity					
From 25-28 wk	1.113	1.132	1.134	0.009	0.985
From 29-32 wk	1.106	1.102	1.099	0.009	0.606
From 33-36 wk	1.118 [a]	1.114 [ab]	1.112 [b]	0.001	0.029
From 37-40 wk	1.117[a]	1.115[ab]	1.112[b]	0.001	0.089
From 25-40 wk	1.122	1.116	1.115	<0.001	0.695
Haugh unit					
From 25-28 wk	87.69	89.25	89.44	1.243	0.580
From 29-32 wk	84.54[b]	89.44[a]	89.84[a]	0.972	0.015
From 33-36 wk	88.07	88.99	88.34	0.712	0.664
From 37-40 wk	85.87	88.21	86.27	1.002	0.284
From 25-40 wk	86.54[b]	88.97[a]	88.47[a]	0.539	0.042
Yolk weight (g)					
From 25-28 wk	22.22	21.85	21.11	0.395	0.209
From 29-32 wk	22.91	23.04	23.26	0.306	0.736
From 33-36 wk	23.14[c]	24.44[a]	23.70[b]	0.154	0.003
From 37-40 wk	22.90	23.33	23.21	0.309	0.623
From 25-40 wk	22.79	23.16	22.82	0.104	0.080
Shell weight (g)					
From 25-28 wk	9.20	9.37	9.74	0.258	0.384
From 29-32 wk	9.51	9.69	9.61	0.178	0.775
From 33-36 wk	9.33	9.66	10.11	0.231	0.132
From 37-40 wk	8.74	9.18	8.78	0.151	0.147
From 25-40 wk	9.19	9.48	9.56	0.111	0.127
Shell thickness (mm)					
From 25-28 wk	0.411	0.427	0.429	0.005	0.099
From 29-32 wk	0.369	0.384	0.386	0.009	0.386
From 33-36 wk	0.339	0.325	0.339	0.006	0.022
From 37-40 wk	0.304	0.321	0.337	0.007	0.042
From 25-40 wk	0.348	0.364	0.372	0.004	0.014

[a-c] Means with different superscripts within the same row differ significantly (P<0.05).

References

Abeer El-Shenway, M., and A. Mosaad Soltan. 2015. Effect of dietary probiotic and/or prebiotic supplementation on growth performance, carcass traits and some serum biochemical alterations in broiler chickens. J. Anim. Sci. Adv., 5:1480-1492.

Abdelqader, A., A.R. Al-Fataftah, G. Das. 2013. Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. Anim. Feed Sci. Technol., 179:103-111.

Amani, W., H.M.A. Youssef, H.M. Ali Hassan, and M.A. Mohamed. 2013. Effect of probiotics, prebiotics and organic acids on layer performance and egg quality. Asian J. Poult. Sci., 7:65-74.

Awad, W.A., K. Ghareeb, S. Abdel-Raheem, and J. Bohm. 2009. Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weight, and intestinal histomorphology of broiler chickens. Poult. Sci., 88:49-55.

Card, L.E., and M.C. Nesheim, 1972. Poultry Production. 11th ed. Lea and Febiger, Philadelphia, USA.

Chen, Y.C., and T.C. Chen. 2004. Mineral utilization in layers as influenced by dietary oligofructose and inulin. Int. J. Poult. Sci., 3:442-445.

Collins, M.D., and G.R. Gibson. 1999. Probiotics, prebiotics, and symbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin.Nutr., 69:1052S-1-57S.

Kalavathy, R., N. Abdullah, S. Jalaludin, M. Wong, and Y.W. Ho. 2009. Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. J. Sci. Food Agric., 89:482-486.

Li, L., C.L. Xu, C. Ju, Q. Ma, K. Hao, Z.Y. Jin, and K. Li. 2006. Effect of a dried *Bacillus subtilis* culture on egg quality. Poult. Sci., 85:364-368

Li, W.F., I.R. Rajput, X. Xu, Y.L. Li, J. Lei, Q. Huang, and M.Q. Wang. 2011. Effects of probiotic (*Bacillus subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck. Inter. J. Poult. Sci., 10:583-589.

Mikulski, D., J. Jankowski, J. Naczmanski, M. Mikulska, and V. Demey. 2012. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poult. Sci., 91:2691-2700.

Miles, R.D. 1993. Manipulation of the microflora of the gastrointestinal tract: Natural ways to prevent colonization by pathogens. In: Biotechnology in the Feed Industry. Proc. Of Alltech's Ninth Annual Symposium, T.P. Lyons, Ed. Alltech Technical Publications. Nicholasville, Ky, USA., pp:133-150.

Mohammadian, A., S.M. Mehdizadeh, H.Lotfollahian, F. Mirzaei, and H. Noroozian. 2013. Influence of dietary probiotic (Biomin IMBO) on performance of laying hen. Agric. Sci., 4:23-26.

Nekoubinm, H., and M. Sudagar. 2012. Assessment of the effects of symbiotic (Biomin Imbo) via supplementation with artificial diet (with different protein levels) on growth performance and survival rate in grass carp (Ctenopharyn godon Indella). World J. Zoology., 7:236-240.

Ramasamy, K., N. Abdullah, S. Jalaludin, M. Wong, and Y.W. Ho. 2009. Effects of Lactobacillus cultures on performance of laying hens and total cholesterol, lipid and fatty acid composition of egg yolk. J. Sci. Food Agric., 89:482-486.

Patterson, J.A., and K.M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci., 82:627-631.

Patterson, J.A., and K.M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci., 82:627-631.

SAS Institute. 2001. SAS User's Guide. Version 8.02 ed. SAS Institute Inc., Cary, NC.

Scholz-Ahrens, K.E., P. Ade, B. Marten, P. Weber, and W. Timm. 2007. Prebiotics, probiotics and synbiotics affect mineral absorption, bone mineral content and bone structure. J. Nutr., 137:838-846.

Swiatkiewicz, S., J. Koreleski, and A. Arczewska. 2010. Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. Czech J. Anim. Sci., 55:294-304.

Tang, S.G.H., C.C. Sieo, R. Kalavathy, W. Z. Saad, S.T. Yong, H.K. Wong, and Y.W. Ho. 2015. Chemical compositions of egg yolks and egg quality of laying hens fed prebiotic, probiotic, and symbiotic diets. J. Food Sci., 0:C1-C10.

Wells, R.G. 1968. Egg Quality, A Study of Hen's Egg. Ed. Carter, T.C., British Egg Marketing Board Symposium, Edinburgh. pp. 207-249.

Zarei, M., M. Ehsani, and M. Torki. 2011. Dietary inclusion of probiotics, prebiotics and symbiotic and evaluating performance of laying hens. Am. J. Agric. Biol. Sci., 6:249-255.

Zhang, Z.F., and I.H. Kim. 2014. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor content in broilers. Poult. Sci., 93:364-370

O-03-8

Effect of Dried Tomato Meal (Solanum lycopersicum) in Diet on Performance and Egg Quality of Native Chickens

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INTRODUCTION

Native chickens contribute a lot to household nutrition and income in rural areas of the tropics (Norris et al. 2007). But, improving nutrition for increasing egg and meat production in native chickens in Indonesia is critical. It was hardier than imported breeds on free range when little or no food is supplied by the owner (Henuk and Bailey, 2014).

Productivity of native chicken breeds may be doubled with improved diets and management conditions (Chowdhury et al., 2006). But, the native chickens have not attained their full production potential due to exposure to risks that influence against their survival and productivity under extensive management conditions. (Faruque et al, 2013).

Tomato pomace was a good source of protein, vitamins and minerals but may be limited in energy due to the high non-starch polysaccharides content. The wet tomato pomace contains 33% seed, 27% skin and 40% pulp, while the dried pomace contains 44% seed and 56% pulp plus skin (Sogi and Bawa, 1998). Dried tomato pomace (DTP) contains 10% moisture, 20.77% crude protein, 1760 Kcal/kg ME, 39.8% crude fiber, 7.3% ether extract, 4.24% ash, 0.5% calcium and 0.45% phosphorus (Jafari et al., 2006). The limiting factors of DTP in poultry diets are low energy and high fiber contents (Squires, et al., 1992). DTP contain remarkable amounts of α -tocopherol (Bordowski and Geisman, 1980), lutein, β -carotene, and lycopene, which could contribute to a darker yolk color that is desirable for the consumers (Mlodowski and Kuchta, 1998).

Habanabashaka et al. (2014) reported that up to 6% tomato waste meal can be added in laying hen diets without any adverse effect on egg quality and compromising egg production rate. This inclusion level also showed to be beneficial via enhancing yolk colour score and lycopene concentration and reducing egg yolk cholesterol content. The degree of yolk color is an important criterion in table eggs for consumption as well as manufacturing of egg-containing market food products (De-Groote, 1970). The color of egg yolks is produced by oxycarotenoids, as xanthophylls pigments, derived from the feed ingredients (Zahroojian et al., 2011). Vasupen et al. (2013) reported that feeding laying hens diets containing tomato pomace at inclusion 10% did not affect egg production, egg weight, feed consumption and efficiency of the hens. There are limited studies on the effects of dried tomato meal supplementation in birds, especially native chickens. It is therefore, the experiment was conducted to evaluate the effect of dried tomato meal in diet on performance and egg quality of native chickens.

MATERIALS AND METHODS

One hundred of native chickens (36 weeks of age) were allocated into five experimental diets and each was divided into four replications using a completely randomized design. Based diet was formulated to contain 53% corn, 10% rice bran, 10% fish meal, 6% CaCO3, Top Mix 0.5%, NaCl 0.5% and 20% commercial diet. Tomato meal was included in four experimental diets at levels of 2, 4, 6, 8% to substitute based diet. Treatments were: R0 = 100% based diet (BD) + 0% tomato meal (TM); R1 = 98% BD + 2% TM; R2 = 96% BD + 4% TM; R3 = 94% BD + 6% TM; and R4 = 92% BD + 8% TM. Chemical composition of tomato meal were: 16.73% crude protein, 1.53% fat, 30.94% crude fiber, 0.98% Ca, 1.20% P, and 2416 Kcal/kg ME. Feed and water were provided *ad libitum*. Chemical composition of the diets were shown in Table 1.

The study was conducted over a period of 8 weeks. Data were collected on feed intake (FI), egg weight (EW), Hen-day egg production (HDP), egg mass (EM), FCR, egg shell weight ESW), egg shell thicknes (EST), egg yolk weight (EYW) and egg yolk color (EYC). Hen-day egg production was calculated as: (number of eggs-produced $\times$ 100) / (number of hens $\times$ number of hens in production). Yolk colour was determined using the yolk colour chart. Egg shell membrane was removed carefully and manually from the broken egg shell and the thickness of the shell measured using a micro-meter screw gauge (An et al. 2010). Data collected were subjected to one-way analysis of variance Treatment means were compared using Duncan's multiple range test (Snedecor and Cochran, 1967) using software IBM SPSS 22.

RESULTS AND DISCUSSION

The results of performance and egg quality of native chickens fed dried tomato meal in diets were shown in Table 2. Results showed that tomato meal could be used with inclusion levels up to 8% to native chicken diets having no detrimental effect on egg weight and egg shell thickness. Moreover, it was found that tomato meal had effects on the feed intake, Hen-day egg production, egg mass, FCR, egg shell wieght, egg yolk weight and egg olk color of native chickens.

Leke et al. (2015) in previous study reported that tomato meal can be used as an alternative feedstuff in laying hen diets to substitute based diet, at inclusion levels up to 8% without negative effects on egg quality. Studies by Nobakht and Safamehr (2007) indicated that feeding of dried tomato pomace increased feed intake, egg production, egg weight and eggshell weight. Feed conversion ratio of reference diet, dried tomato pulp were better than other treatments. Some authors have found that supplementing dried tomato pomace in laying hens diet did not influence performance parameters but increase yolk color value (Mansoori et al., 2008). In a study by Calislar and Uygur (2010), dried tomato pulp had a significant effect on the egg shape index and egg yolk index, whereas, dried tomato pulp had no significant effect on the albumen index and Haugh unit. This result is similar to those reported by Mitsuhiro et al. (1994) who found a significant increase in egg mass was observed with reference diet, 2% red pepper and 5% dried tomato pulp compare to the control diet. In current study, egg shell thickness was not affected by dietary treatments.

Jafari et al. (2006) reported no significant differences in egg shell thickness and Haugh unit of laying hens fed on diets containing dried tomato pulp compared to hens fed on a control diet. This result is similar to those reported by Yannakopoulos et al. (1992), Nobakht and Safamehr (2007) and Mansoori et al. (2008), that the dietary addition of dried tomato pomace did not have any significant effect on FI. However, Jafari et al. (2006) and Calislar and Uygu (2010) found that DTP resulted in greater FCR. It has been shown that feeding hen diets containing DTP at inclusion rates up to 10% increased EP (Nobakht and Safamehr, 2007). EW was not affected by dietary treatments, a finding which is in agreement with the previously reported data (Jafari et al., 2006; Mansoori et al., 2008). It was observed that the dried tomato meal used in this study did not exhibit any negative effects on the egg quality. These discrepancies in results may be attributed to tomato variety, levels of dietary supplementation with tomato by-product, tomato processing conditions, and breed of native chickens.

CONCLUSION

It can be concluded that tomato meal can be used in native chicken diets up to 8% without negative effects on performance and egg quality.

KEYWORD : Egg, Chicken, Native, Performance, Tomato

Table 1. Chemical Composition of the Diets

Nutrients	Diets				
	R0 (0% TM)	R1 (2% TM)	R2 (4% TM)	R3 (6% TM)	R4 (8% TM)
Crude protein (%)	17.34	17.30	17.29	17.27	17.26
Fat (%)	5.35	5.12	5.04	4.96	4.89
Crude fiber (%)	3.76	5.39	5.93	6.47	7.03
Ca (%)	2.93	2.81	2.77	2.73	2.69
P (%)	0.62	0.65	0.66	0.67	0.68
ME (Kcal/kg)	2742	2722	2715	2709	2702

Table 2. Effect of Dried Tomato Meal in Diet on Performance and Egg Qualiy of Native Chickens

Variable	Treatments					SEM	P Value
	R0	R1	R2	R3	R4		
Feed Intake	75.93^{a}	75.90^{a}	76.91^{ab}	77.85^{bc}	78.18^{c}	0.235	.000
Egg Weight (g)	39.88	40.08	40.01	41.11	41.51	0.228	.059
HDP (%)	57.76^{a}	64.01^{c}	62.49^{b}	62.44^{b}	62.31^{b}	0.476	.000
Egg Mass (g/hen/day)	40.10^{a}	44.66^{b}	43.40^{b}	44.15^{b}	44.94^{b}	0.414	.000
FCR	1.89^{b}	1.70^{a}	1.77^{a}	1.76^{a}	1.74^{a}	0.016	.000
Egg Shell Weight (g)	3.62^{b}	3.35^{a}	3.38^{a}	3.62^{b}	3.73^{b}	0.041	.002
Egg Shell Thickness (mm)	0.35	0.35	0.35	0.34	0.36	.001	.268
Egg Yolk Weight (g)	11.43^{a}	11.52^{a}	11.98^{ab}	12.46^{b}	11.88^{ab}	0.119	.031
Egg Yolk Color	10.75^{a}	11.56^{b}	11.58^{b}	11.90^{b}	12.38^{c}	0.121	.000

Notes: $^{a-c}$Means in a row with different superscripts are significantly different at the *P*-value shown

[1]SEM = pooled standard error of mean (n=5)

REFERENCES

An, S.Y., Y.M. Guo, S.D. Ma, J.M. Yuan, and G.Z. Liu. 2010. Effects of different oil sources and vitamin E in breeder diet on egg quality, hatchability and development of the neonatal offspring. Asian-Australasian J. of Anim. Sci. 23(2): 234-239.

Bordowski, D.L., J.R. Geisman. 1980. Protein content and amino acid composition of protein of seeds from tomatoes at various stages of ripeness. J. Food Sci. 45: 228-229.

Calislar, S., and G. Uygur. 2010. Effects of dry tomato pulp on egg yolk pigmentation and some egg yield characteristics of laying hens. J. of Anim. and Vet. Adv. 9(1): 96-98.

Chowdhury, S.D., S. Ahmed, and M.A. Hamid. 2006. Improved feeding of desi chicken reared in confinement. The Bangladesh Vet. 23: 29-35.

De-Groote, G. 1970. Research on egg yolk pigmentation and its practical application. World's Poult. Sci. J. 20: 435-441.

Habanabashaka, M., M. Sengabo, and I.O. Oladunjoye. 2014. Effect of Tomato Waste Meal on Lay Performance, Egg Quality, Lipid Profile and Carotene Content of Eggs in Laying Hens. Iranian J. of Appl. Anim. Sci. 4 (3): 555-559.

Henuk, Y.L., and C.A. Bailey. 2014. Husbandry Systems for Native Chickens in Indonesia. Proceedings of The 16th AAAP Animal Science Congress, November 10 – 14, 2014. University of Gadjah Mada, Yogyakarta. Pp 759-762.

Faruque, S, M.S. Islam, M. A. Afroz, and M. M. Rahman. 2013. Evaluation of the performance of native chicken and estimation of heritability for body weight. J. of Bangladesh Academy of Sci. 37 (1): 93-101.

Jafari, M, R.R. Pirmohammadi, and V. Bampidis. 2006. The use of dried tomato pulp in diets of laying hens. Int. J. Poult. Sci. 5: 618-622.

Leke, J. R., J. S. Mandey, F. J. Nangoy. 2015. Nutrients and cholesterol of eggs affected by dried tomato meal in laying hens diet. IJASEIT Vol.5, No. 3.

Mansoori B, M. Modirsanei, and M.M Kiaei. 2008. Influence of dried tomato pomace as an alternative to wheat bran in maize or wheat based diets, on the performance of laying hens and traits of produced eggs. Iran. J. Vet. Res. 9 (4): 341-346.

Mlodowski, M, and M. Kuchta. 1998. Using carotenoid pigments from tomato pulp to improve egg yolk color in laying hens. Rocz. Nauk. Zootech. 25: 133-144.

Nobakht, A, and A.R. Safamehr. 2007. The effect of inclusion different levels of dried tomato pomace in laying hens diets on performance and plasma and egg yolk cholesterol contents. J. Anim. Vet. Adv. 6(9): 1101-1106.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical Methods. 6th Ed. Iowa State Univ. Press, Ames, IA.

Sogi, D.S, and A.S. Bawa. 1998. Dehydration of tomato processing waste. Indian Food Packer, 52:26-29.

Squires, M.W, E.C. Naber, and V.D. Toella. 1992. The effect of heat, water, acid and alkali treatment of tomato cannary waste on growth, metabolizable energy value and nitrogen utilization of broiler chicks. Poult. Sci. 71: 522-529.

Vasupen, K., S. Wongsuthavas, S. Bureenok, B. Saenmahayak, K. Ampaporn, and C. Yuangklang. 2013. Effect of tomato pomace and fibrolytic enzyme on egg production and egg quality. Int. J. of Biol. Biomol. Agric. Food and Biotech. Engineer. 7(1): 38-40.

Yannakopoulos, A..L, A.S. Tserveni-Gousi, and E.V. Christaki. 1992. Effect of locally produced tomato meal on the performance and the egg quality of laying hens. Anim. Feed. Sci. Technol. 36:53-57.

Zahroojian, N., H. Moravej, and M. Shivazad. 2011. Comparison of marine algae (Spirulina platensis) and synthetic pigment in enhancing egg yolk colour of laying hens. Br Poul. Sci. 52 (5): 584-588.

O-03-9

Digestibility Value of Broiler Diet Containing Fresh Mulberry (Morus alba) Leaves

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INTRODUCTION

Since feed cost is a major expense in poultry production (Chang, 2005; Ustundag and Zdogan, 2015), the search for cheap, locally available and equally nutritive feed source to partially substitute poultry diet has never been more pressing (Wong and Tan, 2009).

Mulberry (*Morus alba*) leaves have a great potential as an alternative protein source for poultry due to rich in protein, calcium and ascorbic acid and also contain carotene, vitamin B1, folic acid, folinic acid and vitamin D (Schmidek et al., 2002; Sarita et al., 2006), metabolizable energy contents and negligible anti-nutritional factors like tannic acid (Al-Kirshi et al., 2010; Olmo et al., 2012; Simol et al., 2012). Compared to soybean as the main source of protein in commercial poultry feed, mulberry leaves have higher amino acids content (Al-Kirshi et al., 2010). Besides the nutritive value, mulberry leaves are known to possess anti-microbial, anti-oxidant, anti-cancer, anti-stress, and immuno-modulatory activities (Yang et al., 2012; Devi et al., 2013).

Mulberry leaves contain high crude fiber and deoxynojirimycin, which in poultry feed serve as antinutrition to prevent carbohydrate digestibility; therefore it is limited to use as feed. Due to the high fibre content in mulberry, most researchs have tested it as food source for animals whose digestive system can handle fibrous diet. Fresh mulberry leaves have been fed to herbivores such as dairy cattle (Malisetty and Jatoth, 2013), goats (Omar et al. 1999), sheep (Kandylis et al., 2009) and rabbits (Bamikole et al., 2005). If mulberry leaf meal is introduced up to 20% in diets for growing pigs, a decreasing effect on total tract digestibility indices should be encountered (González et al., 1998). Has et al. (2013) reported that there was a decline in final body weight and digestibility of nutrients as long the increased use of mulberry leaves in broiler's feed.

There was limited information about effect fresh mulberry leaves in diet of broilers. The study was conducted to evaluate the effect of fresh Mulberry (*Morus alba*) leaves (FML) in diet on nitrogen retention, metabolizable energy corrected for nitrogen, crude fiber, fat, Ca and P digestibility of broilers.

MATERIALS AND METHODS

A total of 20 birds at age of 6 weeks old were used for 7 days of preliminary period and 3 days of data collection period using a completely randomized design with four treatments and five replications. The dietary treatment was including levels of 0, 2, 4, and 6% of fresh mulberry leaves in the diets. One bird from each experimental unit was put in individual cage for excreta and endogenous collection by using total collection method on 35 days old. Excreta was collected in three days, then sample was analyzed in laboratory to measure nutrient content and digestibility.The variables were nitrogen retention (NR), metabolizable energy corrected for nitrogen (AMEn), crude fiber, fat, Ca and P digestibility. Data were analyzed by one-way analysis of variance. Finally data used for calculation of AME, AMEn with equations as follow: 1. AME = [(F x GE) - (E x GE)] / F; 2. AMEn = [(F x GE) - (E x GE) - (NR x K)] / F (Zarei. 2006). The digestibility values for crude fibre (CF), fat, Ca and P digestibility were calculated as nutrient intake minus nutrient excreted divided by nutrient intake multiplied by hundred. Data were subject to one-way analysis of variance. The treatment means were compared using Duncan's multiple range test (Snedecor and Cochran, 1967) using software IBM SPSS 22.

RESULTS AND DISCUSSION

The effect of dietary treatments on crude fiber digestibility, fat digestibility, Ca and P digestibility, N retention and metabolic energy is showed in Table 3. Results showed that the supplementation of mulberry in diets have significant decreased ($P<0.05$) to NR and AMEn and fat digestibility, but the value of that variables were still in good category. Then, there was highly significant increased for Ca digestilibity, however there were no significant differences beetween treatments for crude fiber and P digestibility. The values of NR showed a significant difference between R0 and R1 diets. And, there was no significant difference between R1, R2 and R3 of diets. The values of AMEn showed that R0 diet had significant higher than those of R1, R2 and R3. However, between R1, R2,

and R3 had no significant differences. Treatments R0-R2 for Ca digestibility was similar and significant increased for R3 treatments. Difference occurred because mulberry leaves treatments had lower digestibility than control. This result was not similar to those reported by Vu et al. (2011), that apparent digestibilities of mulberry leaves were dry matter 66.4%, organic matter 71.8%, crude protein 58.2%, ether extract 49.4%, crude fiber 50.8%, NDF 58.4%, and ADF 52.9%. This difference was due to effect of increasing total crude fiber on diet mulberry leaf; accordingly total fiber in diet R0 was lower than that of other treatments. Ironkwe and Oruwari (2012) found that high crude fiber caused an increasing digestion rate in the gastrointestinal tract and therefore reducing digestion time and nutrients absorption by gastrointestinal membrane. Jiménez et al. (2013) also reported that increasing total fiber in diet significantly affected nutrients retention (dry matter, organic matter and nitrogen).

Crude fiber digestibility in this study was not significantly affected by dietary treatments. And the value of the digestibility was lower than that Alu et al. (2012) reported, that crude fiber digestibility of broiler chickens fed with high fiber such as ground nut shells ranged from 45-55%. However, Onimisi (2008) reported digestibility of crude fiber broilers fed low fiber (2%) ranged from 62.4% - 74.5%.

The decreasing of AMEn n this study maybe was caused by mulberry antinutrition called 1-deoxynojirimycin (DNJ), that could affect energy source absorption by preventing polysacaride hidrolysis and decreasing metabolic energy. Oku et al. (2006) and Yatsunami et al. (2011) reported that DNJ from mulberry could block α -glycosidase activity which hydrolyzes polysaccharide into plain molecule.

CONCLUSION

It can be concluded that mulberry fresh leaves in level up to 6% can be used as an alternatives feedstuff of broiler diet based on the digestibility value.

KEYWORD : Broiler, Digestibility, Fresh, Mulberry

Table 1. Chemical Composition of Based Diet and Mulberry Leaves

Nutrients	Based Diet	Mulberry Leaves		
		Fresh	Dry Weight	Dry Matter
Water (%)	-	65.2	-	-
Dry Matter (%)	-	34.8	93.49	-
Crude Protein (%)	20.58	7.09	19.06	20.39
Crude Fat (%)	5.66	0.31	0.82	0.88
Crude Fiber(%)	60.29	15.43	41.44	44.33
NFE (%)	7.78	5.25	16.79	17.95
Ash (%)	5.64	1.05	2.83	3.03
Ca (%)	1.77	0.15	0.41	0.44
P (%)	0.41	5.83	15.68	16.77
GE (Kcal/kg)	4962	1663	4359	4663

Table 2. Nutrients of the Experimental Diets

Diets	R0	R1	R2	R3
Based Diet (%)	100	98	96	94
Fresh Mulberry Leaves (%)	-	2	4	6
Total	100	100	100	100
Nutrients:				
Crude Protein (%)	20.58	20.58	20.57	20.57
Crude Fiber (%)	7.78	7.99	8.19	8.39
Crude Fat (%)	5.66	5.56	5.46	5.37
NFE (%)	60.29	59.97	59.65	59.33
Ca (%)	1.77	1.79	1.82	1.84
P(%)	0.41	0.41	0.42	0.42
Ash (%)	5.64	5.87	6.09	6.31
GE (Kcal/kg)	4962	4956	4950	4944

Table 3. Effect of Fresh Mulberry Leaves in Diet on the Digestibility

Variables	Treatments				SEM**	pValue
	0% FML	2% FML	4% FML	6% FML		
N Retention *	89.81[b]	85.97[a]	84.51[a]	84.09[a]	0.735	.011
AMEn (Kcal/kg) *	4024[b]	3518[a]	3452[a]	3361[a]	77.99	.003
CF Digestibility (%)	31.10	30.36	31.29	30.44	0.193	.232
Fat Digestibility (%)	96.74 [c]	93.07 [b]	92.70 [b]	88.70 [a]	0.598	.000
Ca Digestibility (%)	73.63 [a]	73.22 [a]	73.54 [a]	76.99 [b]	0.440	.001
P Digestibility (%)	32.85	32.40	32.77	33.75	0.595	.892

Notes: CF = crude fiber; FML = fresh mulberry leaves
* Dady, *et al*. (2016)
** SEM = standard error of means

REFERENCES

Alu, S.E., M.M. Adua, H.I. Damulak, R.S. Umar, A.D. Abubakarand, and U.D. Matthew. 2012. Growth rate and nutrient digestibility by broilers fed alkali-treated birds groundnut (*Arachis hypogea*) shell meal-based diets. Scholarly J. Agric. Sci. 2 (10): 231-237.

Al-Kirshi, R.A., A.R. Alimon, I. Zulkifli, A.Q. Sazili, W.M. Zahari, and M. Ivan. 2010. Utilization of mulberry leaf meal (*Morus alba*) as protein supplement in diets for laying hens. Italy J. Anim. Sci. 9: e51.

A.O.A.C, 1990. Association of official analytical chemists, official methods of analysis. 15th Edition. Washington, DC

Bamikole, M.A., M.I. Ikhatua, U.J. Ikhatua , and I.V. Ezenwa. 2005. Nutritive value of mulberry (*Morus spp.*) leaves in the growing rabbits in Nigeria. Pak. J. Nutr. 4: 231-236.

Chang,J. 2005. Malaysia Poultry and Products Annual 2005. GAIN Report Number: MY5036. USDA Foreign Agricultural Service, Kuala Lumpur.

Dady, Z., J. S. Mandey, M. R. Imbar, dan M. N. Regar. 2016. Nilai retensi nitrogen dan energi metabolis ransum menggunakan daun murbei (Morus alba) segar pada broiler. Jurnal Zootek ("Zootek" Journal) Vol. 36(1): 42-50.

Devi, B., N. Sharma, D. Kumar, and K. Jeet. 2013. *Morus alba* Linn: A phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences, 5 (2): 14-18.

Gonzalez, C., R. Tepper, and J. Ly. 1998. An approach to the study of the nutritive value of mulberry leaf and palm oil in growing pigs. Revista Cientifica, ISSN 0798-2259.

Has, H., V.D. Yunianto, and B. Sukamto. 2013. The Effectivity of Fermented Mulberry Leaves with Rumen Liquor as Broiler Feed on Final Body Weight, Dry Matter and Crude Fiber Digestibility, and Metabolic Energy. Animal Production 15(3): 173-179.

Ironkwe, M.O., and B.M. Oruwari. 2012. Effect of replacement levels of maize with plantain peel in broiler finisher diets. Bulletin of Environment Pharmacology and Life Sci. 1 (4): 39-42.

Jiménez, E.M., M. Frikha, A. de Coca Sinova, J. García, and G.G. Mateos. 2013. Oat hulls and sugar beet pulp in diets for broilers; Effects on growth performance and nutrient digestibility. Anim. Feed Sci. Tech. 182: 33–43.

Kandylis, K., I. Hadjigeorgiou, and P. Harizanis. 2009. The nutritive value of mulberry leaves (*Morus alba*) as a feed supplement for sheep. Trop. Anim. Health Prod. 41: 17-24.

Malisetty, V., and N. Jatoth. 2013. Effect of Supplementation of Mulberry Hay with Sorghum Straw on Nutrient Utilisation and Growth in Crossbred Calves. IOSR J. of Agric. and Vet. Sci. (IOSR-JAVS). 2 (2): 54-59.

Oku, T., M.Yamada, M. Nakamura, N. Sadamori, and S. Nakamura. 2006. Inhibitory effects of extractives from leaves of Morus alba on humanand rat small intestinal disaccaridase activity. Brit. J. Nutri. 95 (5): 933-938.

Olmo, C., Y. Martinez, E. Leon, L. Leyva, M. Nunez, R. Rodriguez, A. Labrada, M. Isert, C. Betancur, M. Merlos, and G. Liu. 2012. Effect of mulberry foliage (*Morus alba*) meal on growth performance and edible portions in hybrid chickens. Int. J. of Anim. and Vet. Adv. 4 (4): 263-268.

Omar, S.S., C.M. Shayo, and P. Uden. 1999. Voluntary intake and digestibility of mulberry (*Morus alba*) diets by growing goats. Trop. Grasslands. 33: 177-181.

Sarita, S., K. Rashmi, T. Anju, and R. Prakash. 2006. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). Int. J. of Food Sci. and Nutr. 57: 305-313.

Schmidek, A., R. Takahashi, A.N. de Medeiros, and K.T. de Resende. 2002. Bromatological composition and degradation rate of mulberry in goats. In: Mulberry for Animal Production, FAO Animal and Health Production Paper 147, Sanchez MD ed (FAO, Rome), pp. 207-212.

Simol, C.F., A. A. Tuen, H. H. Ahmad Khan, J. K. Chubo, P. J. H. King, and K. H. Ong. 2012. Performance of chicken broilers fed with diets substituted with mulberry leaf powder. African J. of Biotech. 11 (94): 1610-1611.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical Methods. 6th Ed. Iowa State Univ. Press, Ames, IA.

Ustundag, A.O., and M. Ozdogan. 2015. Usage Possibilities of Mulberry Leaves in Poultry Nutrition. Scientific Papers. Series D. Anim. Sci. Vol. LVIII, 2015.

Vu, C.C., M. W. A. Verstegen, W. H. Hendriks, and K. C. Pham. 2011. The Nutritive Value of Mulberry Leaves (*Morus alba*) and Partial Replacement of Cotton Seed in Rations on the Performance of Growing Vietnamese Cattle. Asian-Aust. J. Anim. Sci. 24 (9): 1233 – 1242.

Wong, H.K, and S.L.Tan. 2009. Status of the poultry industry in Malaysia. In: Food Security Malaysia, A.C. Soh and H.S. Yong, eds. (Akademi Sains Malaysia, Kuala Lumpur), pp.13-23.

Yang, X.Y., L. Yang, and H. Zheng. 2010. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food and Chemical Toxicology 48: 2374-2379.

Yatsunami K., K. Murata. and T. Kamei. 2011. Ideoxynojirimycin content and alfa-glucosidase inhibitory activity and heat stability of 1-deoxynojirimycin in silkworm powder. Food and Nut. Sci. 2: 87-89.

Zarei, A. 2006. Apparent and true metabolizable energy in Artemia meal. Int. J. of Poult. Sci. 5(7): 627-628.

O-03-10

EFFECTS OF SUPPLEMENTAL EFFECTIVE MICROORGANISM ON PERFORMANCE, SOME HISTOLOGICAL AND BLOOD PARAMETERS OF BROILER*

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Introduction

Feed additives including antibiotics have been widely used in poultry industry for several decades. The ban of antibiotics use as feed additives has led to investigations for alternatives to antibiotics. EM products originated in Japan after being discovered by Dr. Teruo Higa, a professor of horticulture. EM products have been on the market since 1983 and is now produced in over 156 countries. EM is a mixture of groups of anaerobic and aerobic beneficial microorganisms (lactic acid bacteria, photosynthetic bacteria, yeasts).

Studies have already shown that the use of EM could improve growth parameters (feed intake, weight gain, feed conversion ratio) and/or gut histology and microbiology in broilers (Esatu ve ark., 2011, 2012; Simeamelak ve ark., 2012; Wondmeneh ve ark., 2011). However, there is still lack of information regarding the efficacy and beneficial effects of EM in broiler chicks. Sokol et al. (2009) showed also that supplemental EM increased cholesterol, albümin and LDH activity but there was no effect on the body weight gain. Safalaoh and Smith (2001) suggest that the effectiveness of supplemental EM decreased serum cholesterol content of broilers, although Safalaoh (2006) were unable to confirm this observation statistically.

The present study was conducted to investigate the effects of effective microorganism (EM) supplied by drinking water and/or spraying onto poultry litter on performance, intestine histology and serum ALT, AST, glucose, cholesterol and triglycerides concentration of broiler chicks.

Material and Methods

Eighty-one thousand, 1-day-old broiler chicks were randomly divided into three treatment groups of 27000 birds each. The groups were 1) control, 2) EM supplied by drinking water, 3) EM supplied by drinking water + by spraying onto poultry litter. The basal diets (starter: 0-10 d, grower 11-21 d, finisher 22-42 d) were based on maize and soybean meal. Throughout the experiment, which lasted 6 weeks, feed and water were given ad libitum. Light was provided 24 hours each day throughout.

At the end of the experiment, when chickens were 42 days of age, all birds were slaughtered. During the slaughtering process, 50 birds from each group were randomly chosen to take blood samples into plain tubes. After collecting blood samples, they were analyzed with commercial kits (Shenzen Mindray Bio-Medical Electronics, China) for ALT, AST, glucose, cholesterol, and triglyceride levels. Jejunal samples were cut 2 cm length and were fixed in 10% buffered formalin saline and were prepared for routine histological study as mentioned by Higa (1993).

The data obtained in the experiment were analysed using the ANOVA procedure of SAS (1996) and treatment means were separated using Tukey's Honest Significant Difference test.

Results and Discussion

The results with respect to performance, blood and histological parameters of broilers are shown in Tables 1, 2, 3 and 4. The results obtained in the experiment showed that EM application affected performance parameters significantly ($P<0.05$). Serum glucose, cholesterol and triglycerides were affected by *EM treatments ($P<0.01$)* while having no effects serum ALT and AST concentrations. At the same time, EM supplied by drinking water + by spraying onto poultry litter significantly improved intestinal villi length ($P<0.01$) compared to the other groups. Total bacteria counts of poultry house floor were not influenced by the EM application.

Conclusions

In conclusion, effective microorganism supplementation to drinking water and/or spraying onto poultry litter tended to increase broiler growth performance and improved intestinal villi length of broiler chicks, while leading increased mortality due to faster weigh gain.

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KEYWORD : broiler, effective microorganism, growth performance, intestine histology, blood parameters

Table 1. Effect of supplemental EM in drinking water and/or spraying on to poultry litter on performance of broiler chicks

Performance Parameters	control	EM by dw	EM by dw + spraying	P
Body weight (kg/chick)	2.40 b	2.38c	2.50a	0.001
Feed conversion ratio (fi/bw)	1.66c	1.78a	1.68b	0.001
Mortality (%)	4.41c	6.85b	7.99a	0.001

[a, b]:means in the same row with different letters are significantly different (P<0.05).

Table 2. Effect of supplemental EM in drinking water and/or spraying on to poultry litter on some blood parameters of broiler chicks

Serum Parameters	control	EM by dw	EM by dw + spraying	SEM[c]	P
ALT (U/L)	12.1	11.1	10.3	1.02	0.771
AST (U/L)	381.6	409.0	474.1	21.4	0.197
Glucose (mg/dL)	159.6b	189.0a	190.1a	5.03	0.012
Triglycerides (mg/dL)	110.5b	126.9a	129.9a	2.14	0.011
Total Cholesterol (mg/dL)	40.3b	54.4a	41.2b	3.00	0.005

[a, b]:means in the same row with different letters are significantly different (P<0.05).
[c] SEM: standart error of means.

Table 3. Effect of supplemental EM in drinking water and/or spraying on to poultry litter on histological characteristics of broiler chicks

Histological parameters	Control	EM by dw	EM by dw + spraying	SEM[c]	P
Crypt depth (μm)	193.3	199.0	183.9	3.25	0.160
Villus height (μm)	1062.5b	1055.8b	1255.7a	11.32	0.001

[a, b]:means in the same row with different letters are significantly different (P<0.05).
[c] SEM: standart error of means.

Table 4. Effect of supplemental EM in drinking water and/or spraying on to poultry litter on total bacterial counts of poultry house floor

Bacterial counts	Control	EM by dw	EM by dw + spraying	SEM[c]	P
Initial total bacterial counts (cfu/g)	$3.67x10^3$	$3.60 x10^3$	$3.2 x10^3$	0.35	0.843
Final total bacterial counts (cfu/g)	$5.27 x10^6$	$5.47 x10^6$	$4.47 x10^6$	0.34	0.493

[a, b]:means in the same row with different letters are significantly different (P<0.05).
[c] SEM: standart error of means.

REFERENCES

HIGA, T., 1993: Revolution for helping world. Sukjai publishing, Bangkok. 199.

SAS 1996: Applied statistics and the SAS programming language. Third Editions.

SAFALAOH ACL, 2006: Body weight gain, dressing percentage, abdominal fat and serum cholesterol of broilers supplemented with a microbial preparation. Afr. J. Food Agric. Nutr. Dev., 6 (1):1-10.

SAFALAOH ACL, SMITH GA (2001) Effective microorganisms (EM) as an alternative to antibiotics in broiler diets: effect on broiler growth performance, feed utilisation and serum cholesterol. Proceedings of the 6th Kyusei Nature Forming Proceeding, Pretoria, South Africa, pp 150-155.

SOKÓŁ', R., M. MICHAICZYKI, A. SPODNIEWSKA', D. BARSKI, D., 2009: The influence of administering "effective microorganisms" to pull et s on chosen haematological and biochemical blood indexes. Pol. J. of Vet. Sci., 12(4): 519-522.

SIMEAMELAK, M., D. SOLOMON, T. TAYE, 2012: Evaluation of effective microorganisms on production performance of rhode island red chicks. Global J. of Sci. Frontier Res. Agri. and Vet. Sci. 12 (10): 23-29.

WONDMENEH, E., T. GETACHEW, T. DESSIE, 2011a: Effect of effective microorganisms (EM®) on the growth parameters of fayoumi and horro chicken. Int. J. of Poult. Sci. 10 (3):185-188.

WONDMENEH, E., A. MELESSE, T. DESSIE, 2011b: Effect of effective microorganisms on growth parameters and serum cholesterol levels in broilers. Afri. J. of Agri. Res. 6 (16): 3841-3846.

O-04-1

How do Thai consumers respond to quality of pork with different marbling levels?: a preliminary study in different age ranges

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INTRODUCTION

Marbling or intramuscular fat (IMF) level found in pork lean is related to eating quality of pork (Brewer et al. 2001). An increase in the amount of marbling has generally been accepted to positively influence sensory quality of pork. Tenderness, juiciness, oiliness, and overall liking ratings were positively correlated with marbling (Cannata et al. 2010). But Moeller et al. (2010) found no influences of marbling level on consumer perception for juiciness and tenderness. Although marbling has been shown to influence tenderness, its contribution to tenderness varies (Brewer et al. 2001; Cannata et al. 2010; Verbeke et al. 1999) reported that 2.0 to 4.0% marbling was optimal for pork palatability. It is always an interest for industries to understand consumer preferences. Knowing how marbling level may affect Thai consumer perception on pork eating quality and purchase intent can be useful information for pork production management and marketing. However, it has also been recognized that as individual ages and gains life experiences, their needs and interests evolve. Age is a process which naturally changes an individual's needs and interests, and consequently their consumer behavior (Agriculture and Agri-Food Canada, 2012). It is, therefore, an objective of this study to investigate the response of Thai consumers with three different age ranges on quality of pork LD with three different marbling levels as well as their purchase intent.

MATERIALS AND METHODS

At 24-h postmortem, nine boneless pork *Longissimus dorsi* (LD) muscles were fabricated from left side of Duroc castrated males (approximately 110.0 ± 10.0 kg BW) at a commercial packing plant (Lopburi, Thailand). Marbling level and color score were subjectively evaluated approximately between the 10^{th} and 11^{th} ribs by trained personnel based on the US National Pork Board (2011) marbling and color standard. After categorized into three marbling levels as low (score=1 or 2), medium (score=3 or 4), and high (score=5 or 6), the LD muscles with color score 3 were trimmed to approximately 2.0-mm thick fat cover. Each trimmed LD was vacuum packaged and dipped into hot water at 85.0 ± 0.5℃ for 2 sec. (Ultravac 2100 and Ultra shrink 2818, UltraSource, USA) before placing into stylofoam containers with ice (3.0 ± 2.0 ℃ , EBI 20, Ebro datalogger, Germany) for transportation to Meat Technology Research Network Center (Bangkok, Thailand). Upon arrival, vacuum packaged LD muscles were stored in a walk-in chiller (2.0 ± 1.0℃ , EBI 20, Ebro datalogger, Germany). On the next day, each LD was cut into ten 3.5-cm thick steaks, which were individually vacuum packaged (K-Nylon/LLDPE, Packmart, Thailand) and frozen (-20.0 ℃) until evaluation. For visual evaluation, each LD steak at each marbling level was cut and allowed to bloom for 24-h before photography (Canon EOS 700D, Japan). The three marbling level LD steak images were displayed on iPad mini tablets (Apple Inc., USA) for consumer visual evaluation. On the evaluation day, LD steaks were cooked in electronic broiler ovens (EO-42K, Sharp Corporation, Japan) at 180.0 ± 5.0℃ until core temperatures reached 71.0 ± 1.0℃ (Testo 176T4, Testo Inc., Germany) and cut across and along the muscle fibers into 1.3-cm^3 cubes. LD muscle cubes were placed in closed high density polyethylene bags to prevent dehydration and kept warm at 54.0 ± 1.0℃ in a water bath (Labec, Laboratory Equipment PTY. LTD, Australia) for consumer testing. Eighty-five consumers were randomly recruited from Faculties of Agricultural Technology and Agro Industry, King Mongkut's Institute of Technology, Bangkok Thailand. They were asked for demographic questions and screened for their pork eating preferences. Pork eating quality, including tenderness, juiciness, oiliness, overall flavor, and overall liking, was evaluated using a nine-point hedonic scale, where 9 is extremely like and 1 is extremely dislike. Water and crackers were served between samples for palate cleansing. After eating quality evaluation, consumers visually evaluated the 3 images for their liking responses (nine-point scale) and purchase intent (five-point scale). The orders of serving and image viewing were in random. The experimental

design was a Completely Randomized Design. Influences of marbling level and consumer age range on liking scores and purchase intents were analyzed using Analysis of Variance according to a 3x3 factorial treatment structure. Significant differences between treatments or interaction means were reported at $p<0.05$.

RESULTS AND DISCUSSION

Demographic profile and pork consumption habits of consumers

Among 85 recruited consumers, 69.40% were females and 30.60% were males. Members of households were primarily 4-6 persons. The majority age of consumers were between 26-45 years old (42.35%). Household incomes were in the ranges of <฿7,500 (20.00%), ฿7,501 to 18,000 (27.04%), and ฿24,001 to 35,000 (18.80%). The large majorities were either college graduates (40.00%) or post-college graduates (37.60%). For pork consumption habits, most consumers (68.20%) consumed pork 2-3 and 4-5 times per week, whereas nearly one-third (28.20%) of the panel participants consumed pork every day. In addition, 76.50% of the respondents liked to eat healthy food. Only 82 (96.50%) respondents reported to like eating pork and actually continued to participate in consumer sensory evaluation.

Effect of marbling level and age range on consumer response of pork LD eating quality.

The results (Table 1) showed that marbling level influenced ($p<0.05$) consumer responses on tenderness, juiciness, oiliness, overall liking, and visual marbling liking level, but did not affect their responses on ($p>0.05$) overall flavor. Pork LD with high marbling level obtained a higher ($p<0.05$) liking scores on tenderness, juiciness, oiliness, overall liking, and visual marbling liking than those with low marbling level. According to Cannata et al. (2010), tenderness, juiciness, oiliness, and overall liking ratings of pork LD were positively associated with marbling level. They found an increase in liking scores with an increase in marbling level ($p<0.05$). Consumer evaluation (n=150) on pork quality showed that tenderness and juiciness were positively correlated with marbling level (Brewer et al., 2001). Likewise, Fortin et al. (2005) found that initial tenderness (r=-0.31), softness (r=-0.32), and shear force (r=-0.41) were correlated ($p<0.01$) with IMF when evaluated by a trained panel. According to Smith and Carpenter (1974) cited by Savell and Cross (1988), based on "bite theory", decreasing of marbling results in lowering the bulk density by replacing protein with lipid. Fat is less resistant to shear value than coagulated protein. The reduction of bulk density is, therefore, accompanied by raising in tenderness. No influence ($p>0.05$) of consumer age range main effect was observed on their liking responses on pork LD eating quality, visual marbling liking, and purchase intent. But both marbling level and age range of consumers had a combined influence ($p<0.05$) on their purchasing decisions based on their visions on images of pork LD with different marbling levels.

Consumers with the ages of 45 years old preferred to buy ($p<0.05$) low and medium marbling LD more than those with high marbling. For consumers ages between 26-45 years old, no difference ($p>0.05$) on their purchase intent rating scores on each marbling level was found. Verbeke et al. (2005) indicated that most consumers had high awareness of the relation of food and health and reported strong feelings to have control over their health. In their study, older people >55 years provided a significantly higher food-health awareness score than other age groups. Age is a process which naturally changes an individual's needs and interests (Agriculture and Agri-Food Canada, 2012).The uncertain decision whether to buy or not to buy high marbling pork of Thai consumers >45 years old in our study could be due to their higher food-health awareness.

CONCLUSION

Level of marbling in lean pork affected consumer liking on tenderness, juiciness, oiliness, overall liking, and visual marbling level liking, but did not affect overall flavor liking. Consumers gave higher scores on tenderness, juiciness, oiliness, overall liking, and visual liking on high marbling LD than low. Based on image viewing of low, medium, and high marbling level LD steaks, consumers younger than 25 years old would definitely buy high marbling pork. But those older than 45 years old would buy low and medium marbling. Consumers seemed to prefer eating quality of marbled pork, but their purchase intent varied depending on marbling level and age range.

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KEYWORD : marbling level, consumer response, pork loin

Table 1 The effect of marbling level (ML) of pork *Longissimus dorsi* muscles and consumer (n=82) age range on consumer rating scores on pork eating quality, visual marbling liking, and consumer purchase intent.

Attributes	Marbling level (ML)			Age (years)			SEM	p-valve		
	Low	Medium	High	<25	26-45	>45		ML	Age	ML*Age
tenderness[1]	6.67[a]	6.85[ab]	7.29[b]	6.65	6.98	7.17	1.48	**0.03**	0.11	0.89
juiciness[1]	6.30[a]	6.51[ab]	6.94[b]	6.56	6.50	6.70	1.57	**0.03**	0.73	0.96
oiliness[1]	5.94[a]	6.31[ab]	6.62[b]	6.19	6.29	6.38	1.59	**0.03**	0.78	0.92
overall flavor [1]	6.33	6.54	6.78	6.45	6.55	6.65	1.62	0.23	0.77	0.82
overall liking[1]	6.56[a]	6.96[ab]	7.17[b]	6.86	6.76	7.07	1.50	**0.04**	0.44	0.99
visual marbling liking[1]	6.47[a]	7.09[b]	7.09[b]	6.58	7.05	7.03	1.47	**0.02**	0.08	0.25
purchase intent[2]	3.95	4.16	3.84	3.96	4.01	3.98	1.14	0.21	0.96	**0.02**

[a b] Means in a row with different letters are significantly different ($p<0.05$).
[1] Consumer acceptability was assessed by tenderness, juiciness, oiliness, overall flavor, overall liking and visual marbling liking ratings provided on a 9-point verbal hedonic scale, where 9 = like extremely and 1 = dislike extremely.
[2] Purchase intent was assessed by a buy or not buy rating scales provided on a 5-point verbal hedonic scale, where 5 = definitely buy, 4 = probably buy, 3 = might or might not buy, 2 = probably not buy, and 1 = definitely not buy.
SEM = Standard Error of Mean

Table 2 Means of purchase intent rating scores[1] of consumers (n=82) with three different age ranges after viewing images of pork LD muscles containing low, medium, and high marbling levels

Age range (years)	Marbling level (ML) [1]			Average	SEM
	Low	Medium	High		
<25	3.57[cd]	4.00[bc]	4.31[a]	3.96	0.11
26-45	4.03[bc]	4.24[ab]	3.76[bc]	4.01	0.11
>45	4.25[ab]	4.25[ab]	3.45[d]	3.98	0.18
Average	3.95	4.16	3.84		
SEM	0.13	0.09	0.15		

[a b c d] Means with different letters are significantly different ($p<0.05$).
[1] Purchase intent was assessed by a buy or not buy rating scales provided on a 5-point verbal hedonic scale, where 5 = definitely buy, 4 = probably buy, 3 = might or might not buy, 2 = probably not buy, and 1 = definitely not buy.
SEM = Standard Error of Mean

REFERENCES

Agriculture and Agri-Food Canada. 2012. Global consumer trends: age demographics. International markets bureau: Market analysis report. Canada.

Brewer, M.S., L.G. Zhu and F.K. McKeith. 2001. Marbling effects on quality characteristics of pork loin chops: Consumer purchase intent, visual and sensory characteristics. J. Meat Sci. 59:153-163.

Cannata, S., T.E. Engle, S.J. Moeller, H.N. Zerby, A.E. Radunz, M.D. Green, P.D. Bass, and K.E. Belk. 2010. Effect of visual marbling on sensory properties and quality traits of pork loin. J. Meat Sci. 85: 428-434.

Fortin, A., Robertson, W.M., and Tong, A.K.W. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. J. Meat Sci. 69: 297-305.

Moeller, S.J., Miller, R.K., Edwards, K.K., Zerby, H.N., Logan, K.E. and Aldredge, T.L. 2010. Consumer perceptions of pork eating quality as affected by pork quality attributes and end-point cooked temperature. J. Meat Sci. 84: 14-22.

National Pork Board. 2011. Official color & marbling quality standards. National Pork Board Des Moines: USA.

Smith, G.C. and Carpenter, Z.L. 1974. Eating quality of animal products and their fat content. Proceedings of the Symposium on Changing the Fat Content and Composition of Animal Products. Washington, D.C.: National Academy of Sciences Cited by Savell, J.W. and Cross, H.R. 1988. The role of fat in the palatability of beef, pork and lump In Designing food: Animal product option in the marketplace. Washington. National academy press.

Verbeke, W., Oeckel van, M.J., Warnants, N., Viaene, J. and Boucque, Ch.V. 1999. Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. J. Meat Sci. 53: 77–99.

Verbeke, W., S. De Smet, I. Vackier, M. J. Van Oeckel, N. Warnants and P.V. Kenhove. 2005. Role of intrinsic search cues in the formation of consumer preferences and choice for pork chops. J. Meat Sci. 69: 343–354.

O-04-2

THE ROLE OF FORAGES IN REDUCING HOUSEHOLD LABOUR DEMANDS OF CATTLE FEEDING DURING THE DRY SEASON IN CAMBODIA

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Objective

Dry season conditions in Cambodia pose a significant challenge to smallholder cattle raising in rural Cambodia where the majority of households rely on rice straw and stubble produced from the previous rice harvest and collected or grazed native grasses to provide feed for their cattle (Sath et al., 2008). Low in nutritional value and digestibility, native grasses are not only limited in their ability to support adequate animal growth but also result in significant labour demands due to time spent collecting feed and taking cattle to and from grazing areas (Maxwell et al., 2012). Introduced forages, when adopted by smallholder cattle owning households, represent a mechanism for which farmers can improve the quality of nutrition offered to their animals and reduce household labour demands of cattle feeding. Research has shown that households who grow and feed introduced forages experience a number of benefits including increased cattle live weight gain and body condition score as well as increased household income and time savings (Young et al., 2014a, Young et al., 2014b). However, these benefits largely depend on the ability of farmers to grow and feed forages to their cattle especially during the dry season when water availability is limited. For farmers able to utilise introduced forages during the dry season, household labour demands of cattle feeding may be reduced by providing a readily available source of fodder to animals instead of the traditional practices of feeding rice straw and native grasses. Therefore, the objective of the current study is to evaluate the role of forages in reducing household labour demands of cattle feeding during the dry season in Cambodia.

Methodology

Information on dry season cattle feeding practices and household labour inputs was recorded for 120 smallholder cattle owning households at the conclusion of the 'Best practice health and husbandry of cattle, Cambodia' (BPHH) project across six villages in three provinces in Cambodia. Households were classified into 2 groups; adopters (households feeding introduced forages) and non-adopters (households not feeding introduced forages). Information was recorded for each household including number of cattle owned, feeding practices including types of feed provided, responsibilities of household members in sourcing feed for cattle and time spent sourcing feed per day. Types of feed provided included rice straw, cut and carry grass (introduced forages and native grass), grazing on native pastures, crop bi-products (maize stover and soybean straw) and crop residues (rice bran, cereal and feed concentrates). Information regarding source of cut and carry grass and time spent sourcing per day was also recorded including forage plots (farmers own forage plots), 'chamka' areas (areas where fruit and vegetables are grown), field (farmer owned or communal fields), lakeside (area surrounding nearby lake) and bought (cut and carry grass purchased from a neighbour or seller). Data was collated, transferred into Microsoft Excel 2010 and analysed using Genstat 14th Edition (VSN International) with significant differences between groups assessed using one-way ANOVA for population means and the Z-test for population proportions.

Results

The results of the current study are shown in Table 1 and Table 2. Of the 120 households interviewed, 21 households reported feeding introduced forages to their cattle during the dry season and were classified as 'adopter households' whilst the remaining 99 households did not report this practice and were classified as 'non-adopter households' (Table 1). Adopters reported owning more cattle on average (3.67 head per household) compared to non-adopters (2.90 head per household). Whilst adopter and non-adopter households reported similar use of rice straw (95% and 99%), a greater proportion of adopters reported feeding cut and carry grass (95%) to their cattle compared to non-adopters (88%) and a significant reduction ($P < 0.01$) in use of crop bi-products (<1% vs. 29%). There was also a significant difference ($P < 0.05$) in the proportion of adopter households grazing their cattle on native pastures (24%) compared to non-adopters (58%). Forage growing appeared to have a significant

effect on household labour demands where forage growing significantly ($P < 0.01$) reduced the average daily time spent sourcing feed for cattle at the household level by 1.79 hours (Table 1). Adopter households also reported a significantly higher ($P < 0.01$) proportion of adult males involved in sourcing feed (100% vs 73%) and a reduction in daily time spent by adult males performing this activity (2.29 to 1.03 hours). Adult females and children also appeared to benefit from introduced forages where adopters reported a significant reduction ($P < 0.05$) in involvement of women and children (32% to 10%). However no significant differences were identified in the daily time spent sourcing feed for both these groups compared to non-adopters.

Table 1. Number of households, adoption category, number of cattle, feeding practices and labour demands with sample size (*n*) for proportions and standard deviation (S.D.) for variables

Households who reported feeding cut and carry grass to their cattle also reported the source and time spent sourcing per day (Table 2). All adopter households sourced cut and carry grass from their own forage plot (100%) and spent an average of 0.3 hours (approximately 20 mins) performing this activity. Non-adopter households mainly relied on field areas to supply cut and carry grass (53%) and spent an average of 2.30 hours each day on average collecting grass from these areas whilst also utilising chamka (23%) and other communal areas such as a grass area surrounding a nearby lake (21%).

Table 2. Sources of cut and carry grass and time spent sourcing per day with sample size (*n*) for proportions and mean and standard deviation (S.D.) for variables

Discussion

The current study has demonstrated that households who utilise forages during the dry season reduce the daily time spent providing feed to their cattle compared to households that rely solely on traditional feed sources. This time saving is likely due to two reasons including 1) the grazing of cattle on native pastures less common amongst adopter households leading to reduced time spent taking cattle to and from the field and 2) cut and carry grass sourced from forage plots (forages) providing a time saving for households not having to source cut and carry grass from field, chamka and lakeside areas. However, the results of the study demonstrated that these time savings are largely attributed to adult males in the household, who although reported increased involvement in cattle feeding as a result of forages, also reported a reduction in overall time spent sourcing feed per day. For adult females and children in adopter households, forage use resulted in decreased involvement in cattle feeding. However, unlike adult males, daily time spent sourcing feed was not significantly reduced. This may likely be due to a number of reasons with the small sample size ($n = 2$) and the large variation in values reported a likely factor, especially for children where one household reported children spent 5 mins per day feeding 2 cattle whilst another household estimated children spent 5 hours per day feeding 3 cattle. A small number of adopter households ($n = 5$) also reported grazing cattle on native pastures and this may have further affected the time savings reported for children in adopter households who would likely have been responsible for taking cattle to and from the field. Therefore, further research is warranted regarding household the labour benefits of forages for women and children involving a larger sample size and repeat measurements.

Conclusion

The results of the current study indicate that forages play an important role in reducing household labour demands of cattle feeding during the dry season in Cambodia. By providing a readily available source of cut and carry grass, households are able to save time previously spent sourcing native grass from other areas. Whilst the distribution of time savings amongst these households appears to be largely directed to adult males, the results of this study demonstrate that forage feeding results in decreased involvement of women and children in cattle feeding and thus presents opportunity for these household members to spend more time pursuing other activities that improve the social and financial status of their households. For children especially, reduced involvement in sourcing feed for cattle can lead to increased time spent at school (Dimang et al., 2009). However, the ability of households to grow forages during the dry season is an important consideration with water availability limited during the dry season in rural Cambodia. Therefore, future applications of forage technology on-farm must be supported by appropriate solutions to address this issue so that households can continue to benefit from the reduced labour demands of introduced forages.

KEYWORD : Forages, Cattle, Cambodia, Livelihoods

Table 1. Number of households, adoption category, number of cattle, feeding practices and labour demands with sample size (*n*) for proportions and standard deviation (S.D.) for variables

	Overall	Adopter HH	Non-adopter HH	*P*-value
Number of interviewed households	120	21	99	
No. of cattle per household (head)	3.03 (± 2.05)	3.67 (± 3.92)	2.90 (± 1.37)	$P = 0.12$
Type of feed fed to cattle				
Rice straw	98% ($n = 118$)	95% ($n = 20$)	99% ($n = 98$)	$P = 0.09$
Cut and carry grass	90% ($n = 108$)	95% ($n = 20$)	88% ($n = 88$)	$P = 0.17$
Grazing on native pastures	52% ($n = 62$)	24% ($n = 5$)	58% ($n = 57$)	$P < 0.05$
Crop bi-products (excl. rice straw)	25% ($n = 30$)	<1% ($n = 1$)	29% ($n = 29$)	$P < 0.01$
Crop residues	23% ($n = 27$)	33% ($n = 7$)	20% ($n = 20$)	$P = 0.10$
Household member involvement				
Adult male	78% ($n = 93$)	100% ($n = 21$)	73% ($n = 72$)	$P < 0.01$
Adult female	28% ($n = 34$)	10% ($n = 2$)	32% ($n = 32$)	$P < 0.05$
Children	28% ($n = 34$)	10% ($n = 2$)	32% ($n = 32$)	$P < 0.05$
Daily time spent sourcing feed				
Adult male	2.01 (± 1.35)	1.03 (± 1.47)	2.29 (± 1.18)	$P < 0.01$
Adult female	2.02 (± 1.21)	1.25 (± 1.06)	2.07 (± 1.22)	$P = 0.36$
Children	2.49 (± 1.23)	2.54 (± 3.47)	2.49 (± 1.11)	$P = 0.95$
Per household	2.87 (± 2.34)	1.39 (± 2.41)	3.18 (± 2.21)	$P < 0.01$

Table 2. Sources of cut and carry grass and time spent sourcing per day with sample size (*n*) for proportions and mean and standard deviation (S.D.) for variables

	Overall	Adopter	Non-adopter
Total number of responses	118	21	97
Source of cut and carry grass			
Forage plot	18% ($n = 21$)	100% ($n = 21$)	0% ($n = 0$)
Field	43% ($n = 51$)	0% ($n = 0$)	53% ($n = 51$)
Chamka	21% ($n = 25$)	0% ($n = 0$)	26% ($n = 25$)
Lakeside	17% ($n = 20$)	0% ($n = 0$)	21% ($n = 20$)
Bought	<1% ($n = 1$)	0% ($n = 0$)	<1% ($n = 1$)
Time spent sourcing per day (hours)			
Forage plot	0.21 (± 0.42)	0.30 (± 0.41)	0.00 (± 0.00)
Field	2.30 (± 1.08)	0.00 (± 0.00)	2.30 (± 1.08)
Chamka	2.03 (± 1.14)	0.00 (± 0.00)	2.03 (± 1.14)
Lakeside	2.10 (± 1.20)	0.00 (± 0.00)	2.10 (± 1.20)
Bought	0.08 (± 0.00)	0.00 (± 0.00)	0.08 (± 0.00)

References

Dimang, S, Pen, M, Sophal, L, Mom, S, Stuer, W & Savage, D 2009. Improved cattle nutrition increases the time available for children of small-holder farmers in Cambodia to attend school. *Recent Advances in Animal Nutrition*, 17, 192-192.

Maxwell, TW, You, S, Ung, B, Peou, L & Reid, J 2012. The social and other impacts of a cattle/crop innovation in Cambodia. *Agricultural Systems*, 107, 83-91.

Sath, K, Borin, K & Preston, TR 2008. Survey on feed utilization for cattle production in Takeo province. *Livestock Research for Rural Development* 20

Young, JR, O'Reilly, RA, Ashley, K, Suon, S, Leoung, IV, Windsor, PA & Bush, RD 2014a. Impacts on Rural Livelihoods in Cambodia Following Adoption of Best Practice Health and Husbandry Interventions by Smallholder Cattle Farmers. *Transboundary and Emerging Diseases*, 61, 11-24.

Young, JR, Rast, L, Suon, S, Bush, RD, Henry, LA & Windsor, PA 2014b. The impact of best practice health and husbandry interventions on smallholder cattle productivity in southern Cambodia. *Animal Production Science*, 54, 629-637.

O-04-3

Influence of Season on the Yield and Nutritional Composition of Indigofera zollingeriana at Different Cutting Intervals

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Introduction

Indigofera zollingeriana is a valuable shrubby legume which can be utilized as protein supplement for goats. Increasing interests in the use of tree legumes as a supplement to improve the productivity of small ruminants receiving poor quality roughages to improve their nutrition were explored. The potential of *I. zollingeriana* as forage for ruminants reportedly shows great promise. Their deep rooted growth form, ability to respond to low rainfall conditions, drought and high protein levels makes it an agronomically desirable source of feed for dairy goats. Tree legumes tend to have crude fiber concentrations greater than 18%; however, the nutritional compositions of the plants were reportedly dependent upon the stage of growth, maturity and species (Abdullah and Suharlina, 2010; and Hassen *et al*., 2008).

Information on the agronomic characteristics, yield, nutritional composition and proximate chemical analysis of *I. zollingeriana* purposely cultivated to sustain dairy goat production in Central Luzon is wanting. Data generated in this study will be necessary in considering ways to maximize cultivation of the legume plant through synchronization of planting schedules with seasons that favor plant growth.

Objective

The study intended to determine the influence of season on the agronomic characteristics, yield, nutritional composition and proximate chemical analysis of *I. zollingeriana* harvested at different cutting intervals.

Methodology

A. Transplantation of *zollingeriana* seedlings

Seedlings of *I. zollingeriana* (1 to 2 months of age) were transplanted in the forage production area. The seeds were soaked in warm water for 12 hours to ensure seed survival before sowing in seed beds. After one to two weeks, the shoots were transferred to a nursery garden in individual polyethylene bags until planting time. The seedlings were transplanted as they attain 60 cm in height, with an average number of 15 leaves before planting at distances of 0.75m by 0.5m.

B. Monitoring for Agronomic Parameters

Ninety-six (96) plants representing 10% of the total plant population in the designated experimental area (2,500m^2) were pruned 90 days from transplant at the height of 100 cm from soil surface and allowed to re-grow up to the time of succeeding cutting intervals of 45 and 30 days. Data on plant height (PH), herbage yield (HY), dry matter yield (DMY), percent dry matter (%DM) and leaf to stem ratio (LSR) were monitored on the cutting intervals described. A period of two (2) years was spent for gathering the aforementioned parameters done during the wet season (May to November) and dry season (December to April) of 2014 to 2016. Completely Randomized Design (CRD) was applied in the analysis of data while test of mean differences involved the use of ANOVA (SPSS v16 for windows).

Results and Discussion

Table 1 shows the average Plant Height (PH), Herbage Yield (HY), Dry Matter Yield (DMY), Percent Dry Matter (%DM) and Leaf to Stem Ratio (LSR) at 45d and 30d defoliation time during wet and dry season for two consecutive years. Data shows that PH, HY, DMY and LSR were significantly higher ($p<0.05$) at 45d cutting period compared to the 30d cutting period during the wet season while %DM was comparable ($p>0.05$) at both defoliation time. Results demonstrate that PH, HY, DMY and LSR were significantly higher ($p<0.05$) at 45d cutting period compared to the 30d cutting period except for a significantly higher ($p<0.05$) percent DM noted at 30day defoliation during the dry season as compared to the 45d cutting period.

Similar findings were observed by Abdulla and Suharlina (2010) where they found that average plant height of *Indigofera* sp. varied from 175-228 cm and herbage production was reportedly contributed by leaf and stem

formation, affected by cell division and elongation which are sites of high metabolic activity and dry matter accumulation. Dry matter production of both vegetative parts of *Indigofera* sp herbage was significantly affected by defoliation time. The decline in forage quality with maturity is due to increasing lignification of the stem compared to leaf although they remain nutritious and palatable at an advance stage of growth (Man et al., 1995; and Herdiawan (2013). LSR values in legumes are valuable because the leaves are metabolic organs while the quality of stems is largely affected by their structural function (Tjelele, 2006). *Indigofera* sp. can produce an average of 38 to 51 tons of herbage per annum at different times of defoliation (Abdullah et al. 2010) while a 2.6 ton DM/ha herbage production of *Indigofera sp was reported by Hassen et al.(2008).*

Abdullah, (2010) and Hassen et al.(2006) reported that the CP value ranged between (28.76%-29.83%), NDF(49-57%) and OM(43.10-77.63%) content of *Indigofera* sp. herbage. These values were within those reported by CIAT which is >20% for leaves and >10% for stem (Tropical Forages Fact Sheet, 2014).

Calcium and P level found was within those cited by CIAT at 12.7% and more than those reported by Abdullah (2010) at 1.16 to 6.95%. In most circumstances P, K, Mg, Na, Cl, Cu, Co, Fe, Se, Zn and Mo decline as the plant matures (Hassen et al., 2006). Concentrations of mineral elements in forage are dependent upon the interaction of a number of factors, including soil pH, plant species, stage of maturity, and climatic conditions.

Computed Herbage Yield and Dry Matter Yield per Hectare

Figure 1 shows the average estimated herbage yield (HY) and dry matter yield (DMY) per hectare during the wet and dry season for two consecutive years based on 39,900 plants per hectare. The computed average HY during wet season was at 22.97t/ha and 14.18t/ha; and 11.70t/ha and 9.26t/ha for the dry season during the 45d and 30d defoliation time respectively. The computed dry matter yield was at 5.66t/ha and 3.22t/ha for the wet season and 3.54t/ha and 2.19t/ha for the dry season at 45d and 30d cutting period, respectively. Data analysis demonstrated significant differences ($p<0.05$) in HY and DMY between wet and dry season on both defoliation intervals.

Proximate Chemical Analysis

Table 2 shows the proximate chemical analysis of *I.zollingeriana* leaves harvested at 30 and 45 days defoliation times. The average values of DM (24.19 and 24.09%), OM (86.84 and 89.93%) and CP (22.19% and 23%) of *I. zollingeriana* at 30 or 45 days cutting were comparable ($p>0.05$). Significant values ($p<0.05$) of CF (18.05 and 19.01%), EE (2.51 and 3.28), NDF(23.16% and 24%), ADF (19.19% and 20.42%), Ca (9.14 and 12.18%) and P at 1.003 and 1.009 were noted at 45 days defoliation time compared to 30d defoliation time except a significantly higher ($p<0.05$) Ash content in *I.zollingeriana* at 30d cutting.

Conclusion

Data on agronomic characteristic of *I. zollingeriana* gathered on the wet and dry season revealed that there were significant differences ($p<0.05$) between plant height, herbage yield, DM yield, %DM and leaf to stem ratio of *Indigofera* at 45 and 30 days cutting intervals.Proximate analysis on *I.zollingeriana* indicated no significant differences between the 45 and 30 days regrowth in DM, OM and CP. However, significant differences were found in the CF, EE, NDF, ADF, Ca and P. Estimated HY and DM yield suggests the vast potential of *I. zollingeriana* as a forage source which yielded sufficient amount of forage material comparable to the yield of other forage legume species which can support the feed requirement of 22 to 24 heads of lactating does with an average feed consumption of 1 to 1.25kg/DM per day.

KEYWORD : Indigofera zollingeriana, Forage, Yield, Cutting Interval

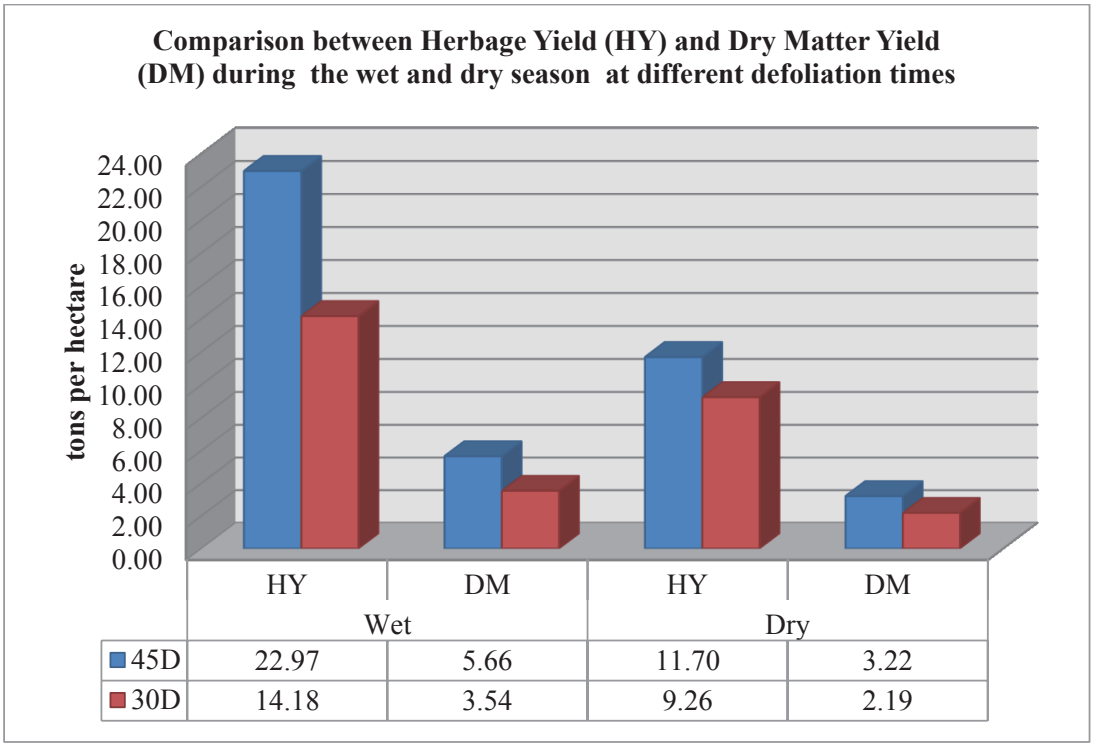

Figure 1. Computed HY and DM for the wet and dry season at 45 and 30 days defoliation time for two consecutive years.

Table 1. Comparison between average Plant Height, Herbage Yield, Dry Matter Yield, %DM and Leaf to Stem Ratio of *I. zollingeriana* at 45 and 30 days cutting period during wet and dry season for a period of two years

PARAMETERS	WET		DRY	
	45D	30D	45D	30D
Plant Height (cm)	208.81(±1.79)^{a}	173.23(±1.07)^{b}	180.53(±0.92)^{a}	162.97(±1.01)^{b}
Herbage Yield (g)	589.10(±24.76)^{a}	363.53(±12.66)^{b}	384.37(±5.97)^{a}	237.54(±7.48)^{b}
Dry Matter Yield (g)	145.20(±5.55)^{a}	90.89(±3.14)^{b}	82.53(±1.59)^{a}	56.24(±1.76)^{b}
Percent (%) DM	25.02(±0.27)	24.41(±0.23)	24.38(±0.20)^{a}	25.94(±0.26)^{a}
Leaf to Stem Ratio	1.00(±0.03)^{a}	0.73(±0.02)^{b}	0.75(±0.02)^{a}	0.54(±0.02)^{b}

Values represent mean (±SEM) on average plant height, herbage yield, dry matter yield, %DM and leaf to stem ratio of *I. zollingeriana* at different defoliation times during wet and dry season for a period of two years ^{a,b} superscripts indicate significant difference ($P<0.05$) in the same row across season.

Table 2. Proximate chemical analysis of *I.zollingeriana* harvested at 30 and 45 days defoliation time

NUTRIENTS	30 DAYS CUTTING	45 DAYS CUTTING
	(PERCENT)	
Dry Matter (DM)	24.19(±0.062)	24.09(±0.442)
Organic Matter (OM)	86.84(±0.903)	89.93(±0.017)
Crude Protein (CP)	22.19(±0.116)	23.00(±0.771)
Crude Fiber (CF)	18.05(±0.095)^{b}	19.01(±0.061)^{a}
Crude Fat (EE)	2.51(±0.185)^{b}	3.28(±0.023)^{a}
Neutral Detergent Fiber (NDF)	23.16(±0.165)^{b}	24.00(±0.012)^{a}
Acid Detergent Fiber (ADF)	19.91(±0.075)^{b}	20.42(±0.110)^{a}
Ash	13.16(±0.020)^{a}	10.07(±0.019)^{b}
Calcium (Ca)	9.14(±0.101)^{b}	12.18(±0.165)^{a}
Phosphorous (P)	1.003(±0.001)^{b}	1.009(±0.001)^{a}

Values represent mean (±SEM) on the proximate chemical analysis *I.zollingeriana* harvested at 30d and 45d cutting interval. ^{a,b} superscripts indicate significant difference ($p<0.05$) in the same row

REFERENCES

ABDULLAH, L. and SUHARLINA. 2010. Herbage yield and quality of two vegetative parts of *I. zollingeriana* at different time of first regrowth defoliation. Med.Pet. 33:44-49. Retrieved on June 10, 2014 from http://journal.ipb.ac.id/index.php/mediapeternakan/article/view/1246/0

CIAT (Centro Internacional de Agricultura Tropical).2001.Tropical Forage Fact Sheet. Retrieved on August 22, 2014 from https://ciat.cgiar.org/crops/tropical-forages

CRUZ, E.M. 2001. Rapid rotational grazing for goats. In: Gray, GD. SPARC Newsletter for Sustainable Control of Helminth Parasites in the Tropics. Issu 6. Special Congress Edition.ILRI, Los Baños, Laguna. September 2001.

HASSEN A., N.F.G. RETHMAN, Z. APOSTOLIDE and W.A. VAN NIEKERK. 2008. Forage production and potential nutritive value of 24 shrubby *I. zollingeriana* accessions under field conditions in South Africa. Tropical Grasslands 2008 Volume 42, 96–103. pp 99-103. Retrieved on December 02, 2014 from www.tropicalgrasslands.asn.au/.../Vol42_02_2008_pp96_103.pdf.

HERDIAWAN, IWAN. 2013. The growth of tree legume fodder Indigofera zollingeriana at various levels of drought stress treatment. JITV 18(4): 260-266.

Retrieved on January 15, 2015 from http://www.researchgate.net/publication/260317247_Influence_of_moisture_stress_on_growth_dry_matter_allocation_and_water_use_and_water_use_efficiency_of_four_Indigofera_species.

MAN N.V., N.V HAO and V.M. TRI.1995.Biomass production of some leguminous shrubs and trees in Vietnam. Livestock Research for Rural Development. Volume 7,Article #14. Retrieved January 21, 2015, from http://www.lrrd.org/lrrd7/2/8.htm

TJELELE, T.J. 2006. Dry matter production, intake and nutritive value of certain *I. zollingeriana* species [Thesis]. Pretoria. M.Inst.Agrar. University of Pretoria.

Retrieved on august 28, 2014 from http://www.repository.up.ac.za/bitstream/handle/2263/23754/06...

TROPICAL FORAGES FACT SHEET. 2014. Fact Sheet *I. zollingeriana* spicata.

Retrieved on Retrieved on august 28, 2014 from *www.tropicalforages.info/key/Forages/Media/Html/I.zollingeriana_spicata.htm*

O-04-4

Enrichment the Nutritive Value of Cassava Top Silage with Molasses and Urea Supplement Using Gas Production Technique

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Abstract

Cassava top was harvested at three months age and chopped to 2-3 cm length for ensiling to study on nutritive value and *in vitro* digestibility. The ensiling study was randomly assigned according to a 3 x 4 x 2 factorial arrangement in a Completely Randomized Design in which the first factor was urea (U) supplementation at U0, U0.5, and U1.0 % and the second was molasses (M) supplementation at M0, M0.5, M1, and M2% of the crop dry matter (DM), while the third factor was Duration (D) time of ensilage at D15 and D30 days, respectively. Samples of each silage were collected and measured for temperature, pH, and chemical composition and subsequently selected to study using *in vitro* gas production technique. The results showed that silages pH was linearly increased with U supplementation while M decreased pH ($P<0.05$). On the other hand, silage temperature were not affected by U, M and D ($P>0.05$). Increasing U supplementation increased crude protein (CP) content of CTS while D15 resulted in higher CP as compared to D30. Increasing level of M supplementation and ensiling at D30 decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the silage ($P<0.05$). The results of the *in vitro* study showed that increasing levels of U and M supplementation increased the *in vitro* True and NDF digestibility ($P<0.05$) while no effect of D of ensiling time was found ($P>0.05$). Furthermore, pH was increased by U supplementation whereas ensiling at D30 resulted in lower pH while protozoal population were similar among treatment. We conclude that supplementation of U and M increased CTS quality and the *in vitro* digestibility without adverse effect on pH. Supplementation of U at 0.5 and M at 2% of the crop DM with the minimum 15 days of ensiling time is recommended for lactating dairy cows and fattening beef cattle.

Introduction

In most parts of the world, forage conservation is a key element for productive and efficient ruminant livestock production. Forage conservation also provides farmers with means of preserving forage when production exceeds that of grazing animals. Silage making increased considerably from the 1960s and is the predominant method of forage preservation in temperate areas of the world (Cheli et al., 2013). Silage is widely used in farms and has a substantial role in animal production systems. High silage quality is a key factor to minimize cost of production and sustained animal health. Increasing use of silage has resulted in continuing efforts to minimize the quality losses (Bartzanas et al., 2013). Ensiling is a crop preservation method based on natural lactic acid fermentation under anaerobic conditions where anaerobic microbes build up organic acids mainly lactic acid by using fermentable carbohydrates (Gollop et al. 2005; Ki et al. 2009). To improve silage preservation and guarantee animal feed quality, silage additives such as chemicals, enzymes, and bacterial agents can be employed. Addition of carbon and nitrogen sources could improve the quality of silage. Wanapat et al. (2013) reported that supplementation of urea and molasses could improve the quality of whole crop rice silage in terms of nutritive value and rumen degradation. Addition of carbon and nitrogen sources could improve the quality of silage and have subsequent effects on rumen degradation characteristic and production in ruminants. It was reported that supplementation of urea (U) and molasses (M) could improve the quality of silage in terms of nutritive value and rumen degradation (Giang et al., 2016; Wanapat et al., 2013; 2014). Addition of carbon and nitrogen sources could improve the quality of silage and as a subsequence effects on rumen degradation characteristic and the production in ruminants. Cassava or tapioca (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropics and sub-tropics. Cassava leaves contain high level of crude protein at about 25% (Wanapat and Kang, 2013). Therefore, the objective of this study was to improve nutritional value of cassava top silage (CTS) using urea and molasses and *in vitro* ruminal fermentation.

Materials and Methods

Cassava top was harvested and immediately chopped at 2-3 cm length and ensiled to the respective

supplementation according to a 3 x 4 x 2 factorial arrangement in a Completely Randomized Design (CRD). Factor A was U supplementation at U0, U0.5 and U1% of the crop DM and factor B was M supplementation at M0, M0.5, M1 and M2% of the crop DM while factor C was D of ensilaged time at D15 and D30 days, respectively. After D15 and D30 days of ensiling, pH and temperature of each CTS were measured using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore) and approximately 200g of each silage were sampled for analysis of chemical composition and kept for study in *in vitro* gas techniques. Samples were dried at 60°C and ground (1mm screen using the Cyclotech Mill, Tecator, Sweden) and analyzed using standard methods of AOAC (1990) for DM, crude protein (CP), organic matter (OM) and ash. Acid detergent fiber (ADF) was determined according to an AOAC method (1990) and was expressed inclusive of residual ash while neutral detergent fiber (NDF) in samples was estimated according to Van Soest et al. (1991) with addition of α -amylase but without sodium sulphite and results are expressed with residual ash.

Strict anaerobic techniques were used in all steps during the rumen fluid transferring and incubation periods. Rumen fluid samples were removed from two dairy steers (1 liter per animal spicy) before morning feeding under vacuum via the rumen fistula into a 2 liters plastic flask and transferred into 2 pre-warmed thermos flasks (1 liter) (Menke et al., 1979; Makkar et al., 1995). The fluid was then transported to the laboratory. Two hundred milligrams of each silage samples were weighed into 60 ml bottle. The bottles with the mixture of substrate treatments were pre-warmed in a water bath at 39℃ for 1 h before filling with 30 ml of rumen inoculums mixture. The bottles were then sealed with rubber stoppers and aluminum caps and incubated in a water bath set at 39°C. All treatments were assigned according to a 3×4×2 factorial arrangement in a CRD with three replications per treatment including triplicates of blank (medium only) in three incubation runs. At 48 h post inoculation a set of samples were determined *for in vitro* true digestibility (TD) according to Van Soest and Robertson (1985). In brief, the content of the bottle was transferred quantitatively to a spoutless beaker by repeated washing with 100 ml neutral detergent solution. The content was refluxed for 1 h and filtered through preweighed Gooch crucibles. The DM of the residue was weighed and *in vitro* TD of feed was calculated based on the following equation: TD = ((DM of feed taken for incubation - NDF residue) × 100)/DM of feed taken for incubation. The portion of 1 ml rumen fluid was collected and kept in a plastic bottle to which 9 ml of 10% formalin solution (1:9 v/v, rumen fluid: 10%formalin) were added and stored at 4 ℃ for measuring total protozoal population according to the method of Galyean (1989) based on the use of a haemacytometer (Boeco, Hamburg, Germany). Rumen fluid were diluted using autoclave distilled water (121°C for 15 minutes) as a medium, 10 time, and counting using 10x10 ocular x objective of microscope.

All obtained data were subjected to the General Linear Models (GLM) procedures of SAS (1998) according to a 3 × 4 × 2 factorial arrangement in CRD. For all parameters, differences among treatments means were contrasted by Tukey's Multiple Comparison Test (Crichton, 1999).

Results

Table 1 presents the result of the nutritive value of CTS affected by U and M supplementation in different duration of time. It shows that increasing U supplementation linearly increased silages pH while M decreased pH ($P<0.05$). On the other hand, silage temperature were not affected by U, M and D ($P>0.05$). Increasing U supplementation increased CP content of silage while D15 resulted in higher CP as compared to D30. Increasing level of M supplementation and ensiling at D30 decreased NDF and ADF of the silage ($P<0.05$). The results from the *in vitro* study are illustrated in Table 2. Increasing levels of U and M supplementation increased the *in vitro* True and NDF digestibility ($P<0.05$) while no effect of D of ensiling time was found ($P>0.05$). Furthermore, fermented pH was increased by U supplementation whereas ensiling at D30 resulted in lower pH while protozoa population were similar among treatments.

Conclusions and recommendations

Based on this study, it could be concluded that supplementation of U and M increased CTS quality and *in vitro* ruminal fermentation. Supplementation of U at 0.5 and M at 2% of the crop DM with the minimum 15 days of ensiling time is recommended for lactating dairy cows and fattening beef cattle.

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KEYWORD : Cassava top silage, Molasses, Nutritive value, Urea, Digestibility

Table 1. Effect of urea, molasses and duration of cassava top ensiling on nutritive values of the silage

Treatments			DM	OM	NDF	ADF	CP	Ash	pH	Tem.
Urea	Molasses	Duration								
0	0	15 days	19.9	93.6	50.4	26.7	24.5	6.4	3.8	28.6
	0.5		18.3	92.7	53.1	26.7	26.3	7.3	3.5	28.4
	1		16.8	92.0	44.5	26.5	23.2	8.0	3.6	28.2
	2		21.5	92.5	39.2	29.8	26.7	7.5	3.1	28.0
0.5	0		19.9	93.6	59.0	43.8	27.9	6.4	4.9	28.8
	0.5		20.8	93.3	53.3	41.6	30.5	6.7	4.3	29.3
	1		20.1	93.0	55.7	36.8	28.4	7.0	4.8	29.7
	2		19.5	93.6	42.7	27.4	31.1	6.4	4.2	29.5
1	0		17.8	93.4	54.9	37.9	32.9	6.6	5.5	29.7
	0.5		21.8	93.4	55.3	38.0	34.1	6.6	5.3	30.1
	1		20.5	92.7	47.2	35.9	33.8	7.3	5.0	30.4
	2		22.5	93.0	46.3	33.8	36.7	7.0	5.0	29.3
0	0	30 days	17.0	94.3	54.9	36.0	22.9	5.7	3.7	31.5
	0.5		21.5	93.2	64.7	36.7	21.9	6.8	3.2	31.5
	1		17.2	93.4	54.1	31.0	20.2	6.6	3.4	31.5
	2		17.4	92.3	36.0	33.1	23.0	7.7	3.2	31.5
0.5	0		19.3	93.8	67.8	39.5	27.1	6.2	4.9	31.5
	0.5		20.1	93.3	58.1	32.6	29.5	6.7	4.7	31.5
	1		17.1	93.7	48.6	28.4	27.5	6.3	4.7	31.5
	2		19.5	93.6	41.5	25.2	29.1	6.4	4.0	31.5
1	0		18.1	94.7	57.8	35.2	31.1	5.3	4.8	31.5
	0.5		18.5	94.2	51.1	28.9	32.9	5.8	4.7	31.5
	1		17.8	93.5	45.2	25.8	33.6	6.5	4.7	31.5
	2		19.8	93.4	41.6	23.0	32.4	6.6	4.4	31.5
SEM			1.49	0.12	1.53	1.20	1.60	0.11	0.87	0.67
Interaction										
Urea			ns	***	**	**	***	***	*	ns
Molasses			ns	***	***	***	ns	***	**	ns
Duration			ns	***	0.07	**	*	***	ns	0.07
Urea × Molasses			ns	***	***	***	ns	***	ns	ns
Urea × Duration			ns	***	**	***	ns	**	ns	ns
Molasses × Duration			ns	***	*	0.06	ns	***	ns	ns
Urea × Molasses × Duration			ns	0.06	*	ns	ns	0.06	ns	ns

DM = Dry matter; M = Molasses; CP = Crude protein; ADF = Acid detergent fiber; NDF = Neutral detergent fiber; Tem. = Temperature

Table 2. Effect of urea, molasses and duration of cassava top ensiling on *in vitro* digestibility, pH and protozoal population

Treatments			Digestibility (48 hour post incubation)		pH	Protozoa
Urea	Molasses	Duration	True	NDF		
0	0	15 days	50.9	26.9	6.79	3.00
	0.5		64.4	37.4	6.79	2.50
	1		66.8	35.2	6.74	2.25
	2		65.8	41.4	6.72	2.25
0.5	0		55.1	30.4	6.79	3.00
	0.5		62.7	41.2	6.80	3.00
	1		67.2	47.5	6.79	3.00
	2		70.6	48.4	6.78	2.50
1	0		61.3	41.3	6.87	3.00
	0.5		68.3	48.7	6.94	2.75
	1		67.7	53.3	6.91	2.00
	2		73.0	53.8	6.90	2.25
0	0	30 days	50.1	32.2	6.61	4.00
	0.5		68.3	39.4	6.68	2.50
	1		68.5	40.2	6.65	2.25
	2		69.3	54.4	6.65	3.25
0.5	0		64.3	36.5	6.75	3.00
	0.5		70.3	44.3	6.74	2.25
	1		71.1	44.6	6.73	2.50
	2		72.5	49.0	6.71	2.00
1	0		56.9	41.0	6.71	2.50
	0.5		65.3	46.8	6.71	2.25
	1		73.3	50.5	6.67	2.25
	2		75.2	54.0	6.69	3.50
SEM			2.74	2.66	0.02	0.29
Interaction						
Urea			*	***	***	ns
Molasses			***	***	ns	ns
Duration			ns	ns	***	ns
Urea × Molasses			ns	ns	ns	ns
Urea × Duration			ns	ns	***	ns
Molasses × Duration			ns	ns	ns	ns
Urea × Molasses × Duration			ns	ns	ns	ns

NDF = Neutral detergent fiber

REFERENCES

AOAC. 1990. Official Methods of Analyses, 15th edn. Assoc. Offic. Anal. Chem, Arlington, VA.

Bartzanas, T., Bochtis, D. D., Green, O., Sørensen, C. G. and Fidaros. D. 2013. Prediction of quality parameters for biomass silage: A CFD approach. Comp. Electr. Agri. 93: 209-216.

Cheli, F., Campagnoli, A. and Dell'Orto, V. 2013. Fungal populations and mycotoxins in silages: From occurrence to analysis. Anim. Feed Sci. Technol. 183: 1–16.

Crichton, N. 1999. Information point: Tukey Multiple Comparison test. Blackwell Science Ltd, J. Clin. Nurs. 8: 299-304.

Galyen, M. 1989. Laboratory Procedures in Animal Nutrition Research. New Mexico State University.

Giang, N.T.T., M. Wanapat, K. Phesatcha, and S. Kang. 2016. Level of *Leucaena leucocephala* silage feeding on intake, rumen fermentation and nutrient digestibility in dairy steers. Trop. Anim. Health Prod. DOI 10.1007/s11250-016-1060-3

Gollop, N., Zakin, V. and Weinberg and Z.G. 2005. Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. J. Appl. Microbiol. 98: 662–666.

Ki, K.S., Khan, M.A., Lee, W.S., Lee, H.J., Kim, S.B., Yang, S.H., Baek, K.S., Kim, J.G. and Kim, H.S. 2009. Effect of replacing corn silage with whole crop rice silage in total mixed ration on intake, milk yield and its composition in Holsteins. Asian-Austral. J. Anim. Sci. 22(4): 516–519.

Makkar, H. P. S., M. Blummel and K. Becker. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Br. J. Nutr. 73: 897-913.

Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D. Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. (Camb.) 92: 217-222.

SAS. 1998. User's Guide: Statistic, Version 6, 12th Edition. SAS Inst. Inc., Cary, NC.

Van Soest, P. and J. B. Robertson. 1985. A laboratory manual for animal science. Cornell University Press, Ithaca, NY, page. 612.

Van Soest, P. J., Robertson, J. B. Lewis, B. A. 1991. Methods of dietary fiber, neutral detergent fiber and non-starch carbohydrates in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Wanapat, M. and Kang, S. 2013. Enriching the nutritive value of cassava as feed to increase ruminant productivity. J. Nutr. Ecol. Food Res. 1(4): 262–296.

Wanapat, M., Kang, S., Khejornsart, P. and Pilajun, R. 2013. Improvement of whole crop rice silage nutritive value and rumen degradability by molasses and urea supplementation. Trop Anim Health Prod. 45(5): 1–5.

Wanapat, M., Kang, S., Khejornsart, P., Pilajun, R. and Wanapat, S. 2014. Performance of tropical dairy cows fed whole crop rice silage with varying levels of concentrate. Trop. Anim. Health Prod. 46(1): 185-189.

O-04-6

Influence of premix containing mangosteen peel powder and banana flower powder on rumen microbial population in swamp buffaloes

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Abstract

Four, rumen-fistulated swamp buffaloes were randomly assigned to receive dietary treatments according to a 4x4 Latin square design to study the effect of mangosteen peel powder and banana flower powder as premix (PM) on feed intake and rumen fermentation efficiency. Four dietary treatments were as follows; T1= non-supplementation (control); T2 = supplementation of PM1 (Containing mangosteen peel powder at 91%) at 100 g/head/day; T3 = supplementation of PM2 (Containing banana flower powder at 91%) at 100 g/head/day; and T4 = supplementation of PM3 (Containing mangosteen peel powder at 45.5% and banana flower powder at 45.5 %) at 100 g/head/day. All animals were fed concentrate mixture at 0.5% of body weight and rice straw was fed *ad libitum.* The results revealed that there was no difference among treatments on dry matter intake and nutrient digestibilities of dry matter, organic matter and crude protein by PM supplementation (P>0.05) whereas neutral detergent fiber and acid detergent fiber digestibility were increased (P<0.05) in buffaloes fed with PM addition. However, protozoal population were remarkably reduced and bacterial population were increased in PM supplemented groups (P<0.05). In conclusion, supplementation of PM resulted in improvement of nutrient digestibilities and microbial population in swamp buffaloes fed on rice straw based diet.

INTRODUCTION

Research and development regarding methane (CH4) production in ruminants have been receiving considerable attention in which mitigation of the rumen CH4 has been the main issue (Boadi and Wittenberg, 2002; Wanapat et al., 2009; Hook et al., 2010; Bodas et al., 2012).Among many approaches, dietary manipulation using plants containing plant secondary compounds such as condensed tannins saponins, as well as using essential oils and minerals have been investigated with potential applications in feeding systems (Patra and Saxena,2009; Wanapat et al., 2009).Mangosteen (Garciniamangostana) peel is a fruit by-product containing a highlevel of condensed tannins (CT) and saponins (SP) whichexert a specific effect against rumen protozoa, while the rest
of the rumen biomass remains unaltered (Poungchompuet al. 2009).Banana flower has been reported to be used as a rumen buffering agent due to its high mineral elements (Kang and Wanapat, 2013; Kang et al., 2014; 2015). The previous findings showed that using banana flower powder (BAFLOP) as buffering agent could enhance ruminal pH and fermentation efficiency in *in vitro* using buffalo and dairy steer rumen fluid (Kang and Wanapat, 2013), and *in vivo* of dairy steer (Kang et al., 2014) and lactating dairy cattle (Kang et al., 2015). However, premix containing mangosteen peel powder and Banana flower powder has not yet been investigated to be used as a rumen enhancer. Therefore, the present study aimed at investigating the effect of premix containing mangosteen peel powder and banana flower powder on feed intake, digestibility and rumen microbial population in swamp buffaloes.

MATERIALS AND METHODS

Four, rumen-fistulated swamp buffaloes with initial average liveweight of 365 ± 15.0 kg, were randomly allocated to receive diets according to a 4 x 4 Latin square design. The treatments were as follows; T1= non-supplementation (control); T2 = supplementation of PM1 (Containing mangosteen peel powder at 91%) at 100 g/head/day; T3 = supplementation of PM2 (Containing banana flower powder at 91%) at 100 g/head/day; and T4 = supplementation of PM3 (Containing mangosteen peel powder at 45.5% and banana flower powder at 45.5 %) at 100 g/head/day, respectively. All animals were fed concentrate mixture at 0.5% of body weight and rice straw was fed *ad libitum.* The experiment was conducted for four periods and each of the four periods lasted for 21 days in length. Feeds offered and refusals were recorded daily throughout the experimental period for dry matter (DM) intake measurement. Feeds and fecal samples were collected by total collection of each individual buffalo during the last 7 days at morning and afternoon feeding. Feeds, refusals and fecal samples were dried at 60 ºC

and ground and analyzed using standard methods of AOAC (1995) for DM, crude protein (CP), organic matter (OM) and ash. Acid detergent fiber (ADF) was determined according to AOAC (1995) method and is expressed inclusive of residual ash. Neutral detergent fiber (aNDF) in samples was estimated according to Van Soest et al. (1991).
Rumen fluid was sampled on the 21st of each period through rumen fistula. First part of the fluid were immediately fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) to measure microbial populations by total direct counts of bacteria, protozoa and fungal zoospores (Galyean, 1989). Another part was cultured for groups of bacteria using the roll-tube technique Hungate (1969), to identify bacterial groups (i.e., cellulolytic, proteolytic, amylolytic, total viable bacterial counts). All data were subjected to ANOVA according to a 4 x 4 Latin square design using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998).

RESULTS AND DISCUSSION

The present results revealed feed intake and DM, OM and CP digestibility were not affected (P>0.05) by PM supplementation while aNDF) and ADF digestibility were increased in PM supplementation groups. These results were also consistent to the findings of Manasri et al. (2012) reported that dietary tannin sources had no effect on DMI in beef cattlewhen used at suitable level (0.05). The supplementation of soapberry fruit - mangosteen peel, containing condensed tannins and saponins, caused changes in ruminal microorganisms and their fermentation end-products in fistulated Holstein Friesian heifers (Poungchompu et al., 2009). Yaghoubi et al. (2010) also reported reduced protozoanumbers in batch culture of mixed rumen microorganisms by flavonoid extracts.

CONCLUSION AND RECOMMENDATION

Based on this study, it could be concluded that supplementation of PM can improvement of nutrient digestibilities and microbial population in swamp buffaloes fed on rice straw based diet. This study suggested that PM containing either in mangosteen peel powder or banana flower powder could be used to manipulate the rumen fermentation efficiency. It has a high potential use as an dietary rumen enhancer.

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KEYWORD : Mangosteen peel powder, Banana flower powder, Swamp buffalo

Table1. Feed ingredient of premix

Item	PM1	PM2	PM3
Mangosteen peel powder, %	91.0	-	45.5
Banana flower powder, %	-	91.0	45.5
Cassava chip meal, %	5.0	5.0	5.0
Urea, %	2.0	2.0	2.0
Sulfur, %	1.0	1.0	1.0
Salt, %	1.0	1.0	1.0

Table 2. Effect of premix (PM)on voluntary feed intake and nutrient digestibility in swamp buffaloes

Items	Control	PM1	PM2	PM3	SEM	P-value
Total DM intake						
kg/day	5.8	5.8	5.7	5.8	0.13	0.56
%BW	1.7	1.7	1.7	1.7	0.18	0.47
Rice straw DM intake						
kg/day	5.0	4.8	4.9	4.9	0.20	0.87
%BW	1.4	1.4	1.4	1.4	0.04	0.40
Apparent digestibility, %						
Dry matter	59.0	58.5	57.9	58.7	1.56	0.45
Organic matter	61.2	60.3	60.1	61.0	1.77	0.33
Crude protein	65.5	67.2	64.8	65.7	2.45	0.72
Neutral detergent fiber	40.5^{a}	45.7^{b}	46.1^{b}	45.9^{b}	1.15	0.001
Acid detergent fiber	40.2^{a}	45.4^{b}	45.7^{b}	45.5^{b}	0.45	0.001

^{a,b,c} Means in the same row with different superscripts differ (P<0.05)

Table 3 Effects of premix (PM) on rumen Protozoa, Fungal zoospore and bacteria populations

Items	Control	PM1	PM2	PM3	SEM	P-value
Total direct counts						
Protozoa, $x10^5$ cell/ml	8.2^{a}	6.5^{c}	7.7^{b}	6.7^{c}	0.56	0.02
Fungal zoospore, $x10^5$ cell/ml	5.4	6.1	6.3	6.1	0.41	0.12
Roll-tube technique, CFU/ml						
Amylolytic bacteria, $\times10^7$	2.9	3.2	3.3	3.2	1.43	0.07
Proteolytic bacteria, $\times10^7$	3.5^{a}	4.2^{b}	4.4^{b}	4.4^{b}	1.01	0.02
Cellulolytic bacteria, $\times10^8$	3.4^{a}	4.3^{b}	4.6^{b}	4.5^{b}	0.36	0.01
Total viable bacteria ,$\times10^8$	5.7	5.6	5.9	5.7	1.77	0.15

^{a,b,c} Means in the same row with different superscripts differ (P<0.05).

REFERENCES

AOAC, 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Boadi, D. and Wittenberg, K.M., 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF6) tracer gas technique. Canadian Journal of Animal Science 82, 201-206.

Bodas, R.N., Prieto, N.R., García-Gonzá lez, R.S., Andrés S., Girá ldez, F.J. and Ló pez, S., 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Animal Feed Science and Technology 176, 78–93.

Hook, S.E., Wright, A.D.G. and McBride, B.W., 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 10 p.

Kang, S. and Wanapat, M., 2013. Using plant source as a buffering agent to manipulating rumen fermentation in an *in vitro* gas production system. Asian-Australasian Journal of Animal Science 26: 1424-1436.

Kang, S., Wanapat, M. and Cherdthong, A., 2014. Effect of banana flower powder supplementation as a rumen buffer on rumen fermentation efficiency and nutrient digestibility in dairy steers fed on high concentrate diet. Animal Feed Science and Technology, 196, 32–41.

Kang, S., Wanapat, M., Cherdthong, A. and Phesatcha, K., 2015. Comparison of banana flower powder and sodium bicarbonate supplementation on rumen fermentation and milk production in dairy cows. Animal Production Science.

Manasri, N., Wanapat, M. and Navanukraw, C., 2012. Improving rumen fermentation and feed digestibility in cattle by mangosteen peel and garlic pellet supplementation. Livestock Science, 148, 291–295.

Patra, A.K. and Saxena, J., 2009. Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. Antonie van Leeuwenhoek 96, 363–375.

Pilajun, R. and Wanapat, M., 2011. Effect of coconut oil and mangosteen peel supplementation on ruminal fermentation, microbial protein synthesis in swamp buffaloes. Livestock Science, 141, 148–154.

Poungchompu, O., Wanapat, M., Wachirapakorn, C., Wanapat, S. and Cherdthong, A., 2009. Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangos-teen peel and soapberry fruit. Archives of animal nutrition 5, 389–400.

SAS., 1998. User's Guide: Statistic, Version 6, 12th Edition. SAS Inst. Inc., Cary, NC.

Van Soest, P. J., Robertson, J. B., Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Wanapat, M., Chanthakhoun, V. and Wanapat, S., 2009. Development of Food- Feed-System for smallholder livestock farmers. Proceedings of Animal Nutrition Association World Conference, 14-17 Feb. 2009, New Delhi, India, pp.24-27.

Yaghoubi, S. M. J., Ghorbani, G. R., Rahmani, H. R & Nikkhah, A. 2010. Flavonoids manipulation of rumen fermentation: An alternative for Monessen? AGS/1508, Agricultural Segment, 1(1):1-2.

O-04-7 Effect of crude glycerin in diets on growth performance, ruminal pH and blood metabolites of Kamphaeng Saen steers

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Introduction

Biodiesel is an alternative and renewable fuel which involves the processing of lipids derived from animal or plant sources into their methyl or ethyl esters. As the production of biodiesel increases so does the production of the main co-product, crude glycerin. Crude glycerin color ranges from light amber to dark brown due to impurities. Crude glycerin is approximately 60 to 85% pure, with the remainder composed of salt, ash, methanol, lipid, and water. Concentrations of impurities are highly variable due to the catalyst used during production, methanol recovery rate, and proportion of remaining lipids. Crude glycerin has an energy value similar to corn (Person, 2014) and has the potential of replacing corn in ruminant diets (Donkin, 2008) since the glycerol can be converted to glucose in the liver of ruminants, providing energy for cellular metabolism (Goff and Horst, 2001). Therefore, this study aimed at evaluation of the effects of crude glycerin in diets on feed intake, growth performance and rumen pH and blood metabolites of Kamphaeng Saen steers.

Materials and Methods

Animal, Housing and Diets

The study was carried out at Kamphaeng Saen beef cattle farm, Department of animal science, Faculty of agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom province, Thailand.

Twenty Kamphaeng Saen steers (25 % Thai native, 25% Brahman and 50% Charolais), with average initial body weight (BW) of 429.1 ± 44.93 kg at 18 months of age were used in the experiment and housed in individual pen (3.0 x 4.0 m) with concrete floor, a feeders and drinkers. They were treated against internal and external parasites using ABEN-15 (7.5 mg/kg BW) and Ivomec-F (1 ml per 50 kg BW), and vaccinated against Foot and Mouth Disease by the local vaccination program. The cross bred steers were submitted to 14 days of adaptation to experimental installation and diets. After adaptation, they were randomly assigned to one of 4 experiments (n=5) using completely randomized design with the feeding period of 180 days. The animals were offered one of TMR dietary treatments containing 10% rice straw and 90% concentrate comprised of 0, 4, 8 and 12% crude glycerin (Table 1) twice a day at 07.00 h and 16.00 h. Clean water was available at all times. Feed refusals were recorded daily for each pen. Amounts of feed offered to animals were calculated according to previous dry matter intake and adjustments were made when needed so that refused feed did not exceed 10% of daily intake.

Sample collection and analysis

Samples of feed and orts were combined at the end of experiment and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash and gross energy (GE) according to the AOAC (1995) procedure. Neutral and acid detergent fiber (NDF and ADF) were determined following the method described by Van soest *et al*. (1991) in order to calculate nutrient intakes. Individual daily feed intakes were recorded throughout the entire experimental to determine dry matter intake (DMI) and nutrient intakes.

The Kamphaeng Saen steers were weighed at the beginning of the experiment and thereafter monthly of feeding period to monitor BW gain. The measured BW change and feed intake were used to determine average daily gain (ADG) average daily feed intake (ADFI) and feed efficiency (G:F).

Blood samples from jugular vein were collected in 5 mL Vacuntainer® tubes (without additives) on last day of experiment at 0, 2, 4 and 6 h after the morning feeding to analyze for blood glucose and blood urea nitrogen. Ruminal fluid was obtained at 0, 2, 4 and 6 h after feeding at the last day of the experiment and was immediately measured for pH using an electric pH meter.

Statistical analysis

The experimental design was completely randomized with four treatments and 5 replications. All data were

analyzed using the Proc GLM and Orthogonal contrast statement were used to separate linear and quadratic effect of crude glycerin inclusion in the diets, with significant at p-value 0<0.05.

Result and Discussion

Composition of the experimental diets

The ingredients and chemical compositions of the experimental diets are presented in Tables 1. The diets contained similar concentrations of DM, CP, Ash, EE, NDF, ADF but varying amount of GE among those diets ranging from 4260 to 4393 kcal kg^{-1}.

Intake and performance

Effect of crude glycerin on feed intake and growth performance of Kamphaeng Saen steers are shown in table 2. Increasing levels of crude glycerin had no effect on dry matter intake (DMI), nutrient intake, final BW, ADG and gain to feed ratio (G:F). These result Similar to Mach *et al*. (2009) who fed feedlot dairy calves with up to 12% of crude glycerin and found no differences in DMI, ADG, and G:F between control and experiment groups**.** Similarly, Chanjula *et al*. (2016) reported from a study in 20 Kamphaeng Saen steers fed diets contained 0, 7, 14 and 21% crude glycerin to replace corn grain in diets that there were no effects of crude glycerin levels on DMI and growth performance. Van Cleef *et al*. (2014) reported no effect of crude glycerin on weight gain and/or feed efficiency even when animals were fed up to 30% crude glycerin. The result of this association may have been synergistic, enhancing the use of crude glycerin as well as other ingredients, thus not interfering with dry matter intake by animal (Van Cleef *et al*., 2014)

Ruminal pH and blood parameters

Effect of crude glycerin on ruminal pH and blood metabolites are presented in Table 3. Mean pH values of ruminal fluid were 6.57, 6.69, 6.56 and 6.57 for diets with 0,4, 8 and 12% crude glycerin in concentrate, respectively and were not significant different. The ruminal pH of cattle fed a forage diet predominantly is generally between 6.2-7.0 higher than those fed diets with greater proportions of concentrates which is between of 5.5-6.5 (Kolver and De Veth, 2002)

Crude glycerin did not affect blood glucose and blood urea nitrogen concentrations. Blood glucose levels in this study were above the normal range of 45 to 75 mg/dL suggested by Kaneko *et al*. (2008). Glycerol is directly absorbed by the rumen epithelium, metabolized in the liver to gluconeogenesis by the action of glycerol kinase enzyme, which converts it into glucose. Part of glycerol can be fermented to propionate in the rumen, which in turn is metabolized to oxaloacetate via the Krebs cycle in the liver and can be used to form glucose by the gluconeogenic pathway. Thus, crude glycerin has potential application as gluconeogenic substrate for ruminants (Krehbiel, 2008). Therefore, it is suggested that the addition of glycerol to the experimental treatments can alter the blood glucose levels and therefore the energy metabolism of the animals, resulting in better utilization of nutrients

BUN is a key indicator of animal protein intake and diet is the primary factor affecting serum urea levels. The BUN values of all treatments were not significant different and were in normal values between 7-14 mg/dl as proposed by González and Silva (2006).

Conclusions

The result from this study demonstrated that up to 12% of crude glycerin could replace cassava chips in diets without any effect on feed intake, growth performance, rumimal pH and blood metabolites. It was concluded that crude glycerin could be a viable source of dietary energy that well-utilized by Kamphaeng Saen steers.

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KEYWORD : Kamphaeng Saen steers, crude glycerin, growth performance, blood metabolite, ruminal pH

O-04-9

Effects of mycosorbent mixed with turmeric (Curcuma longa) powder to ameliorate the toxic effects of mycotoxins contaminated corn in ducks

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ABSTRACT

The present study was to evaluate the effects of hydrated sodium calcium alumino silicate (HSCAS) singly or mixed with turmeric (Curcuma longa) powder (TP) to ameliorate the toxic effects of naturally mycotoxins contaminated corn in Cherry Valley ducks. A total of 180, seven-day-old mixed-sex ducks were randomly allocated to 5 treatments with 3 replications per treatment (12 birds each) for a 42 d feeding trial. Treatments were conducted as followings: 1) basal diet; 2) mycotoxins contaminated corn diet (MCD); 3) MCD supplemented with 0.5% TP; 4) MCD supplemented with 0.2% HSCAS; and 5) MCD supplemented with 0.2% HSCAS + 0.5% TP. At the end of the experiment, growth performance and serum biochemistry parameters were analyzed using statistical method. From the results, the duck fed MCD had lower growth performance and serum biochemistry parameters ($P<0.05$). The ducks fed MCD supplemented with TP, HSCAS or HSCAS + TP showed enhancement in their growth performance and serum enzyme values when compare with MCD ($P<0.05$). The MCD supplemented with 0.2% HSCAS + 0.5% TP showed lesser toxic effects than the MCD supplemented with TP or HSCAS. The results can be concluded by stating that mycotoxins contaminated corn diet when supplemented with turmeric powder and/or HSCAS can ameliorate the toxic effects of mycotoxins in ducks.

INTRODUCTION

The mycotoxins are toxins produced by molds that occur as grain contaminants, such as aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEN) and ochratoxin A (OTA). These are serious problems in livestock and it leads to economic losses (Afsah-Hejri et al., 2013). Aflatoxins (AF) are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticu*s. Aflatoxin B_1 (AFB_1) is widely known as carcinogenic and the most hepatotoxic of the naturally occurring AF (Godfrey et al., 2013). Symptoms of AF poisoning include growth retardation, immunosuppression, hepatotoxicity from aflatoxicosis, etc. (Dhanasekaran et al., 2011). Detoxification approaches in contaminated diets are critically needed and most approaches are by the use of mycosorbents in diets that adsorbs AF in gastrointestinal tract and reduces bioavailability and toxicity in animals (Tengjaroenkul et al., 2013). Several sorbents including clays and modified clays, such as bentonite, zeolite, and hydrated sodium calcium aluminosilicates (HSCAS) are able to detoxify the mycotoxins. Moreover, Herbs can improve an efficiency of mycosorbents in growth performances. Scientists also found that supplementation of turmeric herb in contaminated diet was capable to prevent the hepatotoxic effect of mycotoxin in animals (Prasad and Aggarwal, 2011). According to Gowda et al. (2008) found that supplementation of HSCAS with turmeric have reduced the negative effects of AFB_1 in broilers.

However, study on efficacy of HSCAS mixed with turmeric to ameliorate the toxic effects of AF in duck is very limited. Therefore, objective of this study was to evaluate the effects of singly HSCAS or mixed with turmeric to ameliorate the toxic effects of naturally mycotoxins contaminated corn in ducks.

MATERIALS AND METHODS

Analysis of mycotoxins

The concentrations of natural mycotoxins contamination in corn maize and diets were determined by the levels of AFB_1 and AFB_2 by HPLC (In-house method based on AOAC, 2005) and other mycotoxins that were not shown in this study was because either they had low levels or not detected.

Animals, Experimental design and Management

A total of 180, seven-day-old mixed-sex (6:6 ratio of male to female) Cherry Valley ducks were used in the experiment. The ducks were weighed and randomly assigned to 5 treatments with three replications. Treatments included in the followings: T1) basal diet (normal corn-soybean meal diet); T2) mycotoxins contaminated corn diet (MCD), contaminated diet with AFB_1 30.52 ppb and AFB_2 12.29 ppb; T3) MCD + 0.5% turmeric powder (TP) (Lily

Food Animals Science, Limited, Thailand); T4) MCD + 0.2% HSCAS (ALCA Co., LTD. Bangkok, Thailand); T5) MCD + 0.2% HSCAS + 0.5% TP. The mycosorbents doses used in this study followed the commercial recommendation of HSCAS. The experimental diets were formulated based on the NRC (1994) recommendations. The ducks were reared under open house system, feed and water was provided *ad libitum*.

Growth performance determination

The ducks were daily examined and recorded for mortality rate. Ducks were weighed at 42 day of age, and feed intake was recorded and calculated to body weight gain (BWG), average daily weight gain (ADG), average daily feed intake (ADFI), feed consumption ratio (FCR) coefficient of variation of body weight (CVBW) and survival rate (SVR).

Blood samples and serum biochemical analysis

At 42 day of age, 4 mL of blood samples from 6 birds (2 birds per replication) from each treatment were taken by puncturing the wing vein for analysis of serum biochemistry to determine glucose, cholesterol, total protein, calcium (Ca), phosphorus (P) aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using using Roche/Hitachi cobas c501 automatic analyzer.

Statistical analysis

All data were analyzed using ANOVA for completely randomized designs. Differences among treatments were examined by Duncan's multiple range tests.

RESULTS

Growth performance

Growth performance data of parameters are presented in Table 1. At 42 d, the ducks fed MCD had significantly decreased BWG, ADG, ADFI, SVR ($P<0.05$) and increased FCR and CVBW ($P<0.05$) when compared to control diet. Supplementation of 0.5% TP in MCD significantly increased BWG, ADG, SVR and decreased CVBW ($P<0.05$) of ducks when compared to MCD. Supplementation of 0.2% HSCAS and 0.2% HSCAS + 0.5% TP in MCD significantly increased BWG, ADG, ADFI, SVR ($P<0.05$) and decreased FCR and CVBW ($P<0.05$) when compared with MCD. However, the BWG, ADG and FCR of ducks in supplementation with 0.2% HSCAS + 0.5% TP were better than supplementation with 0.2% HSCAS singly.

Serum biochemistry

Serum biochemical parameters are presented in Table 2. At 42 d, the ducks fed MCD showed significantly decreased levels of glucose, cholesterol, total protein, Ca and P ($P<0.05$) and increased levels of AST and ALT ($P<0.05$) when compared to control diet. Supplementation of 0.5% TP in MCD significantly increased levels of cholesterol and Ca ($P<0.05$) and decreased levels of AST and ALT ($P<0.05$). Supplementation with 0.2% HSCAS and 0.2% HSCAS + 0.5% TP in MCD significantly increased levels of glucose, cholesterol, Ca and P ($P<0.05$) and decreased levels of AST and ALT ($P<0.05$) of ducks when compared to MCD. However, only supplementation of 0.2% HSCAS + 0.5% TP in MCD significantly increased level of total protein ($P<0.05$) when compare to MCD.

DISCUSSIONS

In the present study, results of analysis of mycotoxins in corn or MCD found that the total levels of total AF (B_1, B_2) was 42.81 ppb and AFB_1 was 30.52 ppb, these levels are higher than the US Food and Drug Administration (FDA) action level of 20 ppb of total AF in feed. Similarly, the European Union (EU) specified maximum level of AFB_1 content of 20 ppb in poultry diets (Yang et al., 2014). This study found that the negative effects of MCD significantly decreased growth performance ($P<0.05$) of ducks. Similar results were also noticed in previous reports, AFB_1 contaminated in diets decreased BWG, ADFI, ADG, uniformity and increased FCR and mortality ($P<0.05$) of poultry (Khajarern et al., 2003; Gowda et al., 2008; Han et al., 2008; Zhao et al., 2010; Li et al., 2012; Wan et al., 2013; Chen et al., 2014).

The levels of serum biochemical parameters can be used for evaluation of health or nutrition status and liver injury of animals (Quist et al., 2000). This study found that the adverse effects of MCD significantly reduced levels of glucose, cholesterol, total protein, Ca and P ($P<0.05$) and increased levels of AST and ALT ($P<0.05$) when compared to control diet. Similar results were also noticed in previous reports, AFB1 contamination in diets

reduced levels of glucose, total protein, cholesterol, Ca and P (P<0.05) and increased activities of ALT and AST (P<0.05) of poultry (Gowda et al., 2008; Han et al., 2008; Li et al., 2012; Chen et al., 2014).

In the present study, supplementation with 0.5% TP, 0.2% HSCAS, or both in MCD showed that it ameliorates the adverse effects of MCD and showed enhanced growth performance and levels of serum biochemical parameters when compared to MCD (P<0.05). The results agree with previous studies showing that supplementation of HSCAS reduces the negative effects of AFB1 in broilers (Zhao et al., 2010). However, this study found that the MCD supplemented with 0.2% HSCAS + 0.5% TP showed lesser toxic effects than the MCD supplemented with TP or HSCAS singly. These findings are consistent with those of previous studies by Gowda et al. (2008) reported that supplementation with 0.5% TP to AFB1 diet significantly increased BWG (P<0.05), and supplementation with 0.2%HSCAS + 0.5%TP showed ameliorated negative effects of AFB1 on some of serum biochemical parameters and improved the antioxidant status in broilers.

CONCLUSION

The supplementation with TP, HSCAS, or both showed enhancement in their growth performance and serum biochemical levels (P<0.05). The MCD supplemented with 0.2% HSCAS + 0.5% TP showed lesser toxic effects than supplemented with TP or HSCAS singly. The result can be concluded by stating that supplementation of turmeric powder and/or HSCAS can ameliorate the toxic effects of mycotoxins in ducks.

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KEYWORD : Mycotoxin, Aflatoxin, Turmeric, Mycosorbent, Duck

Table 1. Effect of HSCAS and turmeric powder on growth performance of ducks

Item	T1	T2	T3	T4	T5	SEM
BWG, g	2,492.21^{a}	2,296.67^{d}	2,309.53^{c}	2,453.86^{b}	2,485.60^{a}	4.048
ADG, g	71.21^{a}	65.62^{d}	65.99^{c}	70.11^{b}	71.01^{a}	0.115
ADFI, g	167.59^{a}	165.32^{b}	170.11^{b}	166.97^{a}	170.33^{a}	0.195
FCR	2.35^{c}	2.52^{a}	2.51^{a}	2.38^{b}	2.36^{c}	0.004
CVBW, %	6.83^{c}	11.62^{a}	9.76^{b}	7.13^{c}	7.13^{c}	0.209
SVR, %	100^{a}	94.44^{b}	100^{a}	100^{a}	100^{a}	1.242

$^{a-d}$ Means have different superscripts in same row difference significantly (p< 0.05).

T1: basal diet; T2: mycotoxin contaminated corn diet (MCD); T3: MCD + 0.5% turmeric powder (TP); T4) MCD + 0.2% hydrated sodium calcium alumino silicate (HSCAS); T5: MCD + 0.2% HSCAS + 0.5% TP

BWG: body weight gain; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed consumption ratio; CVBW: coefficient of variation of body weight; SVR: survival rate

Table 2. Effect of HSCAS and turmeric powder on serum biochemistry parameters of ducks

Item	T1	T2	T3	T4	T5	SEM
Glucose, mg/dL	191.67^{a}	162.00^{b}	171.67^{ab}	188.33^{a}	192.33^{a}	6.710
Cholesterol, mg/dL	187.33^{a}	160.00^{b}	175.67^{a}	182.67^{a}	183.33^{a}	3.986
Total protein, g/dL	3.83^{a}	3.57^{b}	3.67^{ab}	3.80^{ab}	3.90^{a}	0.073
Ca, mg/dL	12.10^{a}	10.40^{c}	11.23^{b}	11.43^{b}	11.60^{ab}	0.182
P, mg/dL	8.07^{a}	6.60^{b}	6.87^{b}	7.90^{a}	7.97^{a}	0.218
AST, U/L	27.33^{c}	48.67^{a}	37.67^{b}	29.00^{c}	27.33^{c}	1.513
ALT, U/L	28.67^{c}	63.67^{a}	39.00^{b}	29.67^{c}	28.33^{c}	1.972

$^{a-c}$ Means have different superscripts in same row difference significantly (p< 0.05).

T1: basal diet; T2: mycotoxin contaminated corn diet (MCD); T3: MCD + 0.5% turmeric powder (TP); T4) MCD + 0.2% hydrated sodium calcium alumino silicate (HSCAS); T5: MCD + 0.2% HSCAS + 0.5% TP

Ca: calcium; P: phosphorus; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

REFERENCES

Afsah-Hejri, L., S. Jinap, P. Hajeb, S. Radu, and S. Shakibazadeh. 2013. A Review on mycotoxins in food and feed: Malaysia case study. Compr. Rev. Food Sci. Food Saf. 12:629–651.

Chen, X., N. Horn, P. F. Cotter, and T. J. Applegate. 2014. Growth, serum biochemistry, complement activity, and liver gene expression responses of pekin ducklings to graded levels of cultured aflatoxin B1. Poult. Sci. 93:2028–2036.

Dhanasekaran, D., S. Shanmugapriya, and N. T. and A. Panneerselvam. 2011. Aflatoxins and aflatoxicosis in human and animals. Available from: http://www.intechopen.com/books/aflatoxins-biochemistry-and-molecular-biology/aflatoxins-and-aflatoxicosis-in-human-and-animals

Godfrey S Bbosa, D. K. 2013. Review of the biological and health effects of aflatoxins on body organs and body systems. Chapter 12:239–265.

Gowda, N. K. S., D. R. Ledoux, G. E. Rottinghaus, A. J. Bermudez, and Y. C. Chen. 2008. Efficacy of turmeric (Curcuma longa), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci. 87:1125–1130.

Han, X.-Y., Q.-C. Huang, W.-F. Li, J.-F. Jiang, and Z.-R. Xu. 2008. Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livest. Sci. 119:216–220.

Khajarern, J. M., S. Khajarern, T. H. Moon, and J. H. Lee. 2003. Effects of dietary supplementation of fermented chitin-chitosan (FERMKIT) on toxicity of mycotoxin in ducks. Asian-Australas. J. Anim. Sci. 16:706–713.

Li, Y., Y. H. Liu, Z. B. Yang, X. L. Wan, and F. Chi. 2012. The efficacy of clay enterosorbent to ameliorate the toxicity of aflatoxin B1 from contaminated corn (Zea mays) on hematology, serum biochemistry, and oxidative stress in ducklings. J. Appl. Poult. Res. 21:806–815.

Prasad, S., and B. B. Aggarwal. 2011. Turmeric, the golden spice: From traditional medicine to modern medicine. In: I. F. F. Benzie and S. Wachtel-Galor, editors. Herbal medicine: biomolecular and clinical Aspects. 2nd ed. CRC Press/Taylor & Francis, Boca Raton (FL). Available from: http://www.ncbi.nlm.nih.gov/books/NBK92752/

Quist, C. F., D. I. Bounous, J. V. Kilburn, V. F. Nettles, and R. D. Wyatt. 2000. The effect of dietary aflatoxin on wild turkey poults. J. Wildl. Dis. 36:436–444.

Tengjaroenkul, B., U. Tengjaroenkul, N. Pumipuntu, K. Pimpukdee, S. Wongtangtintan, and P. Saipan. 2013. An in vitro comparative study of aflatoxin B1 adsorption by thai clay and commercial toxin binders. Thai J. Vet. Med. 43:491–495.

Wan, X. L., Z. B. Yang, W. R. Yang, S. Z. Jiang, G. G. Zhang, S. L. Johnston, and F. Chi. 2013. Toxicity of increasing aflatoxin B1 concentrations from contaminated corn with or without clay adsorbent supplementation in ducklings. Poult. Sci. 92:1244–1253.

Yang, Z. B., X. L. Wan, W. R. Yang, S. Z. Jiang, G. G. Zhang, S. L. Johnston, and F. Chi. 2014. Effects of naturally mycotoxin-contaminated corn on nutrient and energy utilization of ducks fed diets with or without calibrin-A. Poult. Sci. 93:2199–2209.

Zhao, J., R. B. Shirley, J. D. Dibner, F. Uraizee, M. Officer, M. Kitchell, M. Vazquez-Anon, and C. D. Knight. 2010. Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poult. Sci. 89:2147–2156.

O-05-4 CpG oligodeoxynucleotide from Streptococcus thermophilus induces type 2 innate lymphoid cells

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OBJECTIVE

Bacterial DNA contains CpG motifs that stimulate Toll-like receptor 9-expressing cells to mount a protective innate immune response. Synthetic CpG oligodeoxynucleotides (CpG-ODNs) derived from bacterial DNA maintain the immunomodulatory properties against infectious agents, cancer, allergies, and inflammatory disorders (Krieg et al., 1995; Hemmi et al., 2000).

Previously, we reported the strong immunostimulatory effects of a CpG-ODN, designated MsST, from the *lac*Z gene of *Streptococcus thermophilus* ATCC19258. MsST strongly induced the production of interleukin (IL)-33 in mouse spleen cells via Toll-like receptor 9 (Shimosato et al., 2010). We also found that orally administered MsST potentially worsened atopic dermatitis, and that MsST was delivered to the gut Peyer's patches of antigen-immunized mice, where they induced systemic IL-33 expression (Wang et al., 2015). IL-33, which is a member of the IL-1 family of cytokines, binds to the IL-1-related receptor protein ST2 and induces the production of inflammatory and Th2 cytokines in mast cells, Th2 cells, basophils, and eosinophils (Imai et al., 2013). However, the mechanism by which MsST-induced IL-33 contributes to the exacerbation of atopic dermatitis remains unclear. Recently, type 2 innate lymphoid cells (ILC2) were identified as a new cell population that expresses ST2. ILC2 can produce large amounts of type 2 cytokines, such as IL-5 and IL-13, in response to IL-33, and it has been suggested that ILC2 are involved in allergic diseases (Moro et al., 2010; Neill et al., 2010; Kabata et al., 2015). Therefore, in this study, we investigated the effects of MsST treatment on ILC2.

METHODOLOGY

Oligodeoxynucleotides: Endotoxin-free phosphorothioate-bound ODNs (PS-ODNs) were synthesized and desalted by Integrated DNA Technologies, Inc. (Coralville, IA). The PS-ODNs were reconstituted in endotoxin-free water and passed through a 0.22- μ m pore microfilter (Nihon Millipore K.K., Tokyo, Japan). The ODN sequences were: B type CpG-ODN, MsST derived from the *S. thermophilus lac*Z gene (5'-CAGGACGTTGTATCACTGAA-3'; Shimosato et al., 2010; Yamamoto et al., 2016), and control GpC-ODN, ODN_{1612} (5'-GCTAGAGCTTAGGCT-3'; Ito et al., 2013).

Mice: Pathogen-free male C57BL6 mice (4 weeks of age) were purchased from Japan SLC (Shizuoka, Japan) and kept under temperature- and light-controlled conditions. The mice were given a standard diet of Labo MR Breeder (Nihon Nosan Co., Kanagawa, Japan) and sterile water *ad libitum*, and were used for the study at 7 weeks of age. All experimental procedures were conducted in accordance with the Regulations for Animal Experimentation of Shinshu University, and the animal protocol was approved by the Committee for Animal Experiments of Shinshu University. Based on national regulations and guidelines, all experimental procedures were reviewed by the Committee for Animal Experiments, and final approval was obtained from the president of Shinshu University.

Cells and cell culture: Spleen cells from non-immunized mice were prepared using standard methods. Cells were cultured in triplicate or quadruplicate wells of a 24-well plate (Nalga Nunc International K.K., Tokyo, Japan) at a final concentration of 1×10^7 cells/well (total of 1 ml/well) in complete RPMI 1640 medium (Sigma, MO) supplemented with 10% fetal calf serum (FCS; Sigma), 100 U/ml penicillin, 100 mg/ml streptomycin, 25 mM HEPES, 1.0 mM sodium pyruvate, non-essential amino acids, and 0.0035% 2-mercaptoethanol. The spleen cells were pre-incubated in medium for 3 h prior to exposure to 3.0 μ M MsST, ODN_{1612}, or phosphate-buffered saline (PBS) for 24 h.

ILC2 analysis: ILC2 analysis was performed as described by Moro et al. (2010). All of the antibodies were purchased from Biolegend (San Diego, CA). The stimulated spleen cells were fixed in 4% paraformaldehyde for

15 min at room temperature. After washing, the cells were stained with biotin-conjugated antibody mixtures for lineage (Lin) markers (CD3 ε, CD4, CD5, CD8 α, TCR β, TCR δ, CD11b, CD11c, CD45R, CD19, Gr-1, Fc ε RI, NK1.1 and Ter-119), FITC-conjugated anti-Sca1, PE-conjugated c-kit, and PE/Cy5-conjugated anti-streptavidin. The $Sca1^+$ and $c\text{-}kit^+$ cell populations among the Lin^- cells were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA). All data were analyzed using FlowJo (Ashland, OR).

Statistical analysis: ANOVA and post hoc tests were performed using a statistical software package (ystat2004. xls; Igakutosho Shuppan, Tokyo, Japan). One-way ANOVA with a post hoc Student-Newman-Keuls test was used to determine the significance of differences in all experiments. Differences were considered to be significant at $P < 0.05$. Values for all experiments are expressed as the mean ± standard error (SE).

RESULTS AND CONCLUSION

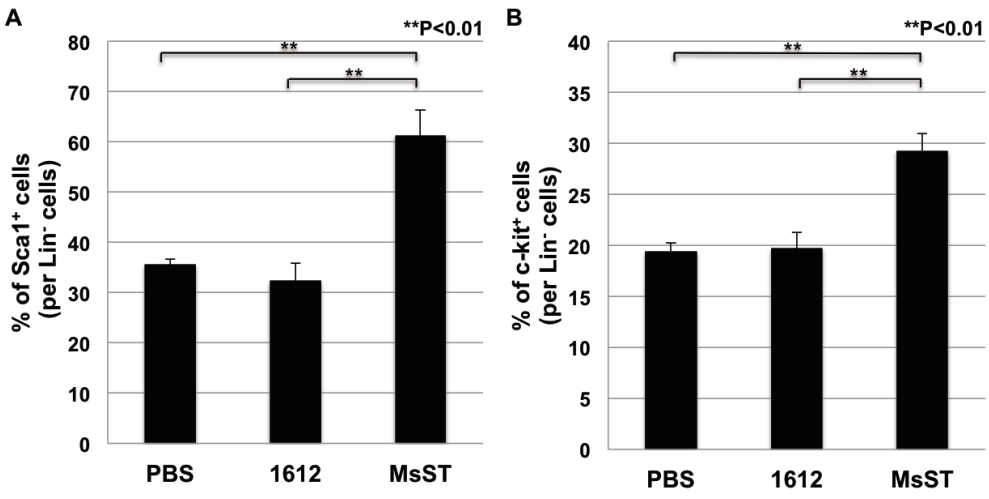
In 2010, Moro et al. were the first to identify a novel cell population that does not express Lin markers (CD3 ε, CD4, CD5, CD8 α, TCR β, TCR δ, CD11b, CD11c, CD45R, CD19, Gr-1, Fc ε RI, NK1.1 and Ter-119), but do express c-kit and Sca1 in fat-associated lymphoid clusters; these cells were named natural helper (NH) cells (Moro et al., 2010). Following this report, Neill et al. (2010) used IL-13 reporter mice to identify and characterize Lin^- $IL\text{-}13^+$ cells that responded to IL-33 or IL-25; these cells were named nuocytes. Nuocytes also do not express Lin markers, but do express c-kit and Sca1 (Neill et al., 2010). In the same year, Price et al. (2010) used IL-4 and IL-13 reporter mice to identify and characterize Lin^- $IL\text{-}13^+$ cells that responded to IL-33 or IL-25; these cells were designated as innate type 2 helper (Ih2) cells. Ih2 cells do not express Lin markers, but do express Sca1 (Price et al., 2010). These cells were all classified as ILC2 according to several common features (Kabata et al., 2015); ILC2 are phenotypically defined as Lin^- cells that express at least Sca1 or c-kit and produce type 2 cytokines.

In this study, we found that the $Sca1^+$ and $c\text{-}kit^+$ cell populations among the Lin^- cells significantly increased in MsST-stimulated spleen cells when compared with ODN_{1612}- or PBS-stimulated spleen cells. This result indicates that MsST induces ILC2 in mouse spleen. Therefore, it is suggested that ILC2 are involved in the MsST-induced exacerbation of atopic dermatitis. In order to further clarify the details, we plan to use allergy model mice to investigate the effects of MsST administration on ILC2 induction *in vivo* in the near future. We propose that a novel immunoregulatory mechanism mediated by CpG-ODNs may induce ILC2. Exploitation of this immunoregulatory property of MsST may prove useful in the design and production of new physiological supplements for foods/feeds.

FIGURE LEGEND

Fig. 1. Analysis of Lin^- $Sca1^+$ cells and Lin^- $c\text{-}kit^+$ cells by flow cytometry. (A) Percentage of $Sca1^+$ cells among the Lin^- cells after 24 h of incubation with 3.0 μ M MsST, ODN_{1612}, or PBS. (B) Percentage of $c\text{-}kit^+$ cells among the Lin^- cells after 24 h of incubation with 3.0 μ M MsST, ODN_{1612}, or PBS. Data are representative and shown as the mean ± SE ($n = 3$) of three independent experiments with similar results. $^{**}P < 0.01$.

KEYWORD : CpG-ODN, Streptococcus thermophilus, ILC2



REFERENCES

Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda and S. Akira. 2000. A Toll-like receptor recognizes bacterial DNA. Nature.408:740-745.

Imai, Y., K. Yasuda, Y. Sakaguchi, T.Haneda, H. Mizutani, T. Yoshimoto, K. Nakanishi and K. Yamanishi. 2013. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proc Natl Acad Sci U S A. 110:13921-6.

Ito, Y., S. Shigemori, T. Sato, T. Shimazu, K. H. Otani, H. Kitazawa and T. Shimosato. 2012. Class I/II hybrid inhibitory oligodeoxynucleotide exerts Th1 and Th2 double immunosuppression. FEBS Open Bio. 3:41-45.

Kabata, H., K. Moro, S. Koyasu and K. Asano. 2015. Group 2 innate lymphoid cells and asthma. 2015. Allergol Int. 64:227-34.

Krieg, A. M., A. K. Yi, S. Matson, T. J. Waldschmidt, G. A. Bishop, R. Teasdale, G. A. Koretzky and D. M. Klinman. 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature. 374:546-9.

Moro, K., T. Yamada, M. Tanabe, T. Takeuchi, T. Ikawa, H. Kawamoto, J. Furusawa, M. Ohtani, H. Fujii and S. Koyasu. 2010. Innate production of T(H)2 cytokines by adipose tissue-associated c-kit(+)Sca-1(+) Lymphoid cells. Nature. 463:540-4.

Neill, D.R., S. H. Wong, A. Bellosi, R. J. Flynn, M. Daly, T. K. Langford, C. Bucks, C. M. Kane, P. G. Fallon, R. Pannell, H. E. Jolin and A. N. McKenzie. 2010. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 464:1367-70.

Price, A. E., H. E. Liang, B. M. Sullivan, R. L. Reinhardt, C. J. Eisley, D. J. Erle and R. M. Locksley. 2010. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. Proc Natl Acad Sci U S A. 107:11489-94.

Shimosato, T., M. Fujimoto, M. Tohno, T. Sato, M. Tateo, H. Otani and H. Kitazawa. 2010. CpG oligodeoxynucleotides induce strong up-regulation of interleukin 33 via Toll-like receptor 9. Biochem Biophys Res Commun. 394:81-6.

Shimosato, T., M. Tohno, T. Sato, J. Nishimura, Y. Kawai, T. Saito and H. Kitazawa. 2009. Identification of a potent immunostimulatory oligodeoxynucleotide from *Streptococcus thermophilus lacZ*. Anim Sci J. 80:597-604.

Yamamoto, Y., S. Shigemori, S. Nigar, K.Oshiro, Y. Wang, T. Sato and T. Shimosato. Development of a simple IgE-independent anaphylactic model using buckwheat antigen and B-type CpG oligodeoxynucleotide from *Streptococcus thermophilus*. 2016. Anim Sci J. 87:710-7.

Wang, Y., Y. Yamamoto, S. Shigemori, T. Watanabe, K. Oshiro, X. Wang, P. Wang, T. Sato, S. Yonekura, S. Tanaka, H. Kitazawa and T. Shimosato. 2015. Inhibitory/Suppressive oligodeoxynucleotide nanocapsules as simple oral delivery devices for preventing atopic dermatitiss in mice. Mol Ther. 23:297-309.

O-05-8

Cognitive and Physiological Effects of Microalgae-DHA Egg Consumption related to the Go/NoGo task in Healthy Thai Men.

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Introduction

Docosahexanoic acid (DHA) is an essential omega-3 fatty acid that the human body cannot efficiently produce by itself; it must be taken in through the diet. DHA is delivered to fetus via the placenta during pregnancy, and infants through breast milk or enriched formula. When infants grow into toddlers and transition to eating and drinking at the table, a nutritional gap is created. Dietary sources of DHA, such as salmon and mackerel, are not commonly found on the plates of children, which leads to less than optimal levels of DHA intake.

Algae, the base of the marine food chain, can be used as a natural source of DHA. The lipid content of microalgae can reach 70% with high concentrations of omega-3 and omega-6 fatty acids. One specific strain, Schizochytrium spp., has been found to contain at least 14% DHA. Microalgae supplementation can be used as a potentially safe, sustainable alternative to fish oil in chicken diet to create functional, DHA-enriched eggs to help address human dietary insufficiencies.

According to the European Union Food Safety Authority (2010), ingesting about 250 mg/day of EPA and DHA is required to obtain the claimed benefit effect as regards eyes, brain, and heart health. Therefore, consuming microalgae-DHA eggs could benefit the brain health of consumers.

DHA may play an important role in autonomic function, attention and inhibition which are the component of executive function, along with skills, e.g., working memory and planning, all of which depend on frontal lobe maturation. The prefrontal cortex region in the brain has been shown to play a major role in cognitive function using contrasts between the activation to Go/NoGo stimuli. (Liddle et al, 2001). DHA is the principle omega-3 fatty acid in mammalian grey matter, representing 15-20% of the total fatty acid composition in the frontal cortex of adult humans. (Carver et al, 2001). DHA-rich frontal lobes are thought to be responsible for executive function and higher-order cognitive activities such as planning, problem solving, and focused attention. (Anderson et al, 1998). There is mounting clinical evidence that the DHA status of humans is positively associated with neurocognitive development particularly on measures of attention and memory. (Lauritzen et al, 2001).

The numerous studies on the relationship between omega-3 fatty acids and central nervous system activity mainly involve pathological situations. (Haag, 2003). It remains to be proven if DHA can change or improve the status of healthy young people, as omega-3 supplementation in healthy subjects has not been widely analyzed. (Fontani et al, 2005). Moreover all of the previous studies were done with an omega-3 supplement, not with functional food.

Materials and Methods

Subjects

Subjects were military drafted soldiers aged between 20-22 years old at the Medical Battalion, Phramongkutklao Hospital, Bangkok, Thailand. The 30 subjects in this study, all men, were randomly divided into two groups of 15 subjects each. The average age of the control group was 21.47 ± 0.19 years and the DHA group was 21.13 ± 0.43 years. There were no significant differences between two groups in education, weight, height, or BMI. All subjects from each group consumed two boiled eggs, either control or DHA eggs every day for 8 weeks. Participants were not instructed to alter any other part of their diet during the 8 weeks.

Eggs samples

The DHA eggs were derived from feeding 2% microalgae (Schizochytrium sp.) in hen diet after 8 weeks. Essential fatty acid in the egg sample for control and DHA egg were analyzed by gas chromatography. Omega 6:3 ration was calculated. The average egg weight in this study was 57.46 gram for control egg and 55.54 gram for DHA egg, respectively.

Experimental procedures

Throughout the 8 weeks study period, the 15 subjects of the DHA group consumed two boiled eggs per day of microalgae-DHA enriched eggs (DHA=278 mg/day), while the 15 subjects of the control group consumed two boiled eggs per day of normal eggs (DHA=51.8 mg/day).

A visual Go/NoGo task requires each subject to respond as fast as possible to the "Go" stimuli by pressing the left mouse click and to the "NoGo" stimuli by pressing the right mouse click. Electroencephalograms (EEG) were performed on each subject of both groups at week 0, week 4 and week 8 by using an Emotiv 14-channel EEG headset. All stimuli was presented by SuperLab Pro v. 2.04 via a monitor screen of notebook computer and adjusted for easy viewing. Regarding the Go/NoGo paradigm, the capital letter of "S" was used as a primer. Subjects had to notice any letter coming after the letter "S". The "Go" condition were, then, as "S-T" and "S-other letters" were named as "NoGo" condition.

Data collection

Brain waves during the Go/NoGo test were recorded by Emotiv14-channels EEG and computed by Emotiv Xavier Control Panel Premium 3.0.0.40. The frequencies were divided into five bands: Delta (

The percentage of hits and the reaction time (RT) to the "Go" condition and the percentage of false alarm to the "NoGo" condition were included for data analysis. When the left mouse was pressed within 200 to 1,000 msec after the "Go" condition (S-T), the response was immediately deemed as a "Correct" response whereas the left mouse response to the "NoGo" condition (S-Other Letters) were scored as the "False Alarm". Reaction time was measured from the onset of the go stimuli to the left-click response.

Data analysis

The data were analyzed by means of split-plot ANOVA, with one between factor and one repeated measures factor over the study period (wk0, wk4 and wk8). The difference was considered significant at p

Results and Discussion

Essential fatty acid composition in the egg

DHA eggs derived from feeding 2% microalgae (Schizochytrium sp.) in hen diet after 8 weeks. The fatty acid profile of eggs of control and DHA group are demonstrated in Table 1.

The level of omega-3 fatty acid of DHA-egg group was 5 times higher than those of control group. In contrast, the omega-6 fatty acid of both groups was not differ significantly. (Figure 1). The Omega 6:3 ratio was found to have reduced from 15:41:1 of control egg to 3.11:1 of DHA-egg group.

Brain wave activation pattern

After consuming eggs for 8 weeks, the power of the slow frequency bands e.g. Delta, Theta and Alpha waves increased with significant difference ($p<0.05$) between baseline and 8 weeks in the DHA-egg group when performing the Go-task. In contrast, the brain waves in the control group did not differ significantly between baseline and 8 weeks. (Figure 2)

This result is consistent with the previous research on the effect of omega-3 on the activity of the central nervous system (Fontani et al, 2005) which recorded an Electroencephalogram (EEG) frequency shift towards the theta and alpha bands in all tests after Omega-3 supplementation (EPA & DHA) for 35 days that was associated with an improvement in attentional and physiological functions, particularly those involving complex cortical processing.

However when performing the NoGo task, the Theta, Alpha, Beta and Gamma waves of the control and DHA groups did not differ significantly between week 0, week 4 and study end (week 8).

Behavioral performance

After consuming eggs for 8 weeks, both the control- and DHA-egg groups showed high performances >90% accuracy with no significant difference in the percentage of correct responses to the go stimuli. Even though the DHA-egg group showed slightly better response. (>95% accuracy)

Perhaps part of the reason for the general improvement of behavioral measures seen in both groups could be attributed to the nutrition-value of both kinds of eggs. One limitation of our study was that the Go/NoGo task may have been too easy, performances was high in both group. And this ceiling effect could mask potential performance-enhancing effects of consuming DHA-eggs.

However the percentage of false alarms in the DHA-egg group was significantly reduced ($p<0.05$) from 0.10 ± 0.04 % at week 0 to almost zero at week 4 and week 8, but not with the control group.

The main finding of the present study was that consuming eggs for 8 - whether control or DHA eggs significantly decreased the reaction time response to Go stimuli ($p<0.05$). The reaction time was significantly faster (22%) in the DHA-egg group (from 486.34 ± 26.0 msec at week 0 to 399.66 ± 23.6 and 380.74 ± 24.1 msec at week 4 and week 8 respectively) compared to 17% faster for the control (normal-egg) group (from 511.85 ± 27.7 msec at week 0 to 451.30 ± 21.2 and 421.61 ± 23.9 msec at week 4 and week 8 respectively). (Figure 3).

These results, obtained from a population of healthy subjects, are in line with Fontani et al. (2005), who showed

that Omega-3 influenced the activity of the central nervous system.
In this study, subjects in the control group received 51.8 mg/day of DHA from two eggs/day, which could have improved reaction times. While the treatment group, which consumed even more DHA from microalgae-DHA eggs (278 mg/day) showed even faster reaction times.

Conclusions

The results of this experiment revealed that manipulation of Omega-3 fatty acids in the egg to optimal level could be possible by supplementing microalgae in hen diet. The influence of microalgae-DHA eggs (2 eggs/day) on the activity of the central nervous system can be seen after 4 to 8 weeks of consumption indexed by the Go/NoGo task.

Suggestions

These results were obtained from a small study group and need further confirmation in a wider group of subjects and, in particular for the possible influences of age and gender. Additionally, the blood analysis to measure DHA content of the red blood cell membranes should be measured to confirm the relationship between consuming DHA-eggs and DHA levels in the blood, as it is a marker of DHA levels in the brain. There are possibility for future research of DHA-egg consumption on the reduction risk of cognitive syndromes e.g. ADHA, Alzheimer's disease etc.

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The authors are grateful for the support from Alltech Inc. for the microalgae (All-G-Rich®) and lab analysis costs of the eggs. We would also like to thank the CP research farm for supplying the layers and feed to produce the control and DHA-enriched eggs.

KEYWORD : DHA egg, Omega-3 fatty acids, Microalgae, Cognitive function, Go-NoGo test

Figure 1: The Omega-6 and Omega-3 fatty acid of the control and DHA-egg groups

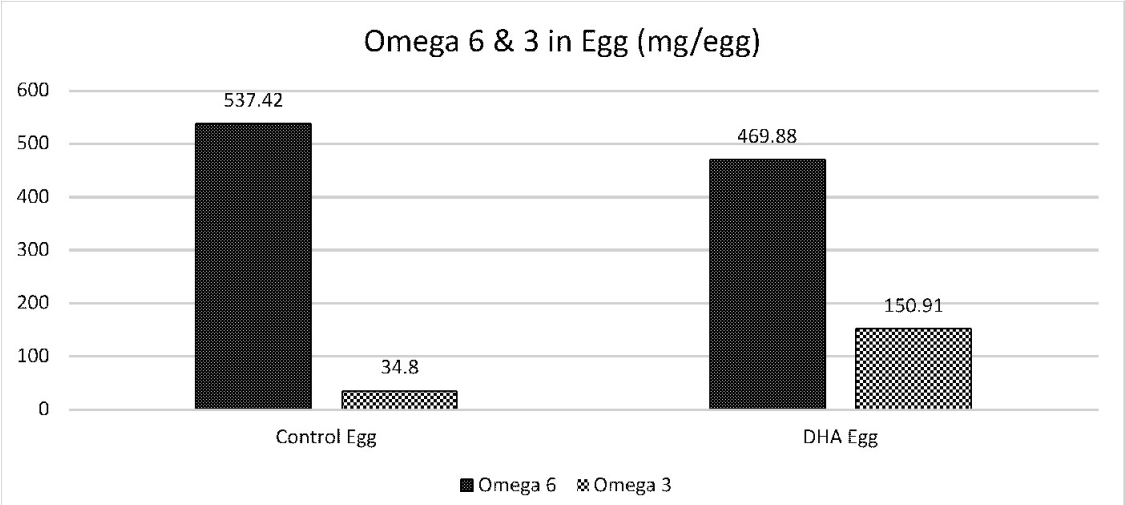

Figure 2: The change in brain waves when performing Attention (Go) test before and after 8 weeks of consuming DHA-enriched eggs.

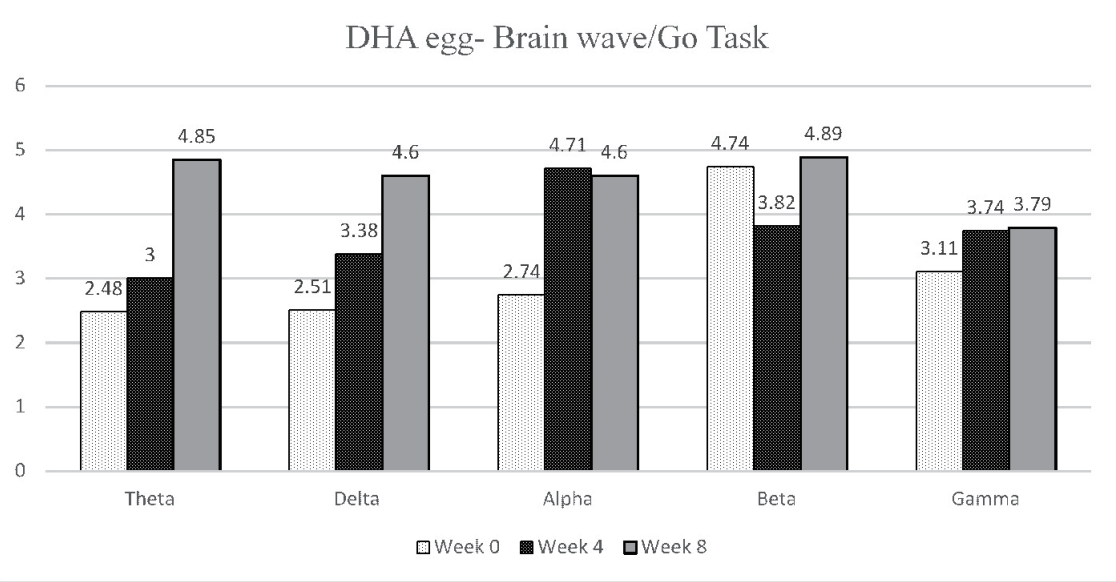

Figure 3. Reaction time to Go stimuli of the control and DHA groups

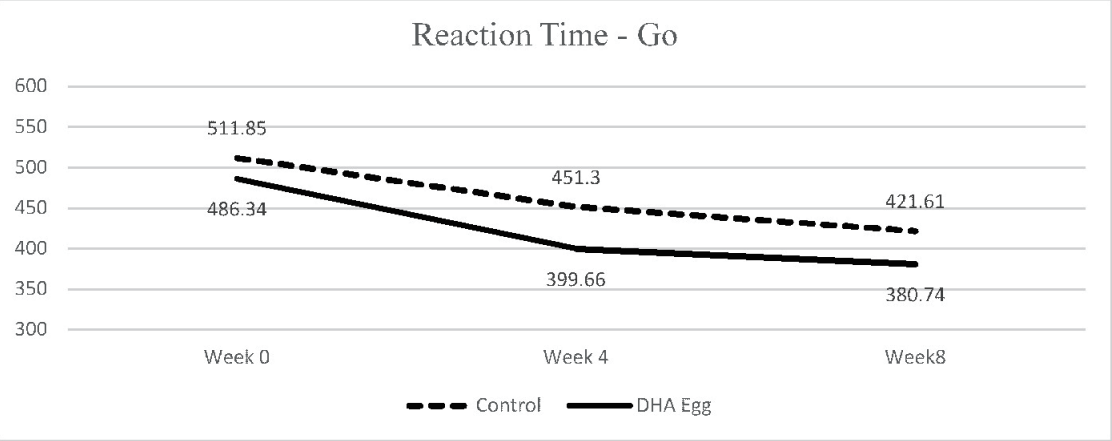

Table 1. Essential Fatty acid composition of the control egg and microalgae-DHA egg

Fatty acid (mg)	**Normal egg**	**DHA egg**
Docosahexanoic acid (DHA) 22:6 (n3)	25.9	139
Eicosapentanoic acid (EPA) 20:5 (n-3)	0	2.15
Alpha Linolenic acid (ALA) 18:3 (n-3)	8.97	9.76
Total omega 3	**34.8**	**150.91**
Linoleic acid 18:2 (n-6)	432.9	405.4
Gamma Linolenic acid 18:3 (n-6)	3.35	2.32
Eicosadienoic acid 20:2 (n-6)	5.87	4.91
Eicosatrienoic acid 20:3 (n-6)	13.11	9.95
Arachidonic acid 20:4 (n-6)	82.19	47.30
Total omega 6	**537.42**	**469.88**
Omega 6:3 ratio	**15.41**	**3.11**
Average egg weight (w/o shell) - gram	57.46	55.54

REFERENCES

Anderson, V., Fenwick, T., Manly, T. & Robertson, I. (1998). Attentional skills following traumatic brain injury in childhood. A componential analysis. *Brain Injury*, 12:937-949

Burns-Whitmore, B., Haddad, E., Sabaté, J. & Rajaram, S. (2014). Effects of supplementing n-3 fatty acid enriched eggs and walnuts on cardiovascular disease risk markers in healthy free-living lacto-ovo-vegetarians: a randomized, crossover, free-living intervention study. *Nutrition Journal*, 13:29

Carver, J.D., Benford, V.J., Han, B. & Cantor, A.B. (2001). The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Research Bulletin*, 56:79-85

Cortical Functions reference. (2012). *Trans Cranial Technologies ltd*. V.10: 1-60

European Food Safety Authority. (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*; 8(3):1461

Fontani, G., Corradeschi, F., Felici, A., Migliorini, S. & Lodi, L. (2005). Cognitive and physiological effects of Omega-3 polyunsaturated fatty acid supplementation in healthy subjects. *European journal of clinical investigation*, 35, 691-699

Haag, M. (2003). Essential fatty acids and the brain. The Canadian Journal of *Psychiatry* 48(3):195-203

Lauritzen, L., Hansen, H.S., Jorgensen, M.H., Michaelsen, K.F. (2001). The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipids Res*. 40:1-94

Liddle, P.F., Kiehl, K.A. & Smith, A.M. (2001, February). Event-related fMRI study inhibition. *Human Brian Mapping*, 12(2):100-109

O-05-10 Sweet Threshold of Lysozyme from Indonesian Kampong Chicken and Cihateup Duck

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Introduction

Lysozyme is a protein that has various functions. Lysozyme consists of 129 amino acid residues with four disulfide bonds. The molecular weight of lysozyme is between 14300-14600 Da with isoelectric point around 10.7. Lysozyme has been used as an antimicrobial agent for various types of food. In 1992, a joint committee between the World Health Organization (WHO) and Food and Agriculture Organization (FAO) declare that lysozyme could be used safely in food. Several studies have also characterized the ability of lysozyme as antioxidant.

Lysozyme is initialized to be studied and developed as sweetener. Lysine, one of the amino acids found in lysozyme, has the ability to generate sweet taste when interacting with sweet receptors on the tongue. Although proteins are generally tasteless, some of them have sweet taste such as thaumatin (van der Wel and Loeve, 1972), monellin (Morris and Cagan, 1972; van der Wel, 1972), brazein (Ming and Hellekant, 1994), Pentadin (van der Wel *et al.,* 1989), mabinlin (Liu *et al.,* 1993), curculin (Yamashita *et a.l,* 1990) and lysozyme (Masuda *et al.,* 2001). The proteins that can give sweet taste into food or pharmaceutical product are known as sweet protein. This protein has sweetness level 100 to 2000 times higher than sucrose.

Lesnierowski and Kijowski (2007) mentioned that lysozyme was found in poultry's egg white, tears, milk, spleen, thymus, pancreas and cauliflower, cabbage and papaya juices. Lysozyme found in chicken eggs was named c-type lysozyme (generated from "chicken" or "conventionali") since it has different structure compared with lysozyme from other poultry. Lysozyme found in geese egg hasa different radical, which is called the g-type (Losso *et al.,* 2002). Furthermore, Prager and Jolles (1996), cited by Losso *et al.* (2002) argued that each poultry had a different lysozyme gene regulator, which made difference the characteristics of the lysozyme.

The purpose of this study was to determine the value of EWL sweet threshold from kampong chicken and cihateup duck eggs

Materials and Methods

Collection of Egg White Fractions of Kampung Chicken and Cihateup Duck

Fresh egg of kampung chicken and Cihateup duck were obtained from field laboratory of Faculty of Animal Science, Bogor Agriculture University (IPB). Eggs collected were in good qualities: egg shell and content were cleaned and freed from blood stains (blood spots), did not contain an embryo and have high viscosity. The eggs used in this study had the same age that is 1 to 3 days after hatched. For comparison, egg of non-Indonesian laying hens (hereafter called commercial laying hens) were also collected and treated as described above. Albumen was then separated and kept for further experiment.

Methods

purification of EWL from kampong chicken and cihateup duck eggs by using ion exchange chromatographyPurification using ion exchange chromatography. The experiment was performed based on Strang (1984) with slight modifications. Briefly, albumen from one egg was filtered and followed by 5 times dilution with 100 mM glycine/NaoH buffer pH 10.0. Two gram of dry carboxymethyl cellulose (CMC) was then added to the bulk and stirred for 15 min to adsorb the EWL. Suspension was then centrifuged at 15,000 g for 5 min and CMC-containing pellet was collected. The pellet was then washed with 100 mM glycine/NaOH buffer pH 10.0, in an equal volume as albumen, followed by centrifugation as before to have washed pellet. The washed pellet was resuspended in glycine buffer as before and then poured into 1 cm diameter column. The first elution was performed by glycine buffer as before. The final elution was performed using glycine buffer containing 0.5 M NaCl. The eluates were collected in 10 mL Falcon tube, so-called fractions, and the presence of proteins in each fractions was monitored using the Agilent 8453 UV-vis spectrophotometer (Agilent Technologies, USA) at 280

nm. The protein concentration of unmodified lysozyme was determined spectrophotometrically by using the absorbance at 280 nm (E1% = 2.63) (Gill andVon Hippel, 1989). EWL were dialyzed for 24h at 4°C with citrate buffer (pH 3.4) and phosphate buffer (pH 7.0).

Sensory Evaluation

The purpose of organoleptic test was to determine the sweet threshold began recognizable by consumers . Five semi -trained test the sweetness of lysozyme. Due to the limited sample , the concentration of lysozyme were very limited. EWL concentration of cihateup duck eggs were 0.365; 0.373; 0.449; 0.536 mg/ml; EWL concentration of commercial laying hens were 0.365; 0.373; 0.384 mg/ml and EWL concentration of kampong chicken were 0.236 ; 0.373 and 0.414 mg/ml . Panelists assessed whether there was a sweet taste in the sample. After each tests, panelist througly rinsed their mouth with distilled water.

Experimental Design

Experimental design used by this study is randomized block design (RBD) with treatment of different types of eggs. The treatments were: (1) commercial laying hens as control (2) kampong chicken and (3) cihateup duck.

Results and Discussion

Based on research , had been done Wulandari *et al.* (2014) , EWL has successfully separated from the commercial laying hens, kampong chicken and cihateup duck purification using ion exchange chromatography. The experiment was performed based on Strang (1984) with slight modifications. This was feasible to be used a single step to purify EWL on the basis of isoelectric point (pI) (10.7) compared to that of other egg white proteins (< 6.5) (Anton *et al*., 2006; Machado *et al*., 2007; Luding *et al*., 2011). The only egg white protein with pI close to EWL`s pI is avidin (10.0). At pH 10, in exception of EWL and avidin, egg white proteins are supposed to be positively charged. While EWL will be considerably negatively charged at pH 10, avidin is supposed to be uncharged. Based on this condition, when carboxymethil-cellulose (-CH2COOH; pKa 3.5-4.5) was dissolved in pH10, these materials are negatively charged. When these materials used as beads upon the purification, the negatively charged of the beads bind to positively charged of EWL, while other proteins did not dissolve and elute as flow-through fractions. Further, complex of EWL-the beads is disrupted by stronger ionic strength solution (NaCl) to obtain beads-free EWL, so called purified EWL.

Ion exchange chromatography at pH 10 successfully purified lysozyme as indicated by a single band corresponding to lysozyme size (~14 kD) free from bands of other proteins (Wulandari *et al,* 2014).

Results of EWL Organoleptic Test Dialyzed with Phosphate Buffer (pH 7)

Results of sweet threshold organoleptic test can be seen at Table 1. Concentration of EWL ranged from 0.236 mg/ml to 0.536 mg/ml. Based on Table 1, EWL fraction has sweet taste. Panelist detected sweet taste from EWL. EWL sweet threshold of cihateup duck was 0.536 mg/ml. EWL sweet threshold of kampong chicken was 0.236 mg/ml. EWL sweet threshold of commercial laying hens was 0.373 mg/ml. Sweet taste EWL was not affected by phosphate buffer (pH 7). Lysozyme is a sweet-tasting protein with a sweetness threshold value of 7 mM (Masuda *et al*, 2005). Maheshahi *et al*, (2007) reported lysozyme (0,11 + 0.02mM); monellin (1.1mM) ; thaumatin (0.3 mM) and aspartame (0.72 mM) equisweet concentration to 0.15 M sucrose. EWL was sweeter than aspartame, but the lowest was compared the other protein sweetener.

Table 1. Results of EWL organoleptic Test Dialyzed with Phosphate Buffer (pH 7)

Results of EWL Organoleptic Test Dialyzed with Citrate Buffer (pH 3.4)

Results of sweet threshold organoleptic test can be seen at Table 2. Concentration of EWL ranged from 0.236 mg/ml to 0.536 mg/ml. EWL sweet threshold of cihateup duck was 0.365 mg/ml. EWL sweet threshold of kampong chicken was 0.236 mg/ml. EWL sweet threshold of commercial laying hens was 0.365 mg/ml. At pH 3.4, the sweet taste was covered by sour taste.

Table 2. Results of EWL organoleptic Test Dialyzed with Citrate Buffer (pH 3.4)

Conclusion

EWL has successfully separated from the commercial laying hens, kampong chicken and cihateup duck using ion exchange chromatography. The sweet threshold of EWL was 0.236 mg/ml. Differences in the pH of buffer dialysis

resulted in differences EWL sweet taste and sweet threshold.

Acknowledgement
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KEYWORD : Egg white lysozyme, Sweet threshold, Kampong Chicken, Cihateup Duck

Table 1. Results of EWL organoleptic Test Dialyzed with Phosphate Buffer (pH 7)

No.	Sample	Code	Concentration (mg/ml)	Panelist				
				1	2	3	4	5
1.	EWL of Cihateup Duck	579	0.365	+				
2.		351	0.449					
3.		738	0.373	+				
4.		167	0.536	+			+	+
5	EWL of Kampong Chicken	235	0.373	+			+	+
6		473	0.414	+		+	+	++
7.		841	0.236	++	+	+	+	+
8.	EWL of commercial laying hens	135	0.365	+	+	+		
9.		652	0.384	++	+		+	+
10.		964	0.373	+	+	+	+	++

Note : (+) : detected sweet taste ; ( ) : not detected sweet taste

Table 2. Results of EWL organoleptic Test Dialyzed with Citrate Buffer (pH 3.4)

No.	Sample	Code	Concentration (mg/ml)	Panelist				
				1	2	3	4	5
1.	EWL of Kampong Chicken	123	0.373					
2.		231	0.414			+		+
3.		312	0.236	+				+
4.	EWL of commercial laying hens	456	0.365	+			+	
5.		564	0.384		+	+	+	+
6.		645	0.373	+			+	+
7.	EWL of Cihateup Duck	789	0.365		+	+		
8.		897	0.449	+	+	+	+	
9.		978	0.373	+	+	+	+	+
10.		798	0.536	+	+	+	+	+

Note : (+) : detected sweet taste ; ( ) : not detected sweet taste

REFERENCES

Anton, M., F. Nau, and Y. Nys. 2006. Bioactive egg components and their potential uses. World Poult. Sci. J. 62:429-438.

Gill, SC and P.H. Von Hippel. 1989. Calculation of protein extinction coefficients from amino acid sequence data. Analytical Biochemistry 182 : 319-326.

Lesnierowski, G and J. Kijowski. 2007. Lysozyme in : R. Huoplahti., R. L-Fandino., M. Anton and R. Schade (Eds). Bioactive Egg Compounds. Springer. Heidelberg, Germany.

Liu, X., Z. Hu., S. Maeda., T. Auchi., K. Nakaya and Y. Kurihara. 1993. Purification, complete amino acid sequence and structure characterization of the heat stable sweet protein, mabinlin II. Eur. J. Biochem, 211: 281-287.

Losso, J.N., S. Nakai, E.A. Charter. 2002. Lysozyme. In : Naidu, A.S (Ed.). Natural Food Antimicrobial System. CRC Press, London.

Luding, Y., S. Shaochuan, Y. Junxian and Y. Kejian. 2011. Isolation of lysozyme from chicken egg white using polyacrylamide-based cation-exchange cryogel. Chin. J. Chem Eng. 19:876-880.

Machado, F. F. M., J. S. R. Coimbra, E. E. G. Rojas, L. A. Minim, F. C. Oliveira and S. S. R. De Cassia. 2007. Solubility and den¬sity egg white proteins: effect of pH and saline concentra¬tion. Food Sci. Technol. 40: 1304-1307.

Maehashi, K., M. Matano., A.Kondo., Y. Yamamoto and S. Udaka. 2007. Riboflavin-binding protein exhibits selective sweet suppression toward protein sweeteners. Chem. Senses 32: 183–190.

Matsuda, T., Y. Ueno and N. Kitabake. 2001. Sweetness and enzymatic activity of lysozyme. J. agric. Food Chem. 49: 4937-4941.

Masuda, T., N. Ide and N. Kitabake. 2005. Effects of chemical modification of lysine residues on the sweetness of lysozyme. Chem. Senses 30: 253 – 264.

Ming, D. and G. Hellekant. 1994.. Brazzein, a new high-potency thermostable sweet protein from Pentadiplandra brazzeana B. FEBS Lett, 355: 106-108.

Morris, J.A. and R.H. Cagan 1972. Purification of moenellin, the sweet principle of Disocoreophyllum cumminsii. Biochem. Biophys. Acta, 261 : 114-122.

Strang, R. H. C. 1984. Purification of egg white lysozyme by ion-exchange chromatograpy. Biochemical education 12: 57-59.

Van der Wel, H. and K. Loeve. 1972. Isolation and characterization of thaumatin I and II, the sweet tasting proteins from Thaumatococcus Danielle Benth. Eur. J. Biochem, 31: 221-225.

Van der Wel, H., G. Larson., A. HAdik., C.M. Hadik, G. Hellekant and D. Gleser. 1989. Isolation and characterization of pentadin , the sweet principle of Pentadinplandra brazzeana Baillon. Chem. Senses, 264 : 6655 – 6659.

Wulandari, Z., C. Budiman., T. Suryati and D. Herawati. 2015. Purification of egg white lysozyme from Indonesian Kampung chicken and duck. Media Peternakan, 38(1):18-26.

Yamashita, H., A. Theeraship., T. Nakaya., Y. Nakamura and Y. Kurihara. 1990. Purification and complete amino acid sequence of a new type of sweet protein with taste –modifying activity, curolin. J. Biol. Chem. 265 : 15770 – 15775.

O-06-2

Effect of Proportion of Fatty Acids from Soybean Oil and Beef Tallow on Fatty Acids Accumulation in Egg Yolk

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Introduction

Differences in fatty acid composition of plant seed oil and animal tallow have been reported in the literature. Soybean oil (SBO) has high amounts of long-chain UFAs (essential fatty acids, especially linoleic acid) (18:2n-6), which are a source of omega-6 polyunsaturated fatty acids (PUFAs). Beef tallow (TAL) has higher contents of stearic acid (18:0) and palmitic acid (16:0)—long-chain, saturated fatty acids (SFAs). Stearic acid (18:0) is catalyzed in the liver by the desaturase enzyme (δ 9-desaturase) to form oleic acid (18:1n-9) (omega-9 polyunsaturated fatty acids (PUFAs) and palmitic acid; in turn to form palmitoic acid (16:1n-7), an alternative precursor when essential fatty acids are deficient (Leonard et al., 2004; Marzouki et al., 1982; Engster et al., 1977). Oleic acid (18:1n 9) in the diet accumulated in the yolk when the hens were fed TAL. SFA accumulation in egg yolks was not significantly different although SFA intake was different. A diet of 10% SBO was high in linoleic acid (18:2n 6) and resulted in enrichment of omega 6 fatty acids in the yolk. The latter is important to endogenous synthesis of PUFA products: n-6 PUFAs are converted to n-3 PUFAs and accumulate in the yolk. The addition of TAL to the diet strongly affects oleic acid (18:1n:9) accumulation in the yolk. Similarly, the addition of SBO to the diet strongly promoted PUFA accumulation in the yolk, especially omega-6 PUFAs.

The current study was to evaluate the fatty acids accumulation of egg yolks of hens fed different fatty acids composition especially the levels of linoleic acid (18:2n-6) and stearic acid (18:0) from proportion of soybean oil and beef tallow.

Materials and Methods

Animals and experimental diets

A total of 30 laying hens (Hisex brown chickens), 36 weeks of age, were put in individual cages (30 x 40 x 40 cm). They were randomly separated into 5 groups (3 replicated) in a room maintained at 32-34 ^{o}C, which were condition appropriate for the hens. Between day 1 to 28, six hens per group were fed a dietary treatment (i.e., a cassava starch - soybean meal diet) with contained six ratio of soybean oil and beef tallow at 100:0, 75:25, 50:50, 25:75 and 0:100 (Tables 1-2). All of the hens were fed and watered ad libitum with 17 hour of light per day. The composition of nutrients (protein, metabolizable energy, vitamins and mineral) was calculated for laying hens as per the NRC (1994) recommendation.

Data collection

Body weight was measured every week. Feed intake, egg production and egg weight were recorded every day from day 1 to 28. On the first to final week of the experiment, 2 eggs per treatment were collected (total 50) and were analyzed the the total fat and fatty acid composition at the ALS Laboratory Co. Ltd., Thailand, with GC analysis (AOAC, 2012).

Statistical analyses

The data on productive performance and fatty acid composition of the yolks were analyzed using an ANOVA for a completely randomized design (CRD) using SAS software (Steel and Torrie, 1980; SAS Institute, 2003). Significant differences among means were compared using the Duncan's New Multiple Range tests procedure.

Results and discussion

The consuming diet of laying hen with containing proportion of soybean oil and beef tallow at 100:0, 75:25, 50:50, 25:75 and 0:100 in diet did not significantly ($p>0.05$) affect on body weight, hen day production, egg weight and egg mass (Table 3). However, feed intake was significant lower ($p<0.05$) in ration 50:50 of soybean oil and beef tallow as 89.87 g/h/d (1-28 day of experiment).

The fatty acids composition of the egg yolks are presented in Table 4. The proportion of stearic acid (18:0) containing graded according to the level of beef tallow in diet had effected to constant of stearic acid (18:0)

accumulation of egg yolk (7.39-8.11% of total fatty acid). The increasing of stearic acid (18:0) content in diet was not affected to increasing content of stearic acid (18:0) in egg yolk. However, increasing of stearic acid (18:0) of hen fed dietary treatments were significantly effected on graded levels of oleic acid (18:1n-9) accumulation in the egg yolk from 27.73% of 100:0 soybean oil : beef tallow to 46.23% of 0:100 soybean oil : beef tallow. Increasing of oleic acid (18:1n-9) in egg yolk were inversely with linoleic acid (18:2n-6) accumulation in egg yolk, according to Sim et al. (1973) reported that changes in oleic acid deposition was inversely related to linoleic acid deposition. These results indicated that linoleic acid was deposited primary at the expense of oleic acid (18:1n-9). Moreover, the stearic acid (18:0) can be desaturated to form oleic acid (18:1n-9), which is a omega-9 fatty acids source for accumulated in egg yolk as shown in Table 4. These differences may be related to the content of saturated and unsaturated fatty acids found in the diets (Oliveira et al., 2001).

Hen fed linoleic acids (18:2n-6) decreasing level from proportion of soybean oil and beef tallow had to decreased of linoleic acid accumulation of egg yolk from 29.73% (100:0 soybean oil : beef tallow) to 7.93% (100:0 soybean oil : beef tallow). The addition of beef tallow in diet (0:100 soybean oil:beef tallow) were contained the minimum of linoleic acid (18:2n-6) as 0.69%. However, the minimal of linoleic acid (18:2n-6) accumulation in egg yolk were contained 7.93% as showed in Table 4.

Arachidonic acid (20:4n-6) and docosahexaenoic acid acid (22:6n-3) levels in the egg yolk of laying hens fed the diet with proportion of soybean oil and beef tallow were significantly the fatty acids composition from the both of fat source. The concentration of linoleic acid (18:2n-6) are important role in fatty acid metabolism and are precursor of the longer chain fatty acid, especially the n-3 and n-6 fatty acids.

For the linoleic acid (18:2n-6) is the precursor of the n-6 fatty acids, the concentrations were higher in egg yolk of laying hen fed diets containing soybean oil, which were significant all lipid proportion ($p<0.05$). The egg yolk of laying hen fed 100:0 of soybean oil and beef tallow was significantly ($p<0.05$) higher amounts the omega-3 (linolenic acid; 18:3n-3 and docosahexaenoic acid (DHA); 22:6n-3) and omega-6 fatty acids (arachidonic acid; 20:4n-6). The results are similar to Grobas et al. (2001), the level of EPA and DHA found in the egg yolks of laying hens fed the diet containing linseed oil, which mainly the PUFAs play several important physiological roles of the body of the chickens.

Conclusions

The proportion of stearic acid (18:0) containing graded according to the level of beef tallow in diet had effected to constant of stearic acid (18:0) accumulation of egg yolk (7.39-8.11% of total fatty acid). The increasing of stearic acid (18:0) content in diet had to respect the increasing of oleic acid (18:1n-9) accumulation in the egg yolk from 27.73% of 100:0 soybean oil : beef tallow to 46.23% of 0:100 soybean oil : beef tallow. Hen fed linoleic acids (18:2n-6) increasing level from proportion of soybean oil and beef tallow had to increased of linoleic acid accumulation of egg yolk from 7.93% (100:0 soybean oil : beef tallow) to 29.73% (100:0 soybean oil : beef tallow. The addition of beef tallow in diet (0:100 soybean oil : beef tallow) were contained the minimum of linoleic acid (18:2n-6) as 0.69% in diet. The concentration of linoleic acid (18:2n-6) are effected to increasing of arachidonic acid (20:4n-6) and docosahexaenoic acid acid (22:6n-3) levels in the egg yolk.

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KEYWORD : Soybean oil, beef tallow, fatty acid, egg yolk, laying hens

Table 1 Ingredients and nutrients composition in diets

Items	Ratio of lipid sources				
Soybean oil	100	75	50	25	0
Beef tallow	0	25	50	75	100
Ingredients, kg/100 kg feed					
Cassava starch	25.00	25.00	25.00	25.00	25.00
Soybean meal (44%)	41.00	41.00	41.00	41.00	41.00
Rice hulls	10.80	10.80	10.80	10.80	10.80
Soybean oil	10.00	7.50	5.00	2.50	0.00
Beef tallow	0.00	2.50	5.00	7.50	10.00
DL-methionine[1]	0.40	0.40	0.40	0.40	0.40
Di-calcium phosphate (P18)[2]	4.00	4.00	4.00	4.00	4.00
$CaCO_3$	7.50	7.50	7.50	7.50	7.50
Salt	0.30	0.30	0.30	0.30	0.30
Premix (vitamins, minerals)[3]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrients calculated,%					
ME poult, kcal/kg	2749	2731	2713	2695	2677
Crude protein	18.32	18.32	18.32	18.32	18.32
Crude fat	11.56	11.56	11.56	11.56	11.56
Ash	6.54	6.54	6.54	6.54	6.54
Calcium	3.85	3.85	3.85	3.85	3.85
Total phosphorus	0.92	0.92	0.92	0.92	0.92
Available phosphorus	0.61	0.61	0.61	0.61	0.61
Lysine	1.07	1.07	1.07	1.07	1.07
Methionine	0.64	0.64	0.64	0.64	0.64
Methionine+Cystine	0.90	0.90	0.90	0.90	0.90

[1] DL-Methionine is 99% methionine, [2] Di-calcium phosphate is contained Ca 24% and P18%, [3] Premix comprises vitamin A 4,800,000 IU, vitamin D_3 800,000 IU, vitamin E_3,200 IU, vitamin K_3 0.80 g, vitamin B_1 0.32 g, vitamin B_2 1.60 g, pantothenic acid 2.00 g, niacin 6.00 g, folic acid 0.20 g, vitamin B_6 0.40 g, choline 48.00 g, biotin 0.006 g, vitamin B_{12} 0.002 g, copper 2.40 g, ferrous 16 g, Zinc 20.00 g, selenium 0.04 g, manganese 24.00 g, iodine 0.32 g, cobalt 0.08 g and a carrier added to 1 kg.

Table 2 Fatty acids composition of experimental diets

Item	Ratio of lipid sources				
Soybean oil	100	75	50	25	0
Beef tallow	0	25	50	75	100
Fatty acids profile, % of total fat					
Lauric acid (12:0)	0.03	0.07	0.12	0.16	0.20
Myristic acid (14:0)	0.08	1.16	2.24	3.31	4.39
Palmitic acid (16:0)	10.80	15.43	20.05	24.68	29.30
Palmitoic acid (16:1)	0.09	0.28	0.46	0.65	0.83
Stearic acid (18:0)	4.07	12.25	20.44	28.62	36.80
Oleic acid (18:1n-9)	24.50	22.63	20.75	18.88	17.00
Linoleic acid (18:2n-6)	52.70	39.70	26.70	13.69	0.69
Linolenic acid (18:3n-3)	6.27	4.75	3.24	1.72	0.20
Arachidonic acid (20:4n-6)	-	0.01	0.02	0.02	0.03
Docosahexaenoic acid (22:6n-3)	-	-	-	-	0.01
Saturated fatty acids (SFAs)	16.10	31.00	45.90	60.80	75.70
Unsaturated fatty acids (UFAs)	83.90	67.68	51.45	35.23	19.00
Mono-unsaturated fatty acid	24.90	23.18	21.45	19.73	18.00
Poly-unsaturated fatty acid	59.00	44.51	30.01	15.52	1.02
Omega-3 fatty acids	6.27	4.76	3.25	1.73	0.22
Omega-6 fatty acids	52.70	39.73	26.75	13.78	0.80
Omega-9 fatty acids	24.80	22.88	20.95	19.03	17.10

Table 3 Production performance of laying hens

Items	Ratio of lipid sources					p-value	SEM
Soybean oil	100	75	50	25	0		
Beef tallow	0	25	50	75	100		
Body weight, g/hen							
Day 1	1712	1702	1691	1676	1721	0.9796	56.560
Day 7	1671	1708	1680	1690	1698	0.9872	49.452
Day 14	1682	1699	1625	1683	1688	0.7942	44.714
Day 21	1687	1708	1613	1670	1670	0.5662	40.566
Day 28	1730	1766	1664	1714	1724	0.7440	52.776
Feed intake, g/h/d							
1-7 day	93.62^{b}	110.88^{a}	93.07^{b}	109.17^{a}	108.07^{a}	0.0029	3.023
7-14 day	94.05	98.12	76.83	105.60	103.17	0.0679	6.484
14-21 day	99.26	99.48	79.79	96.71	100.07	0.0663	4.898
21-28 day	110.19	118.95	109.79	112.12	121.31	0.3890	4.953
1-28 day	99.28^{ab}	106.86^{a}	89.87^{b}	105.90^{a}	108.15^{a}	0.0379	3.870
Hen day production, %							
1-7 day	66.67	80.95	73.81	80.95	71.43	0.7316	8.715
7-14 day	80.95	80.95	83.33	90.48	78.57	0.5856	5.323
14-21 day	85.71	83.33	69.05	85.71	78.57	0.2319	5.429
21-28 day	80.95	92.86	83.33	88.10	85.71	0.2630	3.689
1-28 day	78.78	84.52	77.38	86.31	78.57	0.2314	3.126
Egg weight, g/egg							
1-7 day	56.35	57.80	57.52	58.20	57.47	0.9590	1.778
7-14 day	57.30	58.07	56.62	57.37	57.37	0.8888	0.981
14-21 day	57.39	56.81	54.37	56.75	56.89	0.1619	0.826
21-28 day	58.33	56.91	57.09	27.79	56.03	0.6159	1.060
1-28 day	57.56	57.38	56.47	57.52	57.09	0.9335	1.006
Egg mass, g/h/d							
1-7 day	37.58	46.78	42.41	47.33	41.87	0.7262	5.562
7-14 day	46.41	47.05	47.11	45.06	45.06	0.5927	3.017
14-21 day	49.26	47.26	37.55	44.70	44.70	0.1306	3.147
21-28 day	47.24	52.81	47.47	48.02	48.02	0.2824	2.052
1-28 day	45.12	48.48	43.64	44.91	44.91	0.2444	2.038

^{a,b} Means within rows with no common superscript differ significantly ($p<0.05$).
SEM = standard error of means

Table 4 Fatty acids profile of experiment egg yolk

Items	Ratio of lipid sources					p-value	SEM
Soybean oil	100	75	50	25	0		
Beef tallow	0	25	50	75	100		
Total fat, %	25.90	25.93	28.83	26.70	27.73	0.6507	1.582
Fatty acids profile, g of % fat							
Lauric acid (12:0)	0.09	0.08	0.08	0.07	0.07	0.6832	0.009
Myristic acid (14:0)	0.23^{c}	0.41^{b}	0.55^{b}	0.91^{a}	1.07^{a}	<.0001	0.058
Palmitic acid (16:0)	21.13^{c}	21.23^{c}	22.17^{bc}	22.97^{b}	24.20^{a}	0.0004	0.339
Palmitoic acid (16:1)	1.08^{c}	1.20^{bc}	1.64^{b}	2.23^{a}	2.67^{a}	<.0001	0.152
Stearic acid (18:0)	7.39	8.02	7.42	8.11	7.57	0.0509	0.182
Oleic acid (18:1n-9)	28.97^{e}	32.47^{d}	37.17^{c}	40.63^{b}	46.23^{a}	<.0001	0.454
Linoleic acid (18:2n-6)	29.73^{a}	25.00^{b}	19.80^{c}	13.93^{d}	7.93^{e}	<.0001	0.554
Linolenic acid (18:3n-3)	1.83^{a}	1.28^{b}	0.82^{c}	0.65^{c}	0.26^{d}	<.0001	0.077
Arachidonic acid (20:4n-6)	2.02^{ab}	2.18^{a}	2.16^{a}	1.86^{bc}	1.62^{c}	0.0148	0.076
Docosahexaenoic acid (22:6n-3)	1.39^{a}	1.40^{a}	1.27^{b}	1.13^{c}	0.71^{d}	<.0001	0.038
Saturated fatty acids (SFAs)	30.07^{c}	31.33^{bc}	31.93^{abc}	34.07^{ab}	34.93^{a}	0.0334	0.994
Unsaturated fatty acids (UFAs)	65.40^{a}	63.77^{ab}	63.13^{ab}	60.80^{bc}	59.80^{c}	0.0126	0.960
Mono-unsaturated fatty acid	30.23^{e}	33.90^{d}	39.13^{c}	43.27^{b}	49.40^{a}	<.0001	0.410
Poly-unsaturated fatty acid	35.17^{a}	29.87^{b}	24.03^{c}	17.43^{d}	10.41^{e}	<.0001	0.941
Omega-3 fatty acids	3.26^{a}	2.72^{b}	2.10^{c}	1.79^{d}	0.97^{e}	<.0001	0.086
Omega-6 fatty acids	31.90^{a}	27.20^{b}	21.93^{c}	15.77^{d}	9.44^{e}	<.0001	0.949
Omega-9 fatty acids	29.13^{e}	32.63^{d}	37.43^{c}	40.87^{b}	46.53^{a}	<.0001	0.454

$^{a\text{-}e}$ Means within rows with no common superscript differ significantly ($p<0.05$) and differ highly significantly ($p<0.01$)
SEM = standard error of means

REFERENCES

AOAC. 2012. Association of official analytical chemists. Official Methods of Analysis. 19th ed. Association of Official Analytical Chemists, Alington VA.

Engster, H.M., L.B. Carew and F.J. Cunnningham.1977. Effects of an essential fatty acid deficiency, pair-feeding and level of dietary corn oil on the hypothalamic-pituitary-gonadal axis and other physiological parameters in the male chicken. Presented in part at the annual meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois. April, Federation Proc. 36: 889-900.

Grobas S., J. Mendez, R. Lazaro, C.D. Blas and G.G. Mateos. 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Journal of Poultry Science. 80: 1171-1179.

Leonard, A.E., S.L. Pereira, H. Sprecher and Y.S. Huang. 2004. Elongation of long-chain fatty acids. Journal of Progress in Lipid Research. 43: 36-54.

Marzouki, Z.M.H. and J.G. Coniglio. 1982. Effect of essential fatty acid deficiency on lipids of rat sertoli and germinal cells. Journal of Biology of Reproduction. 27: 312-315.

NRC, 1994. Nutrient requirements of poultry. 9th ed, National Academy Press, Washington DC.

Oliveira, D.D., N.C. Baião, S.V. Cançado, R. Grimaldi, M.R. Souza, L.J.C. Lara and A.M.Q. Lana. 2010. Effect of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. Journal of Poultry Science. 89: 24842490.

SAS Institute. 2003. User's guide, New York.

Sim, J.S., D.B. Bragg and G.C. Hodgson. 1973. Effect of dietary animal tallow and vegetable oil on fatty acid composition of egg yolk, adipose tissue and liver of laying hens. Journal of Poultry Science. 52(1): 5157.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistic, Second Edition., McGrawHill, New York.

O-06-3

Effect of Low Protein Amino Acid Supplementation on Growth Performance and avANT Gene Expression in the Muscle of Betong Chicken

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Introduction

In Thailand, Thai native chickens have become increasingly popular because of their taste and very lean meat. Betong chicken (KU line) is one of the native chickens and is most popular in the southern part of Thailand due to the high quality of the meat and low carcass fat compared to broiler chickens and other Thai breeds (Nirat and Ratana, 1996 and Danvilai, et al., 2014). However, information on nutrient requirements is still limited. Previous researches reported that Betong chickens (KU line) required 17% CP diets and an energy level of 3,000-3,200 ME Kcal/kg, while a high level of dietary protein (23, 21 and 19% CP) did not alter growth performance at 0-42 days of age (Nguyen and Bunchasak, 2005). Putsakul (2010) also reported that the suitable protein and energy level of chickens were 18% CP and 3,000 ME Kcal/ kg at 4-16 weeks of age, and 16% CP and 2,800 ME Kcal/kg at 16-20 weeks of age; but female chickens required 18% CP and 2,800 ME Kcal/kg at 4-16 weeks of age. The data also showed the analysis of investment and return of chickens 0-20 weeks of age to gain a final body weight of 1,700-2,400 kg, founding higher than commercial broiler farming.

Thus, using low-protein diets is one concept for a better financial investment because of the reduced feed cost, which represents more than 70% of the total production cost. However, reducing protein concentration in diets always decreases growth performance and breast muscle proportion, and increases body fat accumulation because of an amino acid imbalance. Thus, amino acid supplementation in a low protein diet is more effective in improving the amino acid balance than supplementation in high protein diets for economic, physiological or environmental reasons (Emmert et al., 2000; Vieira et al., 2004). Furthermore, amino acid supplementation improved feed efficiency (Del-Vesco etal., 2013), and the studies have shown that broilers have worse feed efficiency and produced less ATP (Bottje and Carstens, 2009). Methionine, may influence the expression of the genes involved in energy production in mitochondria. Previous studied found some important protein, involving process of ATP production, adenine nucleotide translocase (ANT) demonstrated the relationship between the expression of genes encoding those proteins with feed efficiency (Ojano-Dirain et al., 2007 and Bottje et al., 2009). Thus, the objectives of this study are to examine the effects of the supplementation of synthetic amino acids to low protein diets on the performance of male and female Betong chickens (KU line) and the expression of the avANT gene in breast muscle.

Materials and Methods

Day-old Betong (KU line) chicks from the same hatch were conventionally raised for 4 weeks under the same conditions (with a use of a basal diet, 18% CP). The male and female birds were distributed for the treatments according to a completely randomised block design (CRBD) (4 replicates of 16 birds per treatments). The treatments consisted of a basal diet (18% CP), low protein diets (16% CP) and 16% CP+ Met at 4-12 weeks; followed by a basal diet (16% CP), low protein diets (14% CP) and 14% CP + Met at 12-18 weeks, with four replicates of 16 birds each, totaling 384 birds. The experimental diets (Table 1) were based on corn and soybean meal, and formulated according to the recommendations of the National Research Council (1994). In order to determine weight gain, all birds were weighed in the beginning, every 2 weeks, and the end of the experimental period. Feed intake was recorded then the feed conversion ratio and feed efficiency were calculated.

At the end of the experimental period, two birds per treatment were sacrificed by neck dislocation and samples of the breast muscle (pectoralis superficialis) were collected and stored in RNAlater Solution (Ambion, USA) at -20℃ until total RNA extraction. All animals were killed by cervical dislocation at the same time. Total RNA was extracted using RNeasy® Fibrous Tissue Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Total RNA concentration was measured using a spectrophotometer at a wavelength of 260 nm. Four hundred nanograms of total RNA were used to prepare complementary DNA (cDNA). Real-time PCR reaction used the fluorescent dye

SYBR GREEN (SYBRs GREEN PCR Master Mix, Bio-Rad, USA). The primers used were: GAPDH forward primer (5'-GAGGGTAGTGAAGGCTG-3') and GAPDH reverse primer (5'-GGGAAGCAGGACCCTTTGTT-3'), avANT forward primer (5'-TATCAGCTGGATGATTGCACAGA-3') and avANT reverse primer (5'-ACATGATATCAGCTCCTTTGCGT-3'). It was initiated with a first denaturation step of 5 min at 95-C, followed by 40 cycles of 94-C for 2 min and 55-C for 1 min. The avANT and GAPDH transcripts were simultaneously quantified for each sample during each real-time PCR run, and negative controls consisted of samples with no template cDNA. A standard curve for quantitation of avANT and GAPDH was constructed using serial dilutions of the PCR product containing avANT and GAPDH, respectively. The amount of avANT transcripts was related to that of GAPDH transcripts. The statistical analysis was carried out using SAS version 9.0 software, and a general linear model (GLM) at a 95% confidence level was used to evaluate the differential mRNA expression between the treatments.

Results and Discussion

The effects of protein levels on growth performance and feed efficiency of the Betong chicks are shown in Table 2. Growth performance of male Betong chickens were significantly higher than those of female Betong chickens ($P<0.05$) the same results reported by Putsakul (2010). However, there were no statistical differences for feed efficiency (FE) between sexes. Moreover, the results showed that methionine supplementation significantly improved the feed conversion ratio (FCR) and feed efficiency (FE) ($p<0.01$); however, there were no statistical differences in body weight (BW) and body weight gain (BWG) between groups throughout the experiment. Surprisingly, feed intake (FI) of the chickens fed with low CP diets (14% CP) was higher than other groups, resulting in the chicken probably improving BW and BWG equal to control (16% CP) and 16% CP + Met diets. The FI of chicken fed with a low CP diet (18% CP, 4-12 weeks and 16% CP, 12-18 weeks) was higher than other treatments, while the FI of control diets was equal to low protein + Met diets.

Aletor et al. (2000) also reported that the FI of broilers fed with low protein levels (CP 15%) was higher than in broilers fed with a high protein diet group (CP 22%). Furthermore, Putsakul (2010) found no significant difference in FI in Betong chickens treated with different protein level diets (16, 18 and 20%). However, many reports have worked on studies on low protein diet in broilers; they found that the FI was low in the chickens treated with a low protein diet (Dean et al., 2006, Iyayi et al., 2014 and Ospia-Rojas (2014) found that the FI of broiler chickens treated with a low protein diet (16% CP) was equal to control group (16% CP).

Quantitative real time-PCR used to evaluate gene expression patterns in muscle in response to the different diets is shown in Figure 1. The data was normalised using the GAPDH gene, the expression of which did not change among the treatment groups and sex throughout the experimental period. The evaluated parameters of growth performance BWG, FCR and FE relative to the mRNA avANT expression in the muscle found that a significant interaction was not observed. Moreover, the expression of the avANT gene of the end of experimental period (week 18) found a decreasing trend from week 14, but no statistically significant difference.

Gasparino (2014) reported that avANT mRNA expression observed for high FE was significantly higher than low FE in the liver female quail, with no significant difference in the muscle. Moreover, no statistical difference was observed among different sources and levels of methionine (supplementation of 0.08% DL-methionine, 0.24% of DL-methionine, 0.11% of MHA-FA and 0.33% of MHA-FA) with regard to the expression of the ANT genes in the muscle and liver (Del Vesco et al., 2013). The results suggested that although ATP production is affects to FE and other productive performance; however, many other physiological systems are probably involved. Metabolic pathways are involved in protein deposition and feed intake control, and also play a key role in FE. Recent studies on muscle growth (Tesseraud et al., 2007 and Zheng et al., 2009) and feed intake control show that the cellular mechanisms with a single signalling pathway with the central nervous system regulating energy acquisition by controlling feed intake (Richards et al., 2010). In addition to their many functions in growth, thyroid hormones have also been shown to participate in the control of avANT mRNA expression.

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KEYWORD : avANT gene expression, Betong chicken (KU-Line), Low-protein diets, Methionine, Growth performance

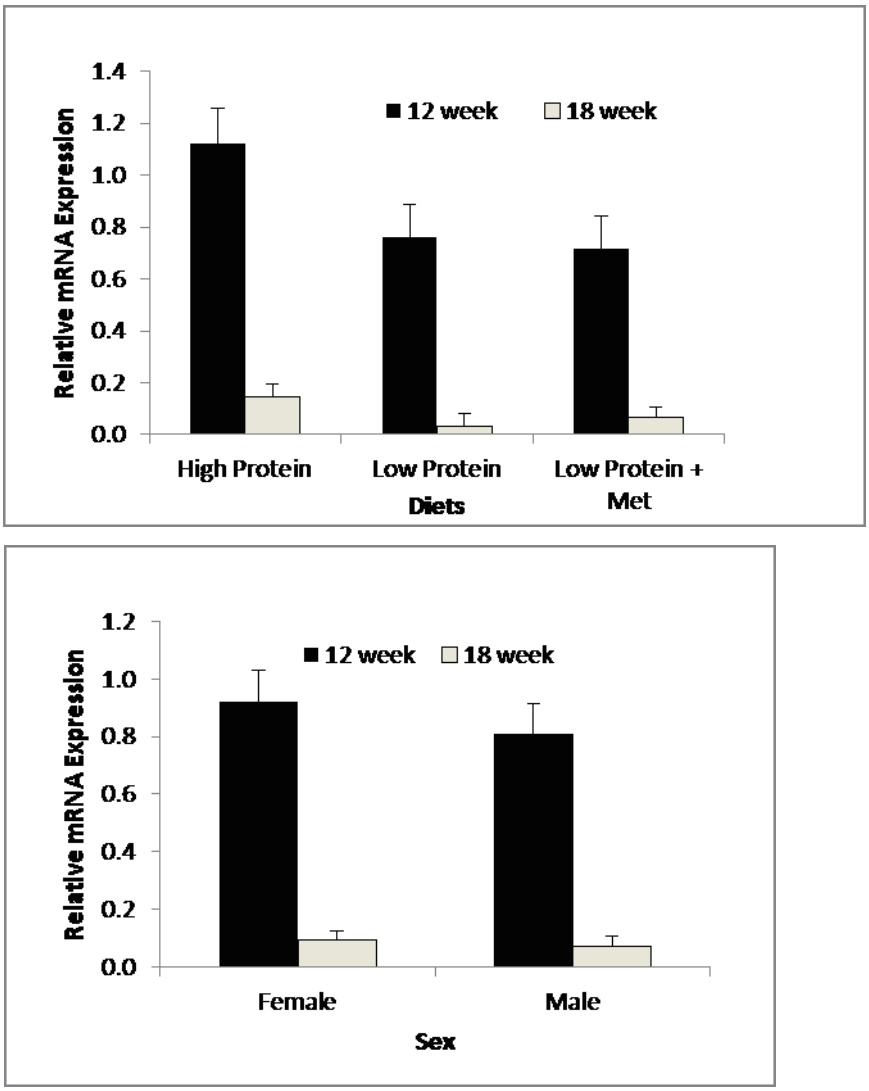

Figure 1. Relative mRNA expression of nucleotide translocase (ANT) in the muscle of at 14 and 18 weeks of age of Betong chickens fed with a control diet, low protein diet + Met and low protein diets. The results are averages a posteriori and standard deviation represented by vertical bars.

Table 1 Composition and nutrient levels in diets

Composition (%)		Experimental diets		
		CP 18%	CP 16%	CP16% + Met
Corn Thai		59.5	66.7	66.8
Oil		6.00	4.81	4.75
Soybean meal (CP 46%)		30.4	24.1	23.9
L-Lysine		0.00	0.19	0.20
DL-Methionine		0.03	0.00	0.09
Monocalcium phosphate		1.21	1.20	1.20
Calcium carbonate		1.93	1.98	1.98
Sodium bicarbonate		0.25	0.50	0.50
Salt		0.43	0.30	0.30
Premix[1]		0.25	0.25	0.25
Total		100	100	100
Nutrients calculated				
Metabolizable energy	(Kcal/Kg)	3,000	3,000	3,000
Protein	%	18.00	16.00	16.00
Lysine	%	0.95	0.95	0.95
Methionine + Cysteine	%	0.61	0.52	0.61
Methionine	%	0.32	0.26	0.35
Calcium	%	1.06	1.05	1.06
Available Phosphorus	%	0.45	0.45	0.45
Sodium	%	0.25	0.27	0.27

Table 2. Effect of additional methionine in low-CP diet on performance of Betong chicken (KU line) during 4–18 weeks of age.

Items Diets	BW (g)	BWG (g)	ADG (g)	FI (g)	FCR	FE
Sex						
4–12 weeks						
Female	1103.4 ± 155.15[b]	803.93±153.06[b]	14.62 ± 2.78[b]	2779.53 ± 271.36[b]	3.63 ± 0.92[b]	0.293 ± 0.07
Male	1422.98 ± 93.08[a]	1070.99 ± 90.68[a]	19.47 ± 1.65[a]	3518.28 ± 375.4[a]	3.34 ± 0.55[a]	0.308 ± 0.04
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	0.4488
12–18 weeks						
Female	1652.38 ± 239.59[b]	1352.92 ± 238.17[b]	13.8 ± 2.43[b]	5884.12 ± 687.4[b]	4.47 ± 0.91[b]	0.233 ± 0.05
Male	2064.08 ± 72.43[a]	1712.08 ± 70.49[a]	17.47 ± 0.72[a]	7598.33 ± 751.4[a]	4.45 ± 0.56[a]	0.227 ± 0.02
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	0.6898
4–12 weeks						
18% CP	1309.23 ± 203.63	982.55 ± 180.72	17.86 ± 3.29	2907.84 ± 405.43[a]	3.03 ± 0.44[a]	0.338 ± 0.05[a]
16% CP	1197.3 ± 220.43	874.85 ± 197.62	15.91 ± 3.59	3541.20 ± 454.11[b]	4.21 ± 0.79[b]	0.246 ± 0.05[b]
16% CP + Met	1283.04 ± 202.71	954.99 ± 178.88	17.36 ± 3.25	2997.69 ± 407.35[a]	3.21 ± 0.37[a]	0.318 ± 0.04[a]
P-value	0.1916	0.2116	0.211	<.0001	<.0001	0.002
12–16 weeks						
16% CP	1904.85 ± 318.75	1,578.16 ± 297.08	16.10 ± 3.03	6215.5 ± 954.2[a]	4.00 ± 0.60[b]	0.255 ± 0.04[b]
14% CP	1814.19 ± 264.75	1,491.74 ± 242.38	15.22 ± 2.47	7579.4 ± 1056.7[b]	5.14 ± 0.66[a]	0.198 ± 0.03[a]
14% CP + Met	1855.65 ± 259.49	1,527.60 ± 237.07	15.59 ± 2.42	6428.8 ± 943.8[a]	4.23 ± 0.42[b]	0.239 ± 0.03[b]
P-value	0.6126	0.6346	0.6342	<.0001	<.0001	0.0077

[a, b,c] within each column, means with different superscript letters are different (P<0.05).

REFERENCES

Aletor, V.A., I. Hamid, E. Neib and E. Pfeffer, 2000. Low-protein amino acid supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole body composition and efficiency of nutrient utilization. J. Sci. Food Agric. 80: 547-554.

Bottje, W.G. and G.E. Carstens, 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. J. of Ani. Sci. 87: 48–63.

Danvilai, W., W. Loongyai and P. Sopannarath, 2014. A study of growth performances and carcasses characteristics of the female broiler, Thai native and betong (KU line) chickens. The 52nd of Kasetsart University Annual Conference. 4-7 Feb 2014, Bangkok, Thailand: 9-16.

Dean, D. W., T. D. Bidner and L. L. Southern, 2006. Glycine supplementation to low protein, amino acid supplemented diets supports optimal performance of broiler chicks. Poult. Sci. 85:288–296.

Del Vesco, A. P., Eliane Gasparino a, A. R. O. Neto, R. M.Rossi, M. A. M.Soares, S. C. C. Silva, 2013. Effect of methionine supplementation on mitochondrial genes expression in the breast muscle and liver of broilers. Livest. Sci. 151: 283-291.

Emmert, J. L., H. M. Edwards, and D. H. Baker, 2000. Protein and body weight accretion of chicks on diets with widely varying contents of soyabean meal supplemented or unsupplemented with its limiting amino acids. Br. Poult. Sci. 41: 204-213.

Gongruttananun, N. and R. Chotesangasa, 1996. A study of growth and carcass yield of Betong chickens compared with those of Native and crossbred Betong x Native chickens. Kasetsart J. (Nat. Sci.), 30: 312-321.

Iyayi, E. A., S. F. A. Aderemi, O. O. Ladele and A. Popoola, 2014. Effects of low protein diets supplemented with high amino acids methionine or lysine on Performance of broilers. Am. J. of Exper. Agri. 4(5): 525-531,

Nguyen,V.T. and C. Bunchasak, 2005. Effect of dietary protein and energy on growth performance and carcass characteristics of Betong chicken at early growth stage. Songklanakarin J. Sci. Technol., 27: 1171-1178.

Nguyen, V.T., C. Banchasak and S. Chantsavang. 2010. Effects of dietary protein and energy on growth performance and carcass characteristics of betong chickens (Gallus domesticus) during growing period. J. Poult. Sci. 9(5): 468-472

Ojano-Dirain C, M. Toyomizu, T. Wing, M. Cooper, 2007. Gene expression in breast muscle and duodenum from low and high feed efficient broilers. Poult. Sci. 86: 372-381.

Ospina-Rojas, I. C., A. E. Murakami, C. R. A. Duarte, C. Eyng, C. A. L. Oliveira and V. Janeiro, 2014. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. British Poul. Sci. 55(6): 766–773.

Putsakul, A., Bunchasak, C., Chomtee, B., Kao-ian, S. and Sopannarath, P. 2010. Effect of dietary protein and metabolizable energy levels on growth and carcass yields in betong chicken (KU Line). The 48th of Kasetsart University Annual Conference. 3-5 Feb 2010, Bangkok, Thailand: 158-166.

Tesseraud, S., S. Coustard-Metayer, S. Boussaid, S. Crochet, E. Audouin, M. Derouet and I. Seiliez, 2007. Insulin and amino acid availability regulate atrogin-1 in avian QT6 cells.

Biochemical and Biophysical Research Communications, 357: 181–186.

SAS. 1996. SAS Procedures Guides, Release 6.3 Edition. SAS Institute Inc., Cary, North Carolina.

Vieira A. A., E. Colombari, L.A. De Luca Jr., D. S. A. Colombari and J. V. Menani. 2004. Cardiovascular responses to microinjection of L-glutamate into the NTS in AV3V-lesioned rats. Brian. Res. 1025: 106-120.

Zheng, Q., Y. Zhang, Y. Chen, N. Yang, W. Xiu-jie and D. Zhu, 2009. Systematic identification of genes involved in divergent skeletal muscle growth rates of broiler and layer chickens. BMC Genomics, 10-87.

O-06-4

Effect of Low Protein Amino Acid-Supplemented Diets on Growth Performance and Carcass Characteristics in Betong Chicken (KU Line)

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INTRODUCTION

The Betong chicken is a meat type strain, which is popular in the Southern region of Thailand. The Betong is considered to produce high quality meat that is softer and tastes better than other Thai native chicks (low carcass fat and high lean meat), but it is not as flabby as broiler's meat (Gongruttananun and Chotesangasa, 1996).

Previous studies have reported that Betong chickens (KU line) required 17% CP diets and an energy level of 3,000-3,200 ME Kcal/kg, while high levels of dietary protein (23, 21 and 19% CP) did not alter growth performance at 0-42 days of age (Nguyen and Bunchasak, 2005). Putsakul (2010) also reported that suitable protein and energy levels of chickens were 18% CP and 3,000 ME Kcal/kg at 4-16 weeks of age and 16% CP and 2,800 ME Kcal/kg at 16-20 weeks of age, but female chickens required 18% CP and 2,800 ME Kcal/kg at 4-16 weeks of age. The data showed the analysis of investment and return of chicken 0-20 weeks of age to gain final body weight 1,700-2,400 kg, founding higher than broiler chickens.

One concept regarding the financial investment is the reduction of feed cost with a low protein diet, which represents more than 70% of the total production cost. However, reducing the protein concentration in diets always decreases growth performance and breast muscle proportion and increases body fat accumulation because of amino acid imbalances. Thus, amino acid supplementation in a low protein diet is more effective at improving amino acid balance than is supplementation in high protein diets due to economic, physiological or environmental reasons (Emmert et al., 2000; Vieira et al., 2004). The effects of insufficient amino acids cause imbalanced amino acids in the blood and reduces feed intake (NRC, 1994). The body weight and growth performance decreased as a result. Low protein diets have been known to increase the deposition of abdominal fat in chickens (Aletor et al., 2000). Thus, low dietary protein leads to decreased body weight gain in animals, which is clearly undesirable in animal husbandry. However, supplementation with essential amino acids (EAA) may restore performance (Kobayashi et al., 2013). Currently, there is limited information on the nutrient requirements (protein and amino acids) of Betong chickens. The objective of this study was to investigate the protein and amino acid requirements of Betong chickens at 4-12 weeks of age for utilisation of feed, high growth performance and carcass characteristics.

MATERIALS AND METHODS

Animals and management

Day-old Betong (KU line) chicks from the same hatch were conventionally raised for four weeks under the same conditions (with the use of a basal diet, 18% CP). Feed and water were provided *ad libitum*. The male and female birds were distributed to the treatments according to a completely randomised block design (CRBD) design (four replicates of 16 birds per treatments).

Experimental diets

The experimental diets were based on corn and soybean meal, and were formulated according to the recommendations of the National Research Council (1994). The treatments consisted of a basal diet (18% CP), low protein diets (16% CP) and low protein with amino acids supplement (16% CP+ Met), as shown in Table 1.

Data collection and sampling

In order to determine weight gain, all birds were weighed at the beginning, every two weeks and at the end of the experimental period. Feed intake was recorded, followed by the feed conversion ratio and feed efficiency. At the end of the experiment, two chickens from each replicate were selected in order to determine the carcass quality according to the following: carcass weight, breast muscle, inner breast weight, abdominal fat and drumstick.

Statistical analysis

All of the data were subjected to analysis of variances using the general linear model (GLM) procedure in the

Statistical Analysis System (SAS: version 9; SAS Institute Inc., Cary, NC, USA). The means of variables were compared according to Duncan's multiple range test when significant differences were observed. The level of significance was established at $p<0.05$.

RESULTS AND DISCUSSION

The effects of protein levels on growth performance and feed efficiency of the Betong chicks are shown in Table 2. At 4-12 weeks of age, the growth performance and feed intake of male Betong chickens were significantly higher than those of female Betong chickens ($p<0.05$), which is the same asPutsakul's (2010) findings.

Some growth performance indicators, body weight, body weight gain and average daily gain, did not show a significant difference among the diets (dietary CP 18%, CP 16% and CP 16%+Met). These results were similar to those studied by Putsakul (2010), reported that there were non-significantly differences in growth performance among the effects of protein level (16%, 18% and 20%). Our results were different from the report by Kobayashi et al. (2013) reported that body weight gain of the low protein diet group was lower than that of the control group in broilers.

The feed intake of the CP 16% diet group (3,541.20 g) in Betong chickens was higher than was the feed intake of CP 18% and CP 16% + Met (2,907.84 and 2,997.69 g, respectively). Aletor et al. (2000) reported that broilers fed with a low protein level (CP 15%) combined with a low protein diet group had a higher feed intake compared to broilers fed with a high-protein diet (CP 22%). The results in this study are contrary to Putsakul's (2010), found that there were non-significant differences in feed intake among the various levels of protein in the diets (16, 18 and 20%) in Betong chicken. Feeding with CP 16% resulted in a high feed intake due to the protein intake of CP 16% being higher than in other groups ($p<0.05$). The value of methionine intake in the CP 16% diet was equal with the value of methionine intake in the CP 18% diet (9.31 g). According to the previous studies, Urdaneta and Leeson (2004) found that the expected protein deposition and growth rate are associated with an optimum requirement of crude protein and amino acids. The feed conversion ratio of those fed the CP18% (3.03) and CP 16% + Met (3.21) diets were presented as being non-significantly different but greater ($p<0.05$) compared to the feed conversion ratio of CP 16% (4.21). Low protein diets had a high feed intake, and therefore, low protein diets had high FCR. The observation was in agreement with Nguyen and Bunchasak (2010) found t that the protein level (15-21%) found in the high protein level diets (21%) group had a FCR that was greater than that found in the low protein level diet (15%) group in Betong chickens at 12 week of age. This was similar to the findings Putsakul (2010) reported. From these results, it can be concluded that under an amino acid imbalance, chickens lose the potential to adjust feed intake to satisfy their amino acid requirements (Bunchasak and Keawarun, 2006).

At the end of the experiment, two birds per each replicate group were randomly selected and slaughtered to record the data on carcass yield, breast meat yield, abdominal fat, and composition of breast meat. The results are shown in Table 3. The carcass in all groups (diets and sex) were not significantly different. In addition, the percentage of breast muscle of male and female chickens was also not significant, while the percentage of breast muscle of chickens fed with CP 16% diets (7.74%) and CP 16% + Met diets (8.71%) was significantly lower compared to those fed with CP 18% (9.14%; $p<0.05$). This is contradictory to Nguyen and Bunchasak's (2010) evaluation of the effects of different protein levels (15%-19%), as they presented no significant difference in breast muscle in Betong chickens.

This study demonstrated that the percentage of abdominal fat in male chickens was 2.44%, which is significantly lower compared to female chickens, which had 2.90% ($p<0.05$), In addition, the effect of the diet showed that those fed with CP 16% + Met (2.12%) were significantly lower compared to those fed with CP 18% diets (2.69%) and CP 16% diets (3.21%; $p<0.05$). According to Nguyen and Bunchasak (2010), abdominal fat of chicks fed with lower levels of dietary was significantly increased, but using a low protein diet supplemented with amino acids may be able to reduce abdominal fat deposition (Aletor et al., 2000). The wings and drumstick were not significant in any group.

CONCLUSION

Betong chickens (KU-Line) fed with low protein diets supplemented with methionine significantly improved growth performance and decreased abdominal fat weight. With nutrition in formulated diets having the optimal balance of essential amino acids, it is expected that there will be a higher growth performance that yields a carcass with more edible meat and less fat.

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KEYWORD : Betong chicken, Low Protein, Amino acid, Performance, Carcass

Table 1 Composition and nutrient levels in diets

Composition (%)		Experimental diets		
		CP 18%	CP 16%	CP16% + Met
Corn Thai		59.5	66.7	66.8
Oil		6.00	4.81	4.75
Soybean meal (CP 46%)		30.4	24.1	23.9
L-Lysine		0.00	0.19	0.20
DL-Methionine		0.03	0.00	0.09
Monocalcium phosphate		1.21	1.20	1.20
Calcium carbonate		1.93	1.98	1.98
Sodium bicarbonate		0.25	0.50	0.50
Salt		0.43	0.30	0.30
Premix[1]		0.25	0.25	0.25
Total		100	100	100
Nutrients calculated				
Metabolizable energy	(Kcal/Kg)	3,000	3,000	3,000
Protein	%	18.00	16.00	16.00
Lysine	%	0.95	0.95	0.95
Methionine + Cysteine	%	0.61	0.52	0.61
Methionine	%	0.32	0.26	0.35
Calcium	%	1.06	1.05	1.06
Available Phosphorus	%	0.45	0.45	0.45
Sodium	%	0.25	0.27	0.27

Vitamin & mineral premix content; Composition per kg: Vitamin A 4,800 IU, Vitamin D3 1,200 IU, Vitamin E 60 IU, Vitamin K3 0.6 g., Vitamin B1 0.6 g, Vitamin B2 2.2 g, Vitamin B6 0.8 g, Vitamin B12 0.04 g, Nicotinic acid 10 g, Pantothenic acid 4.8 g, Folic acid 0.2 g, Biotin,0.048 g, Mn 32 g, Zn 24 g, Fe 16 g, Cu, 32 g, I 0.2 g, Se 0.04 g, Co 0.04 g

Table 2 Effect of additional Methionine in the low CP diet on the performance of Belong chickens (KU line) at 4-12 weeks of age.

Items	Body weight (g)	Body weight gain (g)	Average daily gain (g)	Feed intake (g)	Protein intake (g)	Methionine intake (g)	Feed conversion ratio
Diets							
18% CP	1,309.23	982.55	17.86	2,907.84^{a}	523.41^{b}	9.31^{b}	3.03^{a}
16% CP	1,197.30	874.85	15.91	3,541.20^{b}	566.59^{a}	9.31^{b}	4.21^{b}
16% CP + Met	1,283.04	954.99	17.36	2,997.69^{a}	479.63^{c}	10.52^{a}	3.21^{a}
p-value	0.19	0.21	0.21	<0.05	<0.05	<0.05	<0.05
Sex							
Female	1,103.40^{a}	803.93^{a}	14.62^{a}	2,779.53^{a}	461.96^{b}	8.57^{b}	3.63^{a}
Male	1,422.98^{b}	1,070.90^{b}	19.47^{b}	3,518.28^{b}	584.47^{a}	10.85^{a}	3.34^{b}
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
SEM	41.98	37.50	0.68	101.03	15.59	0.28	0.15

$^{a, b,c}$ within each column, means with different superscript letters are different ($p<0.05$).

Table 3 Observed means on carcass yield characteristics of Belong chickens (KU line) at 12 weeks of age.

Items	Carcass (%)	Breast muscle (%)	Abdominal fat (%)	Wings (%)	Drumstick (%)
Diets					
18% CP	93.40	9.14^{a}	2.69^{b}	9.28	21.39
16% CP	88.42	7.74^{b}	3.21^{a}	8.97	19.65
16% CP + Met	90.41	8.71^{b}	2.12^{c}	9.22	20.28
p-value	0.49	<0.05	<0.05	0.79	0.46
Sex					
Female	89.63	8.86	2.90^{a}	9.16	19.66
Male	91.85	8.20	2.44^{b}	9.16	21.22
p-value	0.52	0.21	<0.05	0.99	0.18
SEM	1.65	0.27	0.12	0.19	0.57

$^{a, b,c}$ within each column, means with different superscript letters are different ($p<0.05$).

REFERENCES

Aletor VA, Hamid I, Neib E, Pfeffer E. 2000. Low-protein amino acid supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole body composition and efficiency of nutrient utilization. J. Sci. Food Agric. 80: 547-554.

Bunchasak, C. and Keawarun, N. (2006). Effect of methionine hydroxy analog - free acid on growth performance and chemical composition of liver of broiler chicks fed a corn– soybean based diet from 0 to 6 weeks of age. Anim. Sci. J. 77: 95-102.

Emmert, J. L., H. M. Edwards, and D. H. Baker. 2000. Protein and body weight accretion of chicks on diets with widely varying contents of soyabean meal supplemented or unsupplemented with its limiting amino acids. Br. Poult. Sci. 41: 204-213.

Gongruttananun, N. and R. Chotesangasa, 1996. A study of growth and carcass yield of Betong chickens compared with those of Native and crossbred Betong x Native chickens. Kasetsart J. (Nat. Sci.), 30: 312-321.in Betong Chicken (KU Line) Kasetsart University conference 48th , Bangkok, Thailand.

Kobayashi H., N. Kazuki, I. Aiko, A. Akane A and K. Masaya. 2013. Effects of low protein diet and low protein diet supplemented with synthetic essential amino acids on meat quality of broiler chickens. Animal Science J. 84,489–495.

National Research Council. 1994. Nutrition Requirement of Poultry.9thed. National Academy of science, Washington D.C

Nguyen, T.V., C. Banchasak and S. Chantsavang. 2010. Effects of Dietary Protein and Energy on Growth Performance and Carcass Characteristics of Betong Chickens (Gallus domesticus) During Growing Period. J. Poult. Sci. 9(5): 468-472

Nguyen,V.T. and C. Bunchasak, 2005. Effect of dietary protein and energy on growth performance and carcass characteristics of Betong chicken at early growth stage. Songklanakarin J. Sci. Technol., 27: 1171-1178.

Putsakul, A., Bunchasak, C., Chomtee, B., Kao-ian, S. and Sopannarath, P. 2010. Effect of Dietary Protein and Metabolizable Energy levels on Growth and Carcass Yields

SAS. 2004. SAS Procedures Guides, Release 9 Edition. SAS Institute Inc., Cary, North Carolina.

Urdaneta-Rincon M, Leeson S. Muscle. 2004. Protein turnover in young broiler chickens fed graded levels of lysine and crude protein. J. Poult. Sci. .;83:1897-1903.

Vieira A. A., E. Colombari, L.A. De Luca Jr., D. S. A. Colombari and J. V. Menani. 2004. Cardiovascular responses to microinjection of L-glutamate into the NTS in AV3V-lesioned rats. Brian Res 1025: 106-120.

O-06-6

Effect of Coconut Oil Supplementation in Diet on Performance, Carcass Quality and Coccidia Control in Broilers

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ABSTRACT: The present study determined the effect of coconut oil supplementation in diet on coccidia control, performance and carcass quality. A total of 360, seven-day-old, mixed-gender of Cobb 500 commercial chicks fed in a pen (4.20 m^2). Feed was divided into two periods according to age i.e., 7-21 (starter phase) and 22-54 days (grower phase). Chicks were randomly allotted in completely randomized design into 5 dietary treatments with 3 replications of 24 chicks. The experiment diets were five groups: 1) control diet; 1% soybean oil (T1), 2) control diet supplemented with 0.05% salinomycin (T2), 3) 1% coconut oil diet (T3), 4) 2% coconut oil diet (T4) and 5) 3% coconut oil diet (T5) respectively. The results showed that during 7-21 days chicks received T4 (1.32) and T5 (1.36) had feed conversion ratio (FCR) lower than T1, T2 and T3 (1.43, 1.49 and 1.58, respectively) ($p<0.05$). During 22-54 days chicks received T2 had feed intake (FI) lower than other groups (2.88 kg/chick/day) ($p<0.05$). Percentage of abdominal fat in chicks fed with T1, T3 and T4 were 0.23, 0.27 and 0.23%, respectively which was lower than T5 (0.58%) and T2 (0.83%) ($p<0.05$). However, the supplementation of coconut oil did not have effect a number of coccidian oocysts ($p>0.05$). In conclusion, the finding indicated that supplementation of 2% coconut oil in broilers diet might be improved performance and seems to be mediated through a changed quality of carcass. Further study needs to be confirmed.

INTRODUCTION

Coccidiosis is protozoan parasites of the genus *Eimeria* that causes a disease of livestock (Chapman et al., 2013). In poultry, outbreaks of coccidiosis characterize high mortality are uncommon but its affect a poor production performance. It is also a predisposing factor for diseases which bring about to enteritis and diarrhea, and may cause significant flock survivor rate (Damer and Fiona, 2014). Thus coccidiosis has an important economic impact on the poultry industry with total estimated annual global losses of about $2.4 billion (Blake and Tomley, 2014).

To control coccidiosis, a number of natural substances have been applied in broilers diet (Quiroz-castañeda and Dantán-gonzález, 2015). Coconut oil is a one of natural substance that may enhance growth performance and control an infection of accidiosis in broilers. The coconut oil consists of lauric acid which comprises about 53% of the fatty acid composition (Faciola and Broderick, 2014). The lauric acid is partly independent of the carnitine transport mechanism into the mitochondria of the liver, and it is rapidly and exclusively oxidized for the production of energy (Rubin et al., 2000). The information suggested that an absorption of lauric acid is governed by physico-chemical properties of both the acid and the bacterial cell surface without any effect on production performance (Galbraith and Miller, 1973). Lauric acid has also been reported to kill viruses and bacteria that are enveloped in a phospholipid membrane. Machmuller et al. (2003) reported lauric acid in coconut oil reduced the average cell concentration of bacteria and ciliate protozoa in the rumen and it could destroy bacteria and protozoa by protozoal population decreased by 68-75% (Kongmun et al., 2011).

However, no information about utilizing coconut oil to control coccidiosis is available in the broilers. Thus, the study was focused on using of coconut oils as an alternative natural substance that may enhance growth performance and control coccidiosis in broilers. If it is compromising, the finding may be used to optimize growth performance, carcass and capability to control coccidiosis in broilers industry.

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MATERIALS AND METHODS

Experimental design

A total of 360, seven-day-old (average weight 137.45 g), mixed-gender of Cobb 500 commercial chicks (CPF (Thailand) Public Co., Ltd.) fed in a pen (4.20 m^2). Chicks were randomly allotted in completely randomized design into 5 treatments with 3 replications. Each treatment consists of 24 chicks maintained in open-sided houses.

Experimental diets

Chicks were randomly allotted to 1 of 5 treatments and raised for 54 d. Feeding was divided into two periods according to age i.e., 7-21 (starter phase) and 22-54 days (grower phase). The diets were based on corn and soybean meal and were supplemented with coconut oil (i.e., 1%, 2%, and 3%). The experiment diets were 5 groups: group 1 control diet (T1), group 2 control diet supplemented with 0.05% salinomycin (T2), group 3 dietary supplemented with 1% coconut (T3), group 4 dietary supplemented with 2% coconut (T4) and group 5 dietary supplemented with 3% coconut (T5), respectively (table 1). The diets were formulated to meet or exceed the nutrient requirements of broiler chickens (NRC, 1994). Feed and water were provided *ad libitum*.

Slaughter and Sample collection

Bird weight and feed intake were recorded on d 21 and 54. These values were used to calculate body weight gain, average daily gain, feed intake and feed conversion ratio for the periods of d 7 to 21, d 22 to 54, and the overall experiment. At the end of the experiment, 54-day-old, randomly chosen birds from each treatment (N=6) were slaughtered to evaluate carcass quality and examination of coccidiosis infection was performed by Mcmaster method (Haug et al., 2006). Sampling and analysis of *E. coli* population (AOAC, 2012) and *Salmonella* spp. (ISO, 2002) was performed by The Central Laboratory (Thailand) Co., Ltd.

Statistical analysis

Data was analyzed by analysis of the variance (ANOVA) based on a completely randomized design. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steeland Torrie, 1980). Comparison among treatments was tested by orthogonal contrast and orthogonal polynomial contrasts were used to test for linear and quadratic effect of level coconut oil 1%, 2% and 3%. Significance was considered at p

RESULTS

Performance

Feed conversion ratio in the starter phases was significant ($p<0.05$), T4 was lowest of feed conversion ratio as compared to the other groups. Grower phases, feed intake was significantly different among the treatment ($p<0.05$), feed intake of T2 was lower than that of every treatment. Meanwhile, supplementation coconut oil in diet was had no effect ($p>0.05$) on average daily gain that showed on table 2.

Carcass

Abdominal fat percentage was significantly different among the treatments ($p<0.01$). The abdominal fat percentage of every treatment was lower than that of the group received 0.05% salinomycin supplement. On d 54, Abdominal fat was linearly ($p<0.05$) decreased as the level of coconut oil increased (1% and 2%) and contrast T1 vs T2,T3,T4,T5 was significantly different among the group ($p = 0.05$). Dressing percentage, breast weight/ eviscerated weight, drumstick weight/eviscerated weight, hip weight/eviscerated weight and wing weight/ eviscerated weight were not affected by supplementation of coconut oil ($p>0.05$) showed on table 3.

Coccidia control

At the end of experiment, d 54, examination of small intestines by counting coccidia oocysts were performed and the results are shown in table 4. Results in table 4 found that effect of coconut oil supplementation in diet was had no effect ($p>0.05$) on coccidiosis control. For detection of intestinal microbes, *E. coli* count was not significantly different and *Salmonella* spp. was not detect.

DISCUSSION

The finding chick performance was affected by feeding on supplementation of coconut oil in diet. There is literature regarding the effect of lauric acid on broiler production. Feed conversion ratio in the starter phases (7-21 d) were significant ($p<0.05$) compliance with Zeitz et al. (2015) found that chick received dietary fats rich in lauric and myristic acid 50% FCR is lower than those who do not supplement ($p<0.05$). In grower phases (22-54 d) has shown chicks received T2 had feed intake (FI) lower than other groups ($p<0.05$) is consistent with reports of Chichlowski et al. (2007) studied the effect of Direct-Fed Microbial (DFM) PrimaLac and salinomycin (SAL) in 18-day-old chicks and found that SAL had feed intake less than the control group compare with enhance PrimaLac

groups. It should also be noted that differences in a broad array of parameters including feed intake were adversely affected by the feeding of salinomycin. It exerts its action by its ability to insert itself into membranes, thereby increasing the intracellular flux of K+ and other cations such as Na+ Ionophores, like salinomycin, do not discriminate between bacterial and mammalian membranes to affect cellular ion transport capacity.

Coconut oil has 62% of medium chain fatty acids (MCFAs) such as lauric acid, having 6-12 carbons atoms, considered to be small molecule as compared to all other oils which consist of long chain fatty acids (LCFAs). When ingested and absorbed into the body. It can be digested in small intestine without using the bile from liver to aid the digestion. MCFAs are directly converted into energy in the liver. As a result, it generates deposit in blood vessels and fat adipose tissue. (Wang et al., 2015).

Early studies on the antimicrobial activity of fatty acids distinguish lauric acid as the most active among the saturated fatty acids in the coccidiosis control. (Fabian, 2015). Lauric acid (C12:0) was most potent, particularly in its monoglyceride form (monolaurin), dilaurin and trilaurin (di and triglycerides) had no activity (Dayrit, 2000). Monolaurin can dissolve in fat and phospholipids (Isaacs et al., 1992), which is a component of the cell walls of protozoa. They were also active against lipid coated viruses as well as fungi and protozoa (Fabian, 2015). However, this study also showed that coconut oil had not effect to *E. coli* and *Samonelola* spp. Consist with Dayrit (2000) reported MCFAs possessed significant activity against gram positive bacteria, but not against gram negatives.

In conclusion, coconut oil supplementation in diet cannot decrease the number of coccidia oocyst count. When used in conjunction coconut oil as an additive in terms of growth, 2% coconut oil is probably appropriate for increase performance and quality of carcass (reduction of abdominal fat) in broilers.

ACKNOWLEDGMENTS

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KEYWORD : Coconut Oil, Performance, Carcass, Coccidia, Broiler

Table 1. Ingredient composition and nutrient content of diets.

Ingredients	Starter diet (7-21d)					Grower diet (22-54d)				
	T1[1]	T2	T3	T4	T5	T1	T2	T3	T4	T5
Coconut oil	-	-	1.00	2.00	3.00	-	-	1.00	2.00	3.00
Soybean oil	1.00	1.00	-	-	-	1.00	1.00	-	-	-
Corn (8% CP)	47.00	47.00	47.00	46.00	45.00	53.00	53.00	53.00	52.00	51.00
Soybean meal (44% CP)	26.80	26.80	26.80	27.70	28.50	20.50	20.50	20.50	21.40	22.20
Full fat soybean meal (36% CP)	20.20	20.20	20.20	19.30	18.50	20.00	20.00	20.00	19.10	28.30
Monodicalcium phosphorus (22% P)	2.40	2.40	2.40	2.40	2.40	2.80	2.80	2.80	2.80	2.80
Limestone	1.14	1.14	1.14	1.14	1.14	1.26	1.26	1.26	1.26	1.26
Sodium choride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Premixed[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.16	0.16	0.16	0.16	0.16	0.22	0.22	0.22	0.22	0.22
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.22	0.22	0.22	0.22	0.22
Choline choride (60 %)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salinomycin 50 (g/100kg)	-	0.05	-	-	-	-	0.05	-	-	-
Total	100.00	100.05	100.00	100.00	100.00	100.00	100.05	100.00	100.00	100.00
Nutrient by calculated										
CP, %	23.00	23.00	23.00	23.00	23.00	20.56	20.56	20.56	20.56	20.56
ME, kcal/kg	3068.65	3068.65	3068.65	3121.49	3174.73	3035.56	3035.56	3035.56	3088.50	3141.74

[1] T1 = control diet; T2 = control diet supplemented with 0.05% salinomycin; T3 = dietary supplemented with 1% coconut oil; T4 = dietary supplemented with 2% coconut oil; T5 = dietary supplemented with 3% coconut oil.

[2] Premixed = Vitamin A : 2,000,000 I.U., Vitamin D3 : 400,00 I.U., Vitamin E : 4,00 I.U., Vitamin K : 400 mg, Vitamin B1 : 200 mg, Vitamin B2 : 1,000 mg, Vitamin B6 : 600 mg, Vitamin B12 : 4,000 µg, Biotin : 5,000 µg, Folic acid : 200 mg, Nicotinic acid : 7,000 mg, Pantotenic : 2,000 mg, Choline choride : 70,000 mg, Cu : 2,000 mg, Mg : 20,00 mg, Zn : 20,000 mg, Fe : 6,000 mg, Iodine : 200 mg, Selenium : 20 mg, Cobol : 100 mg.

Table 2. Effect of dietary source on performance of broilers[1].

Items	Treatment					SEM	ANOVA	p-value			
								Contrast		Polynomial	
	T1[2]	T2	T3	T4	T5			T1 vs T2,T3,T4,T5	T2 vs T3,T4,T5	Linear	Quadratic
Starter phase (d 7 to 21)											
Average dailygain (g/d)	31.01	31.69	26.05	30.98	29.15	1.02	0.26	0.47	0.51	0.29	0.20
Feed intake (g/d)	618.79	659.32	576.66	568.70	554.77	19.17	0.07	0.33	0.57	0.57	0.93
Feed conversion ratio	1.43 ^{ab}	1.49 ^{ab}	1.58 ^{a}	1.32 ^{b}	1.37 ^{b}	0.05	0.04	0.88	0.61	0.04	0.07
Grower phase (d 22 to 55)											
Average dailygain (g/d)	51.79	50.21	54.95	53.18	52.86	0.54	0.87	0.78	0.79	0.68	0.87
Feed intake (g/d)	3051.71^{a}	2881.01^{b}	3099.67^{a}	3109.80^{a}	3125.04^{a}	53.83	0.05	0.97	0.73	0.77	0.97
Feed conversion ratio	1.80	1.75	1.73	1.78	1.81	0.02	0.99	0.83	0.76	0.64	0.94
Overall phase (d 7 to 55)											
Average dailygain (g/d)	43.74	42.87	44.45	44.67	43.93	0.31	0.97	0.92	0.91	0.86	0.85
Feed intake (g/d)	3645.64	3578.95	3513.37	3499.71	3664.60	33.42	0.76	0.52	0.39	0.46	0.61
Feed conversion ratio	1.71	1.71	1.63	1.60	1.72	0.02	0.92	0.76	0.67	0.63	0.68

SEM, standard error of the mean; ANOVA, analysis of variance.
[1] Values are the means of 3 replicates of 24 chicks.
[2] T1 = control diet; T2 = control diet supplemented with 0.05% salinomycin; T3 = dietary supplemented with 1% coconut oil; T4 = dietary supplemented with 2% coconut oil; T5 = dietary supplemented with 3% coconut oil.

Table 3. Effect of dietary source on carcass quality.

Items	Treatment					SEM	ANOVA	p-value			
								Contrast		Polynomial	
	T1[1]	T2	T3	T4	T5			T1 vs T2,T3,T4,T5	T2 vs T3,T4,T5	Linear	Quadratic
Dressing percentage (%)[2]	72.42	67.88	68.44	72.51	71.36	0.99	0.28	0.27	0.21	0.19	0.18
Abdominal fat percentage (%)	0.23^{c}	0.83^{a}	0.27^{bc}	0.23^{c}	0.58^{ab}	0.11	<0.01	0.05	0.10	0.02	0.06
Breast weight (%)	30.81	31.75	30.15	32.61	30.20	16.24	0.54	0.96	0.81	0.53	0.19
Hip weight (%)	11.58	13.68	12.57	12.62	12.83	6.03	0.09	0.18	0.12	0.68	0.21
Drumstick weight (%)	17.46	16.27	15.89	17.61	17.46	4.62	0.26	0.27	0.29	0.78	0.59
Wing weight (%)	14.97	13.42	15.26	14.36	14.76	7.98	0.31	0.39	0.48	0.35	0.47

SEM, standard error of the mean; ANOVA, analysis of variance.
[1] T1 = control diet; T2 = control diet supplemented with 0.05% salinomycin; T3 = dietary supplemented with 1% coconut oil; T4 = dietary supplemented with 2% coconut oil; T5 = dietary supplemented with 3% coconut oil.
[2] Dressing percentage is carcass weight (defeathered and eviscerated) as a percentage of body weight.

Table 4. Effect of dietary source on coccidia and bacteria control

Items	Treatment					SEM	ANOVA	p-value			
								Contrast		Polynomial	
	T1[1]	T2	T3	T4	T5			T1 vs T2,T3,T4,T5	T2 vs T3,T4,T5	Linear	Quadratic
Intestinal content[2]											
Duodenum	3	0	0	0	0	0.60	0.45	0.07	0.08	-	-
Jejunum	6	0	3	6	11	1.83	0.74	0.94	0.71	0.45	0.90
Ileum	32.33	3.00	36.00	23.00	62.67	9.35	0.72	0.95	0.73	0.61	0.59
Cecum	72	3	25	64	81	14.95	0.22	0.33	0.19	0.19	0.75
Intestinal Microbes[3]											
E.coli	0.26x10^{4}	7.07x10^{4}	10.70x10^{4}	1.25x10^{4}	1.04x10^{4}	2.06	0.24	0.24	0.16	0.41	0.98
Salmonella	-	-	-	-	-	-	-	-	-	-	-

SEM, standard error of the mean
[1] T1 = control diet; T2 = control diet supplemented with 0.05% salinomycin; T3 = dietary supplemented with 1% coconut oil; T4 = dietary supplemented with 2% coconut oil; T5 = dietary supplemented with 3% coconut oil.
[2] Unit of coccidia oocysts = oocysts per gram.
[3] Unit of Intestinal Microbes = CFU/g

REFERENCES

AOAC. 2012. International Microbiological Method Committee. In, Official Methods of Analysis of AOAC International 19th Edition. (ed) Latimer, G.W. Jr). 17: 1–279.

Blake, D.P. and F.M. Tomley. 2014. Securing poultry production from the ever-present Eimeria challenge. Trends in Parasitology. 30(1): 12–19.

Bowman, D.D. and R.C. Lynn. 1999. Georgis' Parasitology for Veterinarians. 7th edition. W.B. Saunders Company. Philadelphia. U.S.A. 414 pp.

Chapman, H., R. John, D. Blake, A. Gruber, M. Jenkins, C. Smith, X. Suo and M. Tomley. 2013. A selective review of advances in coccidiosis research. J. Advances in Parasitology. 83: 93–171.

Chichlowski, M., J. Croom, B.W. McBride, L. Daniel, G. Davis and M.D. Koci. 2007. Direct-fed microbial PrimaLac and salinomycin modulate whole-body and intestinal oxygen consumption and intestinal mucosal cytokine production in the broiler chick. J. Poultry Science. 86(6): 1100–6.

Dalloul, R. A. and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. J. Expert Rev. Vaccines. 5: 143-163.

Damer, P. B. and M. T. Fiona. 2014. Securing poultry production from the ever-present *Eimeria* challenge. J. Trends in Parasitology. 30(1): 12-19.

Dayrit, C. S. 2000. Coconut Oil in Health and Disease: Its and Monolaurin's Potential as Cure for HIV/AIDS, XXXVII Cocotech Meeting, Chennai, India, July 25, 2000.

Fabian, M.D. 2015. The properties of lauric acid and their significance in coconut oil. J. Am. Oil. Chem. Soc. 92:1–15.

Faciola, A.P. and G.A. Broderick. 2014. Effects of feeding lauric acid or coconut oil on ruminal protozoa numbers, fermentation pattern, digestion, omasal nutrient flow, and milk production in dairy cows. J. Dairy Science. 97: 5088-5100.

Galbraith, H. and T. B. Miller.1973. Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. J. Appl. Bact. 36(4):647–658.

Isaacs, C. E., R. E. Litov, P. Marie and H. Thormar. 1992. Addition of lipases to infant formulas produces antiviral and antibacterial activity. J. Nutr. Biochem. 3:304-308.

ISO. 2002. Microbiology of food and animal feeding stuffs - horizontal method for the detection of Salmonella spp. Geneva: International Organization for Standardization.

Kongmun, P., M. Wanapat, P. Pakdee, C. Navanukraw and Z. Yu. 2011. Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. J. Livestock Science. 136: 84-92.

Machmuller, A., C. Soliva and M. Kreuzer. 2003. Effect of coconut oil and defaunation treatment on methanogenesis in sheep. J. Reproduction Nutrition Development, EDP Sciences. 43 (1): 41-55.

Quiroz-Castañeda, R. E. and E. Dantán-González. 2015. Control of avian coccidiosis: future and present natural alternatives. J. Bio. Med. Research International, Article ID 430610, 11 pages. From: http://dx.doi.org/10.1155/2015/430610.

Rubin, M., A. Moser, N. Vaserberg, F. Greig, Y. Levy, H. Spivak, Y. Ziv and S. Lelcuk. 2000. Long-chain fatty acids, in long-term home parenteral nutrition: A double-blind randomized cross-over study. J. Nutrition 16: 95-100.

SPSS. 1998. SPSS for Windows, Release 9.0. SPSS Inc., Chicago, IL. U.S.A.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometerial Approach, 2nd ed. McGraw-Hill, New York, U.S.A.

Wang, J., X. Wang, J. Li, Y. Chen, W. Yang, and L. Zhang. 2015. Effects of dietary coconut oil as a medium-chain fatty acid source on performance, carcass composition and serum lipids in male broilers. Asian Australasian J. Anim. Sci. 28: 223-230.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. J. Avian Pathol. 34:159–180.

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The Combination of Coconut Oil and Turmeric Powder Supplementation in Diets on Performance, Carcass and Coccidia Control in Broilers

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INTRODUCTION

Coccidiosis is a gastrointestinal diseases caused by Protozoa (*Eimeria* spp.) and found both in broilers and layer hens. Especially in broilers industry, coccidiosis is important in terms of causing productivity lost (Hady and Zaki, 2012). In the past, broiler production in Thailand usually used antibiotics for prevention and treatment of animals. Nevertheless, main customers of Thailand including European Union (EU) and Japan limited the use of antibiotics in food animals. So, there are many exporters who are trying to produce the antibiotic-free chickens (AFC) in order to meet customer requirements.

The current study used herbal or natural substances to control disease incidence and enhance growth performance in broilers. Turmeric (*Curcuma longa Linn*.) is herbal plant that is widely accepted in pharmaceutical industry. Its therapeutic properties are reported in both humans and animals. Turmeric contains an important component (curcuminoids and essential oil) that can increase growth rate, strengthen immune system, and improve carcass quality and normal flora conditions in digestive tract (Arshami, 2012; Kermanshahi and Riasi, 2006). Coconut oil is another natural substance containing saturated oil (about 90%), and total fatty acid, of which 60% of composition are medium-chain fatty acid (MCFA) that can destroy bacteria and protozoa (Kongmun et al., 2011)

However, no information about utilization of coconut oil and turmeric to control coccidiosis in broilers. Thus, use of coconut oil and turmeric in diet supplement as an antiprotozoal agent and their effects on broiler performance and carcass quality is explored in this study.

MATERIALS AND METHODS

Experimental design

A total of 432, seven-day-old (average weight 140.6 g), mixed-sex Cobb 500 commercial chicks were included in the experiment. Chicks were randomly allotted in completely randomized design into 6 treatments with 3 replications. Each treatment consisted of 24 chicks raised in a pen sized 4.20 m^2.

Experimental diets

Chicks were randomly allotted of 1 to 6 treatments and raised for 54 days. Feeding was divided into two periods according to age i.e., 7-21 (starter phase) and 22-54 days (grower phase). The diets were based on corn and soybean meal and were supplemented with 1%, 2%, or 3% coconut oil and 0.50% turmeric powder. Treatments were group 1 control diet (Con), group 2 control diet supplemented with 0.05% salinomycin (Con+ SC 0.05%), group 3 control diet supplemented with 0.50% turmeric powder (Con+TP 0.50%), group 4 dietary supplemented with 1% coconut oil and 0.50% turmeric powder (CO 1%+TP 0.50%), group 5 dietary supplemented with 2% coconut oil and 0.50% turmeric powder (CO 2%+TP 0.50%) and group 6 dietary supplemented with 3% coconut oil and 0.50% turmeric powder (CO 3%+TP 0.50%), respectively. The diets were formulated to meet or exceed the nutrient requirements of broiler chickens (NRC, 1994). Feed and water were provided *ad libitum*.

Slaughter and sample collection

Bird weight and feed intake were recorded on d 21 and 54. These values were used to calculate body weight gain, average daily gain, feed intake and feed conversion ratio for the periods of d 7 to 21, d 22 to 54, and the overall experiment. Randomly chosen birds from each treatment (N=6) were slaughtered to evaluate carcass quality and examination of coccidiosis infection was performed by Mcmaster method (Haug et al., 2006).

Statistical analysis

Analysis of Variance (ANOVA) was used for the statistical analysis. Multiple comparisons among treatment means were performed by Duncan\'s New Multiple Range Test (DMRT) (Steel and Torrie, 1980). Comparison among treatments was tested by orthogonal contrast (SPSS, 1998).

RESULTS

Performance

The 54-d feeding study showed that use of coconut oil with turmeric powder supplementation in diet had no effect ($p>0.05$) on average daily gain, feed intake or feed conversion ratio during the starter, grower and overall phases (Table 1).

Carcass quality

Abdominal fat percentage was significantly different among the treatments ($p<0.01$). The abdominal fat percentage of every treatment was lower than that of the group received 0.05% salinomycin supplement. Drumstick weight/eviscerated weight showed a significant difference among the groups ($p<0.05$). Dressing percentage, breast weight/eviscerated weight, hip weight/eviscerated weight and wing weight/eviscerated weight were not affected by supplementation of coconut oil and turmeric in diet ($p>0.05$).

Coccidia and bacterial infection control

At the end of experiment, d 54, examination of small intestines by counting coccidia oocysts were performed and the results are shown in table 3. No coccidia oocysts were detected from jejunum of broilers in group 2 (Con+SC), group 3 (Con+TP 0.50%) and group 4 (CO 1%+ TP 0.50%), while group 1 (Con), group 5 (CO 2%+TP 0.50%) and group 6 (CO 3%+TP 0.50%) showed coccidia oocyst count at the average of 5.56, 8.33 and 13.33 OPG, respectively, ($p<0.05$). Coccidia oocysts found in ileum at the lowest number (3.00 OPG) in group 2 (Con+SC), compared to other groups ($p<0.01$), while group 1 (Con), group 3 (Con+TP 0.50%), group 4 (CO 1%+TP 0.50%) group 5 (CO 2%+TP 0.50%) and group 6 (CO 3%+TP 0.50%) showed coccidia oocyst count at the average of 33.67, 41.67, 30.67, 31.33 and 83.33 OPG, respectively. The number of coccidia oocysts in duodenum and cecum are not different among the treatments ($P>0.05$). For detection of intestinal microbes, *E. coli* count was not significantly different and *Salmonella* spp. was not detect.

DISCUSSION

The results of this study indicated that bird performance was not affected by adding coconut oil and turmeric in dietary supplement. The results partially agreed with that of Wang et al. (2015), in which supplementation of coconut oil in a regular chick starter diet reduced feed intake and weight gain, but did not affect feed conversion.

The combination of coconut oil and turmeric supplementation in diets had a marked effect on some carcass characteristics. The abdominal fat percentage was decreased when compared with that of birds receiving only salinomycin in diet supplement. Che Man and Marina (2006) reported that coconut oil is rich in medium-chain fatty acids (MCFA) and exhibits good digestibility when ingested and absorbed into the body. It can be digested in small intestine without using the bile from liver to aid the digestion. Being sent directly to the liver, it generates deposit in blood vessels and fat adipose tissue. The group received 3% coconut oil had abdominal fat percentage higher than that of the other groups. Shinohara et al. (2005) explained that when broiler received a lot of coconut oil (3% in this study) in diet, the excess fatty acid will be stored as fat in the body more than normal. Meanwhile, curcuminoid in turmeric reduces the effect of acetyl-CoA carboxylase enzyme, which functions in the fat synthesis, resulting in abdominal fat decreased (Mehala and Moorth, 2008)

Effects of coconut oil in controlling coccidia infection are due to anti-protozoa effects of monolaurin, which is a glyceride ester of lauric acid contained in coconut oil. Isaacs et al. (1992) reported that coconut oil contains four MCFAs such as lauric acid (C-12, 48-53%) when into the body, they are transformed into corresponding monoglyceride, namely monolaurin, which is able to kill protozoa. Monolaurin can dissolve in lipid and phospholipids, that is a component of the cell membrane of protozoa (Dayrit, 2000). This is consistent with Enig (1996) who found that monolaurin deriving from lauric acid can be absorbed into the outer membrane of protozoa. The similarity of a fatty acid at cell membrane of protozoa then surrenders to the coconut oil, which, being lipid itself, could dissolve and break down, or lyze, the lipid coat of protozoa, thereby penetrating them and literally killing them (Chomchalow, 2011). Likewise, oil in the turmeric essential oil has small molecules and fat-

soluble that can penetrate and damage the cell membranes of protozoa resulting in moving of intracellular fluid to the outside (Boyom et al., 2003; Sarkozi et al., 2007) However, in this study, supplementation of 3% coconut oil with 0.50% turmeric could not decrease coccidia oocyst count in small intestine.

In conclusion, 1% coconut oil and turmeric supplementation in diet can decrease the number of coccidia oocyst count better than diets without supplementation. In terms of growth performance, 1% coconut oil and 0.05% turmeric is appropriate for controlling of coccidiosis and increase performance and quality of carcass (reduction of abdominal fat) in broilers.

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KEYWORD : Coconut Oil, Turmeric Powder, Performance, Carcass, Coccidia

Table 1. Effect of dietary supplement on performance of broilers.

Items	Treatment						SEM	p-value			
	T1[1]	T2	T3	T4	T5	T6		ANOVA	T1 vs T4,T5,T6	T2 vs T4,T5,T6	T3 vs T4,T5,T6
Starter phase (d 7 to 21)											
Average daily gain (g/d)	31.01	31.69	33.03	32.97	33.08	33.33	1.46	0.83	0.23	0.41	0.96
Feed intake (g/d)	44.20	47.09	47.30	46.81	45.72	49.84	1.47	0.23	0.08	0.84	0.93
Feed conversion ratio	1.43	1.49	1.43	1.43	1.38	1.50	0.05	0.63	0.91	0.39	0.95
Grower phase (d 22 to 54)											
Average daily gain (g/d)	53.41	51.78	54.35	57.69	48.23	46.16	2.82	0.14	0.43	0.75	0.30
Feed intake (g/d)	92.48	87.30	87.66	93.54	86.96	89.94	3.53	0.67	0.58	0.50	0.55
Feed conversion ratio	1.80	1.75	1.66	1.69	1.87	2.04	0.14	0.46	0.67	0.49	0.22
Overall phase (d 7 to 54)											
Average daily gain (g/d)	45.60	44.70	46.84	49.10	42.69	41.36	1.82	0.13	0.59	0.89	0.29
Feed intake (g/d)	74.40	73.04	75.17	75.29	73.22	71.50	4.03	0.98	0.82	0.95	0.70
Feed conversion ratio	1.71	1.71	1.67	1.61	1.79	1.81	0.12	0.83	0.83	0.88	0.64

SEM, standard error of the mean

[1]T1=control diet (Con), T2=control diet supplemented with 0.05% salinomycin (Con+SC 0.05%), T3= control diet supplemented with 0.50% turmeric powder (Con+TP 0.50%), T4= dietary supplemented with 1% coconut oil and 0.50% turmeric powder (CO 1%+TP 0.50%), T5= dietary supplemented with 2% coconut oil and 0.50% turmeric powder (CO 2%+TP 0.50%) and T6= dietary supplemented with 3% coconut oil and 0.50% turmeric powder (CO 3%+TP 0.50%)

Table 2. Effect of dietary supplement on carcass quality of broilers.

Item	Treatment						SEM	p-value			
	T1[1]	T2	T3	T4	T5	T6		ANOVA	T1 vs T4,T5,T6	T2 vs T4,T5,T6	T3 vs T4,T5,T6
Dressing percentage (%)[2]	72.42	67.88	70.65	70.83	70.10	69.32	2.06	0.61	0.27	0.30	0.79
Abdominal fat percentage (%)	0.23^{b}	0.83^{a}	0.24^{b}	0.21^{b}	0.17^{b}	0.48^{b}	0.14	<0.01	0.69	<0.01	0.75
Breast weight/eviscerated weight (%)	30.81	31.75	29.98	34.65	36.32	29.74	2.24	0.26	0.30	0.49	0.18
Hip weight/eviscerated weight (%)	17.72	17.46	17.74	19.90	21.59	18.11	1.09	0.09	0.11	0.07	0.11
Drumstick weight/eviscerated weight (%)	14.97^{abc}	13.42^{c}	15.30^{abc}	16.71^{ab}	17.25^{a}	14.22^{bc}	0.80	0.03	0.25	0.01	0.42
Wing weight/eviscerated weight (%)	11.58	14.48	13.13	14.72	16.03	13.88	1.01	0.09	0.01	0.73	0.14

SEM, standard error of the mean

[1]T1=control diet (Con), T2=control diet supplemented with 0.05% salinomycin (Con+SC 0.05%), T3= control diet supplemented with 0.50% turmeric powder (Con+TP 0.50%), T4= dietary supplemented with 1% coconut oil and 0.50% turmeric powder (CO 1%+TP 0.50%), T5= dietary supplemented with 2% coconut oil and 0.50% turmeric powder (CO 2%+TP 0.50%) and T6= dietary supplemented with 3% coconut oil and 0.50% turmeric powder (CO 3%+TP 0.50%)

[2] Dressing percentage is carcass weight (defeathered and eviscerated) as a percentage of body weight.

Table 3. Effect of dietary supplement on coccidia and bacteria control of broilers.

Intestinal content[2]	Treatment						SEM	p-value			
	T1[1]	T2	T3	T4	T5	T6		ANOVA	T1 vs T4,T5,T6	T2 vs T4,T5,T6	T3 vs T4,T5,T6
Duodenum	2.78	0.00	0.00	0.00	0.00	0.00	1.09	0.46	0.06	1.00	1.00
Jejunum	5.56^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	8.33^{ab}	13.33^{a}	3.04	0.04	0.60	0.06	0.06
Ileum	33.67^{bc}	3.00^{c}	41.67^{b}	30.67^{bc}	31.33^{bc}	83.33^{a}	9.60	<0.01	0.17	<0.01	0.55
Cecum	72.22	2.78	69.44	50.00	58.33	94.44	11.81	0.36	0.85	0.02	0.93
Intestinal Microbes[3]											
E. coli	$2.60x10^{3}$	$7.07x10^{3}$	$69.50x10^{3}$	$6.54x10^{3}$	$64.00x10^{3}$	$0.18x10^{3}$	2.56	0.53	0.43	0.38	0.23
Salmonella spp.	-	-	-	-	-	-	-	-	-	-	-

SEM, standard error of the mean

[1]T1=control diet (Con), T2=control diet supplemented with 0.05% salinomycin (Con+SC 0.05%), T3= control diet supplemented with 0.50% turmeric powder (Con+TP 0.50%), T4= dietary supplemented with 1% coconut oil and 0.50% turmeric powder (CO 1%+TP 0.50%), T5= dietary supplemented with 2% coconut oil and 0.50% turmeric powder (CO 2%+TP 0.50%) and T6= dietary supplemented with 3% coconut oil and 0.50% turmeric powder (CO 3%+TP 0.50%)

[2]Unit of coccidia oocysts=OPG; oocysts per 0.2 gram of intestinal tissue.

[3]Unit of Intestinal Microbes =CFU/ml

REFERENCES

Arshami, J., M. Pilevar, M. A. Azghadi and A. R. Raji. 2012. Hypolipidemic and antioxidative effects of curcumin on blood parameters, humoral immunity and jejunum histology in *Hy-line* hens. Avicenna Journal of Phytomedicine. 3: 178-185.

Boyom, F. F., V. Ngouana, P. H. Amvam Zollo, C. Menut, I. M. Bessiere, I. Gut and P. I. Rosenthal. 2003. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. J. Phytochemistry. 64: 1269-1275.

Che Man, Y. B. and A. M. Marina. 2006. Medium chain triacylglycerol. In F. Shahidi (Ed.), Nutraceutical and specialty lipids and their coproducts (pp. 27- 56). Boca Raton: Taylor & Francis Group

Chomchalow, N. 2011. Health and Economic Benefits of Coconut Oil Production Development in Thailand. AU J. T. 14(3): 181-187.

Dayrit, C. S. 2000. Coconut oil in health and disease: Its and monolaurin's potential as cure for HIV/AIDS, XXXVII cocotech meeting, Chennai, India, July 25, 2000.

Enig, M. G. 1996. Health and nutritional benefits from coconut oil: An Important Functional Food for the 21st Century, AVOC (ASEAN Vegetable Oils Club) Lauric Oils Symposium, Ho Chi Min, Vietnam, 25 April 1996.

Hady, M. M. and M. M. Zaki. 2012. Efficacy of some herbal feed additives on performance and control of cecal coccidiosis in broilers. J. APCBEE Procedia. 4: 163-168.

Haug, A., R. B. Williams and S. Larsen. 2006. Counting coccidial oocysts in chicken faeces: A comparative study of a standard McMaster technique and a new rapid method. J. Veterinary Parasitology. 136: 233–242

Isaacs, C. E., R. E. Litov, P. Marie and H. Thormar. 1992. Addition of lipases to infant formulas produces antiviral and antibacterial activity. J. Nutr. Biochem. 3: 304-308.

Kermanshahi, H. and A. Riasi. 2006. Effect of turmeric powder (*Curcuma longa*) and soluble NSP degrading enzyme on some blood parameters of laying hens. J. Poult. Sci. 5: 494-498.

Kongmun, P., M. Wanapat, P. Pakdee, C. Navanukraw and Z. Yu. 2011. Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. J. Livestock Science. 136: 84-92.

Mehala, C. and M. Moorthy. 2008. Effect of *Aloe vera* and *Curcuma longa* (turmeric) on carcass characteristics and biochemical parameters of broilers. Int. J. Poult. Sci. 7: 857-861.

Sarkozi, S., I. Almassy, B. Lukaes, N. Dobrosi, G. Nagy and I. Jona. 2007. Effect of natural phenol derivatives on skeletal type sarcoplasmic reticulum CA2+ ATPase and ryanodine receptor. J. Muscle Res Cell Motil. 28: 167-174.

Shinohara, H., A. Ogawa, M. Kasai and T. Aoyama. 2005. Effect of randomly interesterified triacylglycerols containing medium- and long-chain fatty acids on energy expenditure and hepatic fatty acid metabolism in rats. J. Biosci. Biotechnol. Biochem. 69: 1811-1818.

SPSS for Windows, Release 9.0. 1998. SPSS Inc., Chicago, IL

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometerial Approach, 2nd ed. McGraw-Hill, New York, U.S.A.

Wang, J., X. Wang, J. Li, Y. Chen, W. Yang and L. Zhang. 2015. Histopathological changes in the livers of broiler chicken supplemented with turmeric (*Curcuma longa*). Asian Australasian J. Anim. Sci. 28: 223-230.

O-06-8

Comparative Effect of Corn and Whole Wheat in Diets on Egg Production and Egg Quality of Laying Hens

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INTRODUCTION

Corn is widely cultivated and it is the major constituents of layer diets in Thailand. However, corn production decreases because of the long-term lack of water. Therefore, there appears to be a need for alternative grain sources for pullet and layer rations. Whole wheat makes an excellent replacement for corn in layer feed. Practically, grinding of wheat is probably the highest user of energy in layer feed production (Deaton et al, 1989). Therefore, whole grain feeding, through the reduction of energy consumption for grinding, could significantly lower the feed cost. Thus, whole grain feeding has recently received renewed interest in the commercial poultry industry as a mean of lowering feed manufacturing cost. Therefore, the present study attempts to investigate the effects of whole wheat level in diets of laying hens on egg production.

MATEIALS AND METHOD

Bird housing and management

A total of 192 forty five-week-old Lohmann Brown Classic layers were supplied from the experimental farm of Department of Animal Science, Kasetsart University, Thailand and randomly allocated to battery type wire cages. Four layers were placed in each of 4 consecutive cages with 6 replicates and assigned to receive one of the 2 dietary treatments. Battery cages were equipped with nipple drinkers. Each set of 4 consecutive cages formed one experimental unit in which 16 birds were housed. Laying hens were maintained in an environmentally controlled experimental house with air ventilation and window and received additional artificial light to provide 16 h light and 8 h dark daily. The feed was offered 2 times daily (8 am and 4 pm), all experimental diets were fed *ad libitum* to the laying hens and water was available all the time throughout the experimental period.

Experimental diets

The nutrient composition of experimental diets was performed according to the recommendation of Lohmann Brown Classic. Two experimental diets were formulated to have a different energy source by corn substation of whole wheat 25%. Feed ingredients were obtained from the local feed market and mixed through a horizontal mixer (200 kg capacity) in the feed mixing unit of the department. Experimental diets were formulated isocaloric and isonitrogenous to contain 16.32% CP and 2,725 kcal/kg AME (Table 1). The whole wheat diet was prepared by replacing the ground corn with whole grain wheat, performed according to the recommendation of Lohmann Brown Classic. Other feed ingredients prepared by a commercial feed mill in mash form.

Measurements

Egg production: The birds were weighed at the start (45 week of age) and end (53 week of age) of the trial. Feed intake was recorded weekly and egg production daily. Egg weight was measured daily by weighing all of the eggs collected from the experimental groups. Feed intake was calculated per replicate and period on the basis of the total feed provided minus the total feed leftover. Egg mass was calculated by multiplying egg weight by hen-day egg production percentage. Feed conversion rate was calculated as a gram of feed consumed per day per hen divided by gram egg mass per day per hen.

Egg quality and characteristic: At the end of the trial, all eggs from each replication were weighted, and four eggs from each replication that have weight close to the replication's mean were chosen to analyze egg qualities and characteristic including eggshell breaking strength (by Eggshell Force Gauge Model II, Robotmation Co., Ltd., Tokyo, Japan), eggshell thickness (by electronic digital micrometer), percentage of eggshell, percentage of albumen, percentage of yolk, albumen height (by tripod micrometer) and yolk color (by Roche yolk color fan). Haugh unit (HU) was calculated by the formula $HU = 100\log[H-1.7W^{0.37}+7.57]$ (Eisen et al., 1962) where W refers

to egg weight (g) and H refers to albumen height (mm).

Statistical analysis

T-test was used to compare measured values obtained from the two independent groups on egg production and egg quality. Statements of statistical significance are based on $P<0.05$.

RESULTS AND DISCUSSION

Egg production

Effect of whole wheat levels in diet on egg production of laying hens is presented in Table 3. The results indicate that the level of whole wheat diets did not significantly affect layer egg production. The hens fed diets with whole wheat had no affect on hen-day egg production, egg weights, egg mass, daily feed intake, and FCR compared to the birds in control diet ($P < 0.05$). Similarly, Ciftci et al. (2003) reported that whole wheat did not influence laying-hen performance when 30% corn was substituted by wheat from 27 to 43 weeks of age. Additionally, Lázaro et al. (2003a) and Safaa et al. (2009) reported no differences in productive performance when 50% corn was substituted by wheat in diets of hens from 20 to 44 wk of age. However, Kim et al. (1976) reported that hens fed a corn diet from 21 to 43 weeks of age had higher feed intake and produced heavier eggs than hens fed a wheat diet.

Egg quality

The results indicated that whole wheat did not affect egg shell breaking strength, egg shell thickness, albumen height, yolk weight, shell weight, %albumen, %yolk, %shell, albumen:yolk ratio and Haugh unit but decreased yolk color. Similarly, Pérez-Bonilla et al. (2011) reported a similar reduction in yolk color when corn was substituted by 15, 20, 25% whole wheat in diets. Ciftci et al. (2003) reported that yolk color higher in control diets than whole wheat diets. It is believed that low egg yolk color can be attributed to a lack of carotenoids in whole wheat.

CONCLUSION

It could be concluded that using 25% whole wheat in feed deceased albumen weight and yolk color, but not influenced the production performance of laying hens.

KEYWORD : Corn, Whole Wheat, Laying Hens

Table 1. Composition of the experimental diets.

Ingredients	Control	25% Whole wheat
Corn	57.81	34.77
Whole Wheat	-	25.00
Lard	2.21	2.18
Rice solvent bran	5.00	5.00
Soybean meal 48% CP	22.47	20.54
DL-Methionine	0.16	0.15
Monodicalciumphophate	1.50	1.51
Calcium carbonate	9.66	9.66
Salt	0.17	0.17
Vitamin & Mineral premix[1]	0.25	0.25
Choline Chloride 60%	0.08	0.08
Sodiumbicarbonate	0.70	0.70
Total	100.00	100.00

[1] Vitamin & Mineral premix: Lutavit Mix CNK004 consist of vitamin A 5.0 MIU; D_3 1.2 MIU; E 4,000 IU; K_3 0.6 g; B_1 0.8 g; B_2 2.0 g; B_6 1.2 g; B_{12} 0.0025 g; Nicotinic acid 5.00 g; Pentothenic acid 3.76 g; Folic acid 0.2 g; Biotin 0.036 g; Mn 24.00 g; Zn 20.00 g; Fe 16.00 g; Cu 4.00 g; Iodine 0.8 g; Co 0.08 g; Se 0.04 g; Feed preservative substance 0.4 g and carrier added to 1.00 kg premix.

Table 2. Calculate nutrient content of experimental diets.

Nutrient name	Unit	Control	25% Whole wheat
Metabolizable energy	Cal/kg	2,725	2,725
Crude protein	%	16.320	16.320
Fat	%	3.980	3.525
Crude fiber	%	3.393	4.777
Calcium	%	4.000	4.000
Total Phosphorus	%	0.690	0.703
Available Phosphorus	%	0.370	0.370
Salt	%	0.250	0.250
Arginine	%	1.030	1.025
Isoleucine	%	0.690	0.693
Lysine	%	0.820	0.794
Methionine + Cystine	%	0.700	0.700
Methionine	%	0.430	0.409
Threonine	%	0.600	0.588
Tryptophan	%	0.180	0.193
Valine	%	0.750	0.754
Choline	mg/Kg	400.000	400.000

Table 3. Effect of whole wheat levels in diet on egg production performances of laying hens.

Item	Control	25% Whole wheat	P-value	SEM
Hen-day egg production (%)	70.00 ± 5.33	69.02 ± 4.21	0.77	1.53
Egg weight (g)	65.91 ± 0.75	65.28 ± 1.12	0.28	0.28
Egg mass (g/hen)	46.32 ± 3.69	45.25 ± 2.32	0.63	1.01
Daily feed intake (g/hen)	114.23 ± 2.64	111.87 ± 4.49	0.29	1.08
Feed conversion ratio	2.48 ± 0.20	2.48 ± 0.11	0.98	0.05
Mortality (%)	0.52 ± 1.28	1.04 ± 1.61	0.55	0.41

Table 4. Effect of whole wheat levels in diet on egg quality of laying hens.

Item	Control	25% Whole wheat	P-value	SEM
Shell breaking strength (kg)	3.54 ± 0.16	3.57 ± 0.17	0.80	0.05
Shell thickness (mm)	0.39 ± 0.01	0.39 ± 0.01	0.32	0.00
Albumen height (mm)	7.84 ± 0.52	8.17 ± 0.55	0.39	0.15
Albumen weight (g)	44.80 ± 1.97	42.12 ± 0.60	**0.01**	0.57
Yolk weight (g)	16.34 ± 0.58	16.47 ± 0.79	0.71	0.19
Shell weight (g)	6.53 ± 0.22	6.37 ± 0.11	0.23	0.05
Albumen weight (%)	66.17 ± 1.64	64.85 ± 0.88	0.06	0.40
Yolk weight (%)	24.17 ± 1.20	25.35 ± 0.90	0.06	0.33
Shell weight (%)	9.67 ± 0.47	9.80 ± 0.11	0.32	0.10
Albumen : Yolk ratio	2.75 ± 0.22	2.57 ± 0.12	0.07	0.05
Haugh unit	86.18 ± 2.64	88.87 ± 3.30	0.20	0.88
Yolk color	5.88 ± 0.29	4.79 ± 0.19	**<0.01**	0.17

REFERENCES

Ciftci, I., E. Yenice and H. Eleroglu. 2003. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity and digestive system characteristics. Anim. Feed Sci. Tech. 105: 149-161.

Deaton, J., B. Lott and J. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layers. Poult. Sci. 68: 1342-1344.

Eisen, E., B. Bohren and H. Mckean. 1962. The Haugh unit as a measure of egg albumen quality. Poult. Sci. 41: 1461-1468.

Kim, S., M. Patel, S. Reddy and J. Mcginnis. 1976. Effects of different cereal grains in diets for laying hens on production parameters and liver fat content. Poult. Sci. 55: 520-530.

Lazaro, R., M. Garcia, M. J. Aranibar and G. G. Mateos. 2003. Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. Brit. Poult. Sci. 44: 256-265.

Pérez-Bonilla, A., M. Frikha, S. Mirzaie, J. García and G. Mateos. 2011. Effects of the main cereal and type of fat of the diet on productive performance and egg quality of brown-egg laying hens from 22 to 54 weeks of age. Poult. Sci. 90: 2801-2810.

Safaa, H. M., E. Jimenez-Moreno, D. G. Valencia, M. Frikha, M. P. Serrano and G. G. Mateos. 2009. Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg-laying hens in early phase of production. Poult. Sci. 88: 608-614.

O-06-10 DIETARY FIBER DIGESTIBILITY AND APPARENT METABOLIZABLE ENERGY IN CASSAVA MEAL AND CASSAVA RESIDUE PELLET FED TO BROILERS

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INTRODUCTION

Increasing global demand and prices of corn and soybeans directly affect the feed cost which is the largest cost of livestock production; therefore, strategically solving alternative to corn and soybean meal as feed ingredients leading to the increased use of agricultural by-products as feedstuffs in poultry diets must be developed to reduce diet costs to increase profitability and sustainability of the livestock enterprise. These ingredients are relatively cheap and abundant but in terms of chemical composition, contains high levels of dietary fiber. Cassava tuber is one of the most important food crops grown in the tropics and a significant source of calories for more than 800 million people (FAO, 2013). There is great interest in the use of cassava products and residues as a feed ingredient in monogastric diets not only benefit livestock ad cassava farmers because cassava production potentially increases. Since the energy value of cassava meal is close to that of cereal grains, cassava roots are primarily used as a substitute for corn in diets for poultry (Aina and Fanimo, 1997; Adeyemi et al., 2008). However, utilization of cassava residue pellet is limited due to its low protein content and high dietary fiber concentration, which may negatively affect nutrient digestibility and growth performance (Anugwa et al., 1989; Wu, 1991; Ochetim, 1993; Aina and Fanimo, 1997; Adeyemi et al., 2008; Régnier et al., 2010; Son et al., 2012). However, there is limited information on the effect of simultaneous inclusion of cassava meal and residue pellet on the digestibility of dietary fiber, energy, and apparent metabolizable energy in diets fed to growing broilers. Therefore, this conducted study was to determine the effect of cassava meal (CM) and cassava residue pellet (CRP) in the diet on the apparent total tract digestibilities (%ATTD) of energy and dietary fiber fed to growing broilers and to determine the relationship of these values to the nitrogen-corrected apparent metabolizable energy (AMEn) of the diet and excreta dry matter.

METHODOLOGY *Animals and Experimental Design*

For this experiment, 60 day-old (Cobb 500) male broilers were used. Birds were group-brooded and fed a commercial chick booster diet for 7 days. Afterwards, the birds were randomly allotted to 1 of 5 dietary treatments using a completely randomized design consisting of 12 birds per treatment. Each broiler was housed in a metabolic cage (50 × 60 cm) allowing for total excreta collection captured on aluminum trays directly beneath each cage.

Experimental Diets

Diet**1** was a corn-soybean meal basal diet that served as the control (**Table 1**). The next 4 diets contained 70% of the basal diet and 30% of varying mixtures of prime-quality cassava meal (**CM**) and cassava residue pellet (**CRP**). For Diets**2** to **5**, the cassava mixture were 100%CM, 75%CM:25%CRP, 25%CM:75%CRP and 100%CRP, respectively. Limestone, inorganic phosphate, vitamins, and minerals were added to the diets to meet or exceed NRC (2012) requirements for growing broilers. All experimental diets were in pellet form.

Feeding and Excreta Collection

Birds were fed a common diet for 12 days. Afterwards, birds were weighed and placed into the cages after a 4-h fast. The experimental diets were fed from day 13 to 22 post-hatch. Birds were offered feed and water ad libitum throughout the experimental period. The first 7 days served as an adaptation period. The balance period started on day 20 after a 17-h fast until day 22. Total excretion collection was performed every 24 h during the balance period. On the last day (day 22), birds were fasted for17 h and weighed after fasting. Daily excreta collection was then pooled within a cage, weighed, and stored at -20°C for subsequent analyses.

Chemical Analysis

At the end of the experiment, pooled 3-day excreta samples were lyophilized, wrapped in aluminum foil, and then dried at 65°C in a forced-air oven to constant weight and then finely ground through a 40-mesh sieve prior to analysis. All samples were analyzed for DM by oven drying triplicate samples at 135°C for 2 h (Method 930.15;

AOAC, 2007). Excreta, diet, and ingredient samples were analyzed in triplicate for GE using bomb calorimetry (Model 6200, Parr Instruments, Moline, IL) and CP. All diet and cassava meal samples were analyzed in triplicates for DM (method 930.15; AOAC, 2007), CP (method 990.03; AOAC, 2007), ether extract (method 920.39; AOAC, 2007), crude fiber (method 978.10; AOAC, 2007), and ash (method 942.05; AOAC, 2007). Fecal and diet samples were analyzed in triplicate for ADF (method 973.18; AOAC, 2007) and NDF (Holst, 1973).

Energy and Digestibility Calculations

AME of the basal diet was determined using the direct procedure whereas AME in cassava meal was determined using the difference procedure. AME of the diet was calculated using the following equation:

Correction for zero nitrogen retention was made using a factor of 8.22 kcal per g N retained in the body (Sauvant et al., 2004). N-corrected AME (**AMEn**) was calculated using the following equations:

, where:

ATTD of DM, GE, NDF, ADF, and hemicellulose were calculated using the following equation:

where ATTD is the apparent total tract digestibility, $Nutrient_i$ is the total nutrient intake (g) from d 19 to 22; and $Nutrient_f$ is the total fecal output (g) of the nutrient originating from the diet fed from d 19 to 22 (Adeola, 2001).

Statistical Analysis

Data was analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with bird as the experimental unit. The model included diet as the fixed effect and replicate as the random effect. Least squares means were calculated for each independent variable and the alpha level that was used to determine statistical significance was 0.05. Simple and multiple linear regression analyses were performed using REG procedure of SAS to determine the relationship between ATTD and the concentration of NDF, ADF and hemicellulose with the ATTD of GE and AMEn of the diets.

RESULTS AND DISCUSSION *Chemical Composition of CM and CRP*

To be considered prime-quality, cassava pellets should contain a maximum of 13% moisture, 2% CP, 4% CF, and 2% ash and a minimum of 70% starch (Kanto and Juttupornpong, 2005). CM used in this experiment met the standard specifications for prime-quality cassava pellets (**Table 1**). GE in CM (4,216 kcal/kg DM) was greater than previously published values (4080 to 4,111 kcal/kg DM; Sauvant et al., 2004; Heuzé et al., 2015). The analyzed composition of cassava residue pellet used in the present experiment also conformed with the range of published values (Heuzé et al., 2015). As expected, the concentration of GE and starch in CRP were less and the concentration of CP, ash, crude fat, and crude fiber, NDF, ADF and ADL were greater than those in CM. Based on the analyzed composition of the cassava products, the main fraction of dietary fiber in CRP is lignin and cellulose, as hemicellulose is estimated to be only 3.55%.

Daily Energy Balance

Broilers fed the experimental diets had similar DM intake (**Table 2**). However, broilers fed the diet with 25%CRP:75%CM had greater (P=0.05) GE intake than those fed CRP and 75%CRP:25%CM (**Table 2**). This is due to the differences in GE among the experimental diets. Excreta and GE output was greatest (P<0.001) for broilers fed the diet with 100%CRP whereas broilers fed 100%CM had the least (P<0.001) excreta and GE output. There was increasing excreta and GE output as greater levels of CRP were added to the diet. As a result, ATTD of GE was greatest (P<0.001) in the diet with 100%CM and the least (P<0.001) for the diet with 100%CRP. Likewise, AME of the 100%CM and 25%CRP:75%CM diets were greater (P<0.001) than the 100%CRP and 75%CRP:25%CM diets. The diet with 100%CRP had the least (P<0.001) AME. The increasing level of dietary fiber in the diets with greater levels of CRP may explain the observed increase in excreta output. Insoluble dietary fiber increases fecal bulk (Taghipoor et al., 2012) because it reduces intestinal transit time and diet digestibility. As a result, dietary fiber increased GE output in excreta, which conformed with the results of Wang et al. (2004). Dietary fiber negatively affects energy and nutrient digestibility of the feed (Noblet and Le Goff, 2001; Bindelle et al., 2008), which explains the observed decrease in the AME of the diet when greater levels of CRP were added to PCM.

AME and AMEn of Cassava Products

The difference procedure was used to determine the concentration of AMEn in the cassava products, and a corn-soy basal diet was used for the energy balance experiment. A consequence of using the difference procedure is that reliable results for the test ingredient will be obtained only if the result of the AMEn of the basal diet is accurate. The values for AMEn obtained for the corn-soy basal diet (3,095 kcal/kg DM) in the present experiment are in close agreement with the predicted AMEn using previous data for corn and soybean meal (3,005 kcal/kg DM; Sauvant et al., 2004), which indicates that the AMEn in the cassava products that were calculated are

accurate. The values of AME and AMEn (4,345 and 4,177 kcal/kg DM, respectively; **Table2**) of CM determined in the current experiment were greater than previously published values (Sauvant et al., 2004; Kanto and Juttupornpong, 2005; Heuzé et al., 2015). This is because GE of the CM used in the present experiment was greater than those in previous studies. To the best of our knowledge, there is no previous study that determined the AMEn of CRP in broilers. The AMEn of CRP was 3,009 kcal/kg DM, which is less than corn (3,624 kcal/kg DM; Sauvant et al., 2004) but greater than soybean meal (2,646 kcal/kg DM; Sauvant et al., 2004). The AMEn of CRP was almost 1,200 kcal less (P<0.001) than the AMEn in CM. On both an as-fed and DM basis, CM and 25%CRP:75%CM had greater (P<0.001) AME and AMEn compared with CRP and 75%CRP:25%CM (**Table2**). The AME and AMEn of 75%CRP:25%CM was greater (P<0.001) than CRP. This indicates that increasing proportion of CRP in the blend led to a linear reduction in AMEn, which may be related to the increase in dietary fiber and decrease in starch content in the cassava blends. This conformed with previous studies (Krás et al., 2013; Mateos et al., 2013; Walagumbe, 2013) which showed reduction in energy utilization when broilers are fed with ingredients high in NDF and hemicellulose.

Nutrient Digestibility

The ATTD of DM decreased (P<0.001) with increasing levels of CRP in the corn-SBM basal diet (**Table3**). The 100%CRP diet had greater (P<0.001) ATTD of NDF and ADF compared with the other experimental diets. The ATTD of NDF and ADF of the 75% CRP:25% PCM diet was also greater (P<0.001) than the 25% CRP:75% PCM diet. However, no differences were observed in the ATTD of NDF and hemicellulose between the 75%CRP:25%CM diet and 100%CM diet. The ATTD of hemicellulose of the 100%CRP and 100%CM diets were both greater (P<0.001) than the diets with the cassava blends. The observed decrease in DM digestibility of the diets with increasing levels of CRP was similar to the results of Akinola et al., (2013). In their study, increasing levels of cassava peels in growing pig diets also resulted in a decrease in DM digestibility due to greater dietary fiber levels in the diet. This also corresponded with the observed decrease in ATTD of GE, which may explain the reduction in ATTD of DM. Results of the present study indicate that lignin and cellulose are the major fractions of dietary fiber in cassava products. The ATTD of ADF in the cassava products ranged from 0.2 to 31.3% and the ATTD of hemicellulose ranged between 49.9 to 66%, which indicates the difference in fermentability of the cellulose and hemicellulose fractions. Numerous studies have documented the negative effects of dietary fiber on diet digestibility and energy concentration of the diet (Johnston et al., 2003; Krás et al., 2013; Mateos et al., 2013; Walugembe, 2013). Kirwan et al. (1974) also reported that elevated levels of insoluble fiber (cellulose) in the diet shorten residence time of digesta which may lead to lower nutrient digestibilities, reduce AME and performance of birds.

Relationship of Dietary Fiber Fractions with AMEn of Cassava Products

The ADF and NDF concentration in the cassava products were negatively related (P < 0.01) with ATTD of GE (R^2 = 0.96 and R^2 = 0.92, respectively; **Fig. 1**) and AMEn (R^2 = 0.924 and R^2 = 0.885, respectively; **Fig. 2**). However, the hemicellulose content and the ATTD of ADF, NDF, and hemicellulose of the diet had no linear relationship with ATTD of GE and AMEn of the cassava products. The present results agree with the wide body of evidence on the negative effect of NDF and ADF on energy digestibility and energy value of feed ingredients (Noblet, 2000). The strength of the relationship is slightly lower with NDF compared with ADF, and this is mainly due to the effect of hemicellulose which is the more soluble fraction of dietary fiber. With these results, it is then proposed that more cassava sources of varying levels of dietary fiber be evaluated to develop prediction models that can be used to accurately estimate AMEn in cassava products.

Excreta dry matter

A positive linear relationship (R^2 = 0.81 and 0.89; P<0.01) between ADF and NDF concentration in the cassava products with excreta DM of broilers was observed (**Fig. 3**). However, there were no differences in excreta DM of broilers fed diets with corn-soy only and corn-soy diets with CRP, PCM and their blends (**Table 3**). Field observations suggested that the use of cassava in broiler diets increases incidence of wet droppings, which often becomes a reason for limitations in using cassava products in broilers. The results indicate that eventhough cassava products with higher ADF and NDF increases excreta DM closer to levels of broilers fed corn-soy diets, the actual difference is only minimal. It is not known if a 1% difference in excreta DM is appreciable in practice, but the present experiment indicate that using cassava products in broiler diets do not significantly reduce excreta DM.

CONCLUSION

The % ATTD of GE and AME was greatest in the diet with 100%CM and the least for the diet with 100%CRP. The

AMEn (as DM basis) in CM was greater than the value in CRP fed to growing broilers. The ATTD of DM decreased with increasing levels of CRP in the corn-SBM basal diet. The 100%CRP diet had greater ATTD of NDF and ADF compared with the other experimental diets. The ADF and NDF concentration in the cassava products were negatively related with ATTD of GE and AMEn. A positive linear relationship between ADF and NDF concentration in the cassava products with excreta DM of broilers was observed. There were no differences in excreta DM of broilers fed diets with corn-soy only and corn-soy diets with CRP, CM and their blends. Based on the study, the level of dietary fiber particularly the ADF fraction negatively affected the energy value and nutrient digestibility of diet and feed ingredients.

KEYWORD : Cassava meal, Cassava residue pellet, Energy, Dietary fiber, Broilers

Table 1. Composition (as-fed basis) of experimental diets and cassava product (as DM Basis)

Items	Diet					Cassava Product			
	Basal	100% CRP	75% CRP: 25% CM	25% CRP: 75% CM	100% CM	CRP	75% CRP: 25% CM	25% CRP: 75%CM	CM
Ingredient, %									
Corn	50.42	35.29	35.29	35.29	35.29	-	-	-	-
Soybean meal	44.00	30.80	30.80	30.80	30.80	-	-	-	-
Cassava meal, prime (CM)	-	30.00	22.50	7.50	-	-	-	-	-
Cassava residue pellet (CRP)	-	-	7.50	22.50	30.00	-	-	-	-
Limestone	1.64	1.15	1.15	1.15	1.15	-	-	-	-
Monocalcium phosphate	2.71	1.90	1.90	1.90	1.90	-	-	-	-
DL-Methionine	0.28	0.20	0.20	0.20	0.20	-	-	-	-
L-Lysine	0.13	0.09	0.09	0.09	0.09	-	-	-	-
Vitamin-mineral premix[1]	0.31	0.22	0.22	0.22	0.22	-	-	-	-
Salt	0.50	0.35	0.35	0.35	0.35	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	-	-	-	-
Calculated composition, %						Analyzed Composition, %			
DM	88.90	88.85	90.42	90.75	90.42	89.65	89.27	87.75	87.15
GE, kcal/kg	4,337	4,328	4,382	4,424	4,408	3,952	4,027	4,137	4,216
CP (N × 6.25)	24.90	20.19	20.58	18.62	17.79	3.90	2.49	3.52	3.06
Ash	3.98	6.46	6.03	5.62	9.58	7.66	5.98	3.36	1.95
Crude fiber	3.10	3.89	3.12	3.43	3.41	13.97	11.09	5.91	1.74
Crude fat	3.10	1.78	2.42	2.10	1.37	0.41	0.36	0.50	0.14
NFE	72.35	56.53	58.27	60.98	62.50	74.05	80.08	86.70	93.10
ADF	4.20	6.42	5.14	3.21	2.27	22.07	17.91	8.76	2.07
NDF	4.50	11.23	9.10	7.64	6.09	25.62	23.95	12.63	6.97
Hemicellulose	0.30	4.81	3.96	4.43	3.82	3.55	6.04	3.87	4.90
ADL	-	-	-	-	-	-	15.98	6.12	8.18
Starch (polarimetry)	-	-	-	-	-	-	54.65	71.40	70.75

[1]The vitamin-micromineral premix contained the following quantities of vitamins and micro minerals per kilogram of premix: Vitamin A, 11,000,000 IU/kg; Vitamin D, 5,000,000 IU/kg, Vitamin E, 50,000 mg/kg; Vitamin K, 3,000 mg/kg; thiamine, 2,000 mg/kg; riboflavin, 7,000 mg/kg; pyridoxine, 3,000 mg/kg; niacin, 40,000 mg/kg; pantothenic acid, 15,000 mg/kg; vitamin B12, 15 mg/kg; folic acid, 1,5000 mg/kg; Fe, 92,000 mg/kg; Cu, 7,500 mg/kg; Zn, 60,000 mg/kg; Mn, 50,000 mg/kg; I, 700 mg/kg; Se, 150 mg/kg.

Table 2. Daily energy balance, ATTD (%) of GE, and AME of the diets with CM, CRP and their blends; and AME and AMEn of cassava product fed to growing broilers[1]

Item	Treatments				SEM	*P*-value
	100% CRP	75% CRP: 25% CM	25% CRP: 75% CM	100% CM		
DM intake, g/d	108	107	108	108	0.6	0.58
GE intake, kcal/d	469[b]	470[b]	479[a]	475[ab]	2.5	0.05
Excreta output, g/d	34.3[a]	31.4[b]	27.5[c]	25.8[d]	0.4	<0.001
GE in excreta, kcal/d	118[a]	106[b]	93[c]	87[d]	1.8	<0.001
ATTD of GE, %	74.9[d]	77.4[c]	80.6[b]	81.6[a]	0.4	<0.001
AME of diet, kcal/kg	3,242[c]	3,392[b]	3,565[a]	3,597[a]	17.1	<0.001
AME and AMEn of Cassava product						
As-fed basis						
AME	2,783[c]	3,222[b]	3,729[a]	3,823[a]	50	<0.001
AMEn	2,648[c]	3,076[b]	3,591[a]	3,676[a]	51	<0.001
DM basis						
AME	3,162[c]	3,661[b]	4,238[a]	4,345[a]	57	<0.001
AMEn	3,009[c]	3,446[b]	4,081[a]	4,177[a]	57	<0.001

[1]Data are least square means of 12 replicates per treatment.
[a-d]Values within a row lacking a common superscript letter are different (P<0.05).

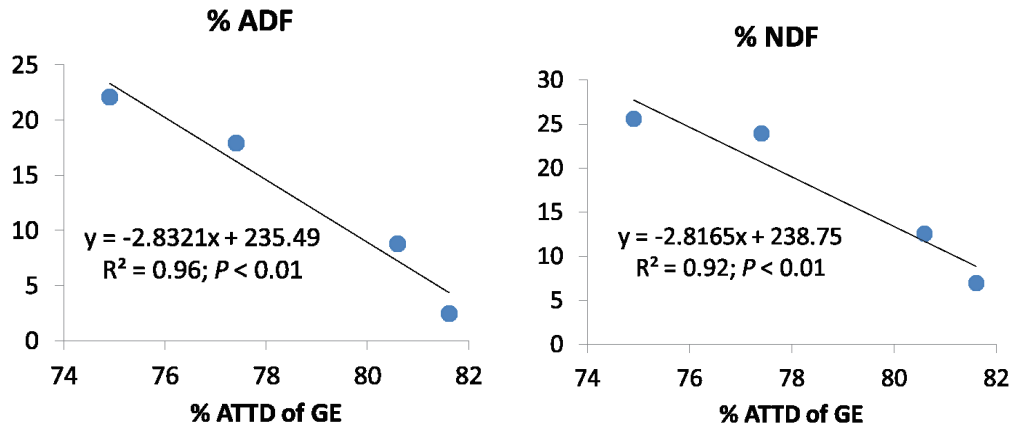

Fig.1. Relationship of ADF and NDF concentration on ATTD (%) of GE of cassava products fed to broilers.

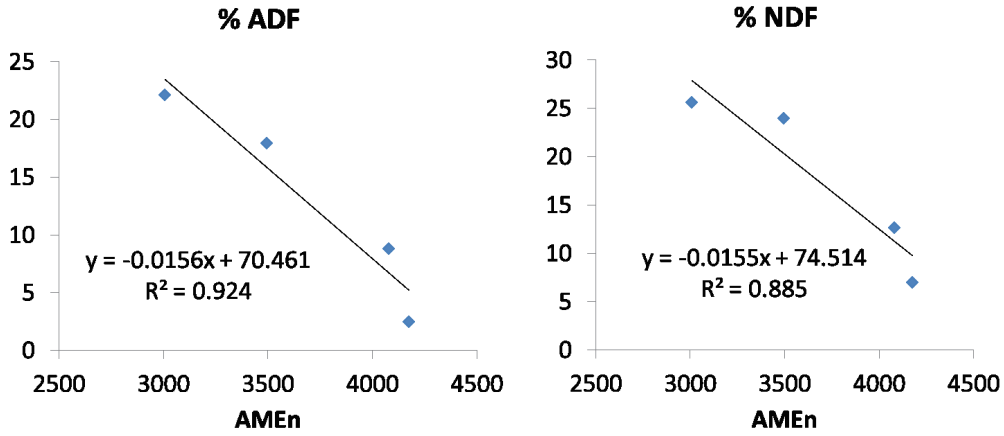

Fig. 2. Relationship of ADF and NDF concentration on AMEn (kcal/kg DM) of cassava products fed to broilers.

Table 3. ATTD of DM dietary fiber fractions of diets with CM, CRP and their blends fed to growing broilers; and excreta dry matter broilers fed corn-soy diets with CM, CRP, and their blends[1]

Item	Corn-soy only	Treatments				SEM	*P*-value
		100% CRP	75% CRP: 25% CM	25% CRP: 75% CM	100% CM		
ATTD of , %							
DM	-	68.4[d]	70.8[c]	74.6[b]	76.0[a]	0.5	<0.001
NDF	-	44.8[a]	39.1[b]	32.3[c]	41.5[b]	1.1	<0.001
ADF	-	31.3[a]	27.1[b]	16.0[c]	0.2[d]	1.5	<0.001
Hemicellulose	-	62.7[a]	54.6[b]	49.9[b]	66.0[a]	2.2	<0.001
Excreta DM, %	18.7	18.8	18.1	17.7	17.5	0.5	0.30

[1]Data are least square means of 12 replicates per treatment.
[a-d]Values within a row lacking a common superscript letter are different ($P<0.05$).

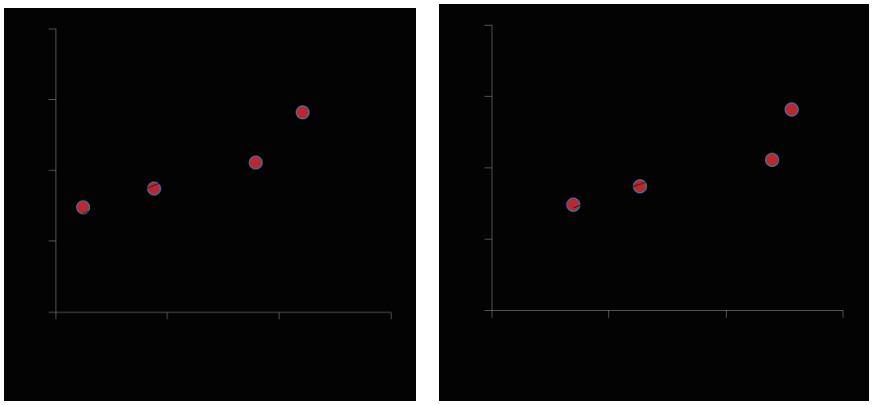

Fig. 3. Relationship of ADF and NDF concentration on excreta DM of broilers fed diets with cassava products.

REFERENCES

ADEOLA, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine Nutrition. 2nd ed. CRC Press, Washington, DC. pp. 903–916.

ADEYEMI, O. A., D. ERUVBETINE, T. OGUNTONA, M. DIPEOLU, J. A. AGUNBIADE. 2008. Feeding broiler chicken with diets containing whole cassava root meal fermented with rumen filtrate. Archivos de Zootecnia. Vol. 57. No. 218, pp. 247-258.

AINA, A. B. J., A. O. FANIMO. 1997. Substitution of maize with cassava and sweet potato meal as the energy source

in the rations of layer birds. Pertanika Journal of Agricultural Science. Vol. 20. No.2/3, pp. 163-167. University Putra Malaysia Press. ISSN: 0126-6128.

AKINOLA, O. S., A. O. FANIMO, J. A. AGUNBIADE, A. SUSENBETH, E. SCHELECHT. 2013. Cassava root peel as a replacement for maize in diets for growing pig: effects on energy and nutrient digestibility, performance and carcass characteristics. Journal of Agriculture and Rural Development in the Tropics and Subtropics. Vol. 114. No. 2, pp. 159-166.

AOAC INTERNATIONAL. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer Jr., ed. AOAC Int., Gaithersburg, MD.

FAO. 2013. Save and grow: Cassava. A guide to sustainable production intensification. www.fao.org/docrep/018/i3278e/

HEUZÉ, V., G. TRAN, D. BASTIANELLI, F. LEBAS, H. ARCHIMÉDE, C. RÉGNIER. 2015. Cassava peels, cassava pomace and other cassava by-products. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/526

HOLST, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J. AOAC. 354 56:1352–1356.

JOHNSTON, L. J., S. NOLL, A. RENTERIA, G. C. SHURSON. 2003. Feeding by-products high in concentration of fiber to nonruminant. In: Third National Symposium on Alternative feeds to livestock and poultry, Kansa City, MO.

KANTO, U., S. JUTTUPORNPONG. 2005. Cassava in animal nutrition: With reference to Thailand cassava. Animal Nutrition Research and Development Center Publication No.1/2548.

KIRWAN, W. O., A. N. SMITH, A. A. MCCONNELL, W. D. MITCHELL, M. A. EASTWOOD. 1974. Action of different bran preparations on colonic function. Br. Med. J. 4, 5938:187.

KRÁS, R. V., A. M. KESSLER, A. M. L. RIBEIRO, J. D. I. HENN, I. I. DOS SANTOS, D. P. HALFEN, L. BOCKOR. 2013. Effect of dietary fiber and genetic strain on the performance and energy balance of broiler chickens. Brazilian Journal of Poultry Science. Vol. 15, No. 1, pp. 15-20.

MATEOS, G. G., E. JIMÉNEZ-MORENO, M. P. SERRANO, R. P. LÁZARO. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. Poultry Science Association, Inc. J. Appl. Poult. Res. 21:156-174. http://japr.oxfordjournals.org

NOBLET, J. 2000. Digestive and metabolic utilization of feed energy in swine: Application to energy evaluation systems. Journal of Applied Animal Research. Vol. 17, issue 1.

NOBLET, J., G. LE GOFF. 2001. Effect of dietary fiber on the energy value of feeds for pigs. Animal Feed Science and Technology. Vol. 90, pp. 35-52. www.elsevier.com/locate/anifeesci

OCHETIM, S. 1993. Fresh cassava as a feed for fattening pigs. American Journal of Animal Science. Vol. 6, No. 3, pp. 361-365.

REAS, B. S. 1999. Use of cassava in swine feeding (performance and economics). ASA Technical Bulletin. Vol. SW22.

RÉGNIER, C., B. BOCAGE, H. ARCHIMÈDE, D. RENAUDEAU. 2010. Effects of processing methods on the digestibility and palatability of cassava root in growing pigs. Animal Feed Science and Technology. Vol. 162, pp. 135-143. www.elsevier.com

SAUVANT, D., J. PEREZ, G. TRAN. 2004. Tables of composition and nutritional value of feed materials. 2nd ed. Wageningen Academic Publ., the Netherlands.

SON, A. R., S. Y. JI, B. G. KIM. 2012. Digestible and metabolizable energy concentrations in copra meal, palm kernel meal and cassava root fed to growing pigs. Journal of Animal Science. Vol. 90, pp. 140-142. www.journalofanimalscience.org

WALUGEMBE, M. 2013. The effect of high and low dietary fiber diets on the performance of two lines of chickens with divergent growth rates. (Master thesis). Iowa State University.

WANG, J. F., Y. H. ZHU, D. F. LI, B. H. JØRGENSEN, B. B. JENSEN. 2004. The influence of different fiber and starch types on nutrient balance and energy metabolism in growing pigs. Asian-Aust. J. Anim. Sci. 2004. Vol. 17, No. 2: 263-270.

WU, J. F. 1991. Energy value of cassava for young swine. Journal of Animal Science. Vol.69, pp. 1349-1353. www.journalofanimalscience.org

O-07-3

Effects of different dietary oils and L-Arginine supplementation in diet on performance, intestinal morphology and serum immunoglobulin level in broiler

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Introduction

Fats and oils are the main energy sources in the broiler ration which promotes growth, in addition dietary protein also regulates growth and reproductive performance. Dietary protein/amino acids are important for the gastrointestinal tract development and absorptive function and enhancement of the immune system (Laudadio et al., 2012 and Wang et al. 2012). Poorghasemi et al. (2015) reported that dietary fat sources had no impact on immune response of broiler. However, Butcher and Miles (2002) stated that proper nutrient has a positive impact on the immune system and concentration of nutrient can influence growth and modify the expression of genes for immune responsiveness. It was observed that the addition of amino acids such as lysine, arginine in the diet improves the immunity against diseases. However, research on the combination of oils and amino acids supplementation in broiler ration on performance, intestinal morphology and immunity of broiler is limited. Therefore, the current research was undertaken with the objective to evaluate the effect of diet containing different dietary sources of oils and L-Arginine (L-Arg) on performance, gut morphology and immune response of broiler.

Materials and Methods

The study was conducted in the experimental poultry unit, Department of Animal Science, Faculty of Agriculture and Institute of Bioscience, Universiti Putra Malaysia (UPM). One hundred eighty, day-old broiler chicks were purchased out of the local hatchery of Malaysia. Each chick was weighed, wing-banded individually and randomly allocated into the five dietary treatments with 6 replications and each replication consist of 6 birds. The basal diet composed of corn and soybean meal with four levels of oil combinations: Palm oil (PO) and Sunflower oil (SO) of which were supplemented with the amino acid, L-Arginine (L-Arg). The dietary treatments were as: T_1, 6% PO (control); T_2, 6% PO + 0.25% L-Arg; T_3, 4% PO + 2% SO + 0.25% L-Arg; T_4, 2% PO + 4% SO + 0.25% L-Arg; and T_5, 6% SO + 0.25% L-Arg. The broiler was vaccinated against infectious bronchitis (IB) and Newcastle disease (ND) at 7 and 14 days, and infectious bursal diseases (IBD) at 21 days of age. Birds were reared under ideal environmental conditions and water and feed were supplied *ad-libitum* during the whole experimental period. Six broiler chickens per treatments (one chicken per replicate) were slaughtered at the 6 weeks of age. Blood and intestinal samples were collected in order to measure serum immunoglobulin level and study for gut morphology of broiler chickens. The villi height and crypt depth was determined according to the procedure of Choe et al. (2012). About 4 to 5.5 cm long segments were collected from the middle part of jejunum and ileum and it was flushed with physiological saline. The collected sample was immediately put into 10% buffer formalin solution until further processing. Intestinal segments were excised about 3.5 mm and transferred into the plastic cassettes and kept in the neutral buffer formalin solution overnight. Then the samples were dehydrated using tissue processing machine and embedded in paraffin wax. After trimming of the intestinal segment at 15 μ m, the sample sectioned at 5 μ m and was placed on a glass slide in hot plate at 60℃ . Then the glass slide was stained by haematoxylin and eosin and examined under the microscope.

At 42 days of age, 2-3 ml blood was collected from six birds per treatment into vacutainer tubes without anticoagulant and serum sample was separated by centrifuging at 3000 rpm for 10 min and stored at -80°C until antibody analysis. The serum total immunoglobulin (IgG and IgM) were measured using ELISA kits (Immunology Consultants Laboratory, Inc., USA) according to manufacture protocol and absorbance were measured at 450 nm. The IgM and IgG concentration were measured using standard curve.

The experiment was carried out by completely randomized design (CRD) and collected data were analysed using PROC GLM of SAS (SAS, 2014). For comparison of treatment means, the Duncan multiple range tests (DMRT) were used at the level of $p < 0.05$.

Results

Effect of different sources of oil and L-Arg on the performance of broiler

Body weight gain and feed conversion efficiency was increased with the increasing level of unsaturated fatty acid sources oil (SO) and supplementation of 0.25% L-Arg compared to control group. Significant higher ($P<0.05$) body weight gain and feed efficiency were found in broiler fed dietary treatments T_4 and T_5 than control and other dietary treatments. However, the treatment group T_2 and T_3 showed intermediate performance. The significant higher ($P<0.05$) body weight gain and better feed conversion of broiler were recorded in bird fed increasing level of (SO) group due to the higher digestibility of unsaturated fatty acid (UFA) than saturated fatty acid (SFA). Similar finding was reported by Velasco et al. (2010) that chicks fed diet containing unsaturated sources fat had the better body weight gain and superior feed conversion ratio than chicks fed saturated fat diet. In addition supplementation of 0.25%L-Arg with different sources of oil enhanced body weight gain of broilers. This finding is agreement with Emadi et al. (2010), who observed increase the dietary arginine increased body weight and feed intake. However, no significant differences were found for feed intake of broilers among treatments.

Effect of added L-Arginine in diet containing different sources of oil on intestinal morphology of broiler

The supplementation of L-Arg in the diet increased intestinal villus length and crypt depth. Longer jejunum villus height and deepest crypt were found in dietary groups of T_4 and T_5 compared to control group and other dietary groups. The changes of the ileum villus height and crypt depth of T_4 and T_5 were significantly higher ($P<0.05$) than control group and other dietary treatments. The pattern of change both jejunum and ileum villus height and crypt depth were found to be similar. However, no differences were observed for jejunum and ileum villus length among the dietary groups of T_3, T_4 and T_5. Increased intestinal villi height provides more surface area for nutrient absorption (Khambualai et al. 2009). The current results are in agreement with Ghiasi et al. (2014). The ileum crypt depth was significantly increased in dietary treatments of T_4 and T_5 compared to control and other dietary groups.

In the current study, villus crypt ratio for jejunum and ileum was increased with the addition of L-Arg with different dietary group, which indicated the positive intestinal function. Moreover, the higher villus height to crypt depth ratio in the broiler reduces the turnover of intestinal mucosa, which requires lower maintenance of intestinal mucosa. This leads to a higher growth performance of the animal. Similar results were reported by Laudadio et al. (2012a).

Effect of added L-Arginine in diet containing different sources of oil on serum immunoglobulin concentration of Broiler

The serum IgM concentration was increased significantly ($P<0.05$) in bird fed with the increasing level of unsaturated fatty acid rich oil (SO) and supplementation of 0.25% L-Arg compared to the bird fed PO diet (T_1). The immunoglobulin IgG showed a significant improvement ($P<0.05$) in the group fed combination of SO and PO (T_4). However, no significant differences were observed in serum IgG concentration between the treatment group T_3 and T_4. The lowest ($P<0.05$) total serum IgM concentration was observed in the control group than other dietary groups at 42 days age of broiler. The supplementation L-Arg increased the serum IgM levels and highest value was observed in broiler fed T_4 (2% palm oil+4%sunflower oil+0.25% L-Arg). No significant differences were found in IgM concentration among the treatment groups except for the control group (T_1). The finding reported by Deng et al. (2005) is in consistent with the present study.

Conclusion

From this study, it can be seen that the combination of saturated, palm oil and unsaturated, sunflower oil and 0.25% L- Arg supplementation had a positive effect on intestinal villus height and crypt depth which increased absorption of more nutrients, resulting good performance of broiler. This dietary supplementation also increased the serum IgM and IgG levels which modulates the immune system of broiler against the diseases.

KEYWORD : L-Arginine, Feed efficiency, Villus length, Crypt depth, oils

REFERENCES

Butcher, G. D. and Miles, R.D. 2002. Interrelationship of nutrition and immunity. VM139. Available online at www. edis.ifas.ufl.edu (accessed 21 April 2010).

Choe, D.W., Loh, T.C., Foo, H. L., Hair-Bejo, M and Awis, Q.S. 2012. Egg production, facecal PH and Microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by lactobacillus plantarum strains. British Poultry Science, 53(1): 106-115.

Deng, K.,Wong, C.W. and Nolan, J.V. 2005. Long-term effects of early life L-arginine supplementation on growth performance, lymphoid organs and immune responses in Leghorn type chickens. British Poultry Science, 46: 318-324.

Emadi, M., Kaveh, K., Bejo, M.H., Ideris, A., Jahanshiri, F., Ivan, M. and Alimon, R.A. 2010a. Growth performance and blood parameters as influenced by different levels of dietary arginine in broiler chickens. Journal of Animal and Veterinary Advances, 9: 70-74.

Ghiasi Ghalehkandi J., Hassanpour S., Ebrahimnazhad Y., Beheshti R. and Maheri-Sis N. 2014.Intestinal morphology of broilers fed diets supplemented with perlite. Journal of Helinic Veterinary Medicine Society, 65(2): 99-108.

Khambualai, O., Ruttanavut, J., Kitabatake, M., goto, H., Erikawa, T. and Yamauchi K. 2009. Effect of dietary natural zeolite including plant extract on growth performance and intestinal history in Aigamo ducks. British Poultry Science, 50: 123-130.

Laudadio, V., Dambrosioa, A., Normannob, G., Khanc, R.U., Nazd, S., Rowghanie, E. and Tufarellia, V. 2012. Effect of reducing dietary protein level on performance responses and some microbiological aspects of broiler chickens under summer environmental conditions. Avian Biology Research, 5 (2), 2012 88–92.

Laudadio , V., Passantino , L., Perillo , A., Lopresti, G., Passantino , A., Khan , R.U. and Tufarelli, V. 2012a. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poultry Science 9: 265–270. doi: 10.3382/ps.2011-01675.

Poorghasemi, M., Seidavi, A., Qotbi, A.A.A., Chambers, J.R., Laudadio, V., and Tufarelli, V. 2015. Effect of dietary fat source on humoral immunity response of broiler chickens. Archiv fur Geflugelkunde, 79. doi : 10.1399/eps.2015.92

SAS Institute Inc. 2014. SAS/STAT® User's Guide Version 9.4 SAS Institute Inc, Cary, North Carolina, USA.

Velasco, S., Ortiz, L.T., Alzueta, C., Rebole, A., Trevino, J. and Rodriguez, M.L. 2010. Effect of inulin supplementation and dietary fat source on performance, blood serum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broiler chickens. Poultry Science, 89:1651–1662.

Wang, K., Cui, H., Peng Xi., Zuo, Z., Fang, J., Deng, J., Deng, Y., Cui, W., and Wu, B. 2012. Effect of deitary vanadium on small intestinal morphology in broilers. Health,4: 667-674. doi:10.4236/health.2012.49105.

O-07-5

Angelica gigas Nakai root powder supplementation improves growth performance and modulates splenic heat shock proteins of broiler chickens

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OBJECTIVE

The present study investigated the effects of varying amounts of *Angelica gigas Nakai* (AGN) root powder supplementation on the growing performance, plasma biochemical profiling and heat shock related gene and protein expression in Ross broiler chickens under normal and heat stressed environment.

METHODOLOGY

Animals and Experimental Design

A total of one hundred ninety-two (192), day old broiler chicks were randomly divided into two groups: control (29℃) and heat stressed (36℃ for 5 hours per day) for 30 days. The two groups were divided into four subgroups having different treatments composed of the control (no AGN feed supplementation), 0.1%, 0.3% and 0.5% AGN root powder supplementation. All of the broiler chickens were fed with a commercial diet and provided with clean and fresh water ad libitum. Growth performance was assessed by determining the average feed intake, daily gain and feed efficiency. At the end of the experimental period, birds were sacrificed and spleen samples were removed for the assessment of mRNA and protein expression of heat shock proteins such as HSF1, HSP90 and HSP70. Plasma biochemical profiling using an automated biochemical analyzer includes measurement of glucose, total cholesterol, total bilirubin and glutamic oxaloacetic transaminase (GOT) while insulin, triiodothyronine (T3), thyroxine (T4) and cortisol using an ELISA kit.

Heat shock procedure

After one week of adaptation, the entire broiler chickens were subjected to normal environmental temperature recommended for broiler chickens (29 ℃) with heat stress group subjecting to the procedure through a heater within the pen regulated at 5 hours at 36℃ for 4 weeks.

Determination of Growth Performance

Body weights were measured at Day 0 and final day of the experimental period. Feed intake and refusals were measured daily to determine the average feed intake, daily gain and feed efficiency.

Sample collection

At the end of the experimental period, two birds from each pen were sacrificed and tissue samples were removed as quickly as possible. Spleen samples were immediately frozen in liquid nitrogen and stored at − 80 prior to the measurement of mRNA and protein expressions.

Total RNA extraction and RT-PCR analysis

One gram of frozen spleen samples in liquid nitrogen were maintained and homogenized into powdered form using mortar and pestle. Total mRNA was extracted from the homogenized tissue samples using RNAiso Plus (Takara Bio Inc., Kusatsu, Japan) to synthesize cDNA from 1μg of total RNA in a 20μL reaction using Maxime RT PreMix Kit (iNtRON Biotechnology, Seongnam, Korea). Polymerase chain reaction (PCR) were initiated by denaturation cycle at 95℃ for 5 min, followed by 30 amplification cycles: 40 s at 95℃ , annealing for 40 s (temperature ranging from 56℃ to 62℃) and extension at 72℃ for 1 min. Oligonucleotide primers of Heat shock factor 1(*HSF1*), heat shock protein 90 (*HSP90*) and *HSP70*, were used. All of the primers used were optimized experimentally. To ensure that mRNA for all samples was equivalent, β -actin was initially subjected to PCR reaction then compared to all gene expression values.

Total protein isolation and Western blot analysis

One gram of frozen spleen samples in liquid nitrogen were maintained and homogenized into powdered form using mortar and pestle. Total protein was extracted from the homogenized tissue by adding protein extraction solution (iNtRON Biotechnology, Korea). The lysates were centrifuged at 15,000rpm for 15 min at 4℃ to determine protein content of the supernatant through a modification of Bradford assay. Diluted 35 μg of the protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis prior to nitrocellulose membranes transfer. The membranes were blocked with 5% skimmed milk and hybridized with anti-

HSF1, Hsp90 and HSP70 polyclonal antibodies. Target proteins were excluded and identified by incubation of the membranes with secondary antibodies conjugated with horseradish peroxidase. To be able to view the exposed target protein, membranes were treated with enhanced chemiluminescence (AB Frontier, Seoul, Korea) then detected on radiographic film.

Statistical analysis

All experiments were performed three times separately and the results were expressed as means ± standard deviation. One-way analysis of variance was used to assess the valid differences between means. It was followed by Duncan Multiple Range Test where p

RESULTS AND DISCUSSION

Heat stress affects biological defense mechanisms such as the immune response in addition to inducing metabolic disorders, leading to a low productivity of chickens (Khajavi et al., 2003). Figure 1 showed that 0.1% AGN root powder supplementation among broiler chickens reared at normal environmental temperature enhances their growth performance as shown by the greatest feed efficiency value. Heat shock procedure of 5 hours per day induces a decreased growth performance as compared to those in the normal environmental temperature as showed in Fig. 2 at the control group. This simply confirmed the negative effect of heat stress on the performance of production animals. However, supplementation of AGN root powder at 0.1% complemented these negative effects as shown by highest ADG and FE among control and treatment groups.

To evaluate the overall health condition of the broiler chickens, plasma biochemical profiling was executed and results were presented in Table 1. Decreased values of all the plasma biochemical parameters among the heat stressed group compared to the normal group were observed. However, in the case of cortisol level, the group reared on an increased environmental temperature showed lower cortisol level rather than those grown in normal temperature. This could be due to the ability of the body for thermoregulation and adaptation especially for chronic conditions. In addition, the treatment of AGN root powder among non heat stressed and heat stressed group enhances the blood biochemical profile for all the parameters even cortisol. This could imply that AGN root powder has the potential to improve the overall health condition of broiler chicken as supported by its previous known property to boost circulation.

The spleen is a peripheral lymphoid organ that plays a major role in the immune response against systemically acquired antigens (Abdul-Careem et al., 2007;Jeurissen, 1991). This organ contains immune cells such as T lymphocytes, B lymphocytes, macrophages, and granulocytes (Jeurissen, 1991). Therefore, various cytokines and small proteins playing important roles in immune responses are expressed in the spleen. RT-PCR and Western blot analysis in figures 3 and 4, respectively, showed dose dependent up regulation of splenic heat shock related genes and proteins such as HSF1, HSP90 and HSP70 for both under thermoneutral circumstances and heat stressed broilers. At the molecular level, thermal stress strongly modifies cell physiology (Bensaude et al., 1996). Cells from all organisms respond to physiologically relevant variations to temperature by rapidly increasing the expression and synthesis of a selected group of proteins, the heat shock proteins (Sonna et al., 2002).

CONCLUSION

Altogether, these findings may suggest that dietary supplementation of AGN root powder improves growth performance and health by modulating the expression of heat shock genes and proteins for both normal and heat stress reared Ross broiler chickens.

KEYWORD : Angelica gigas Nakai root powder, heat shock proteins, heat shock factor, heat stress, broiler chickens

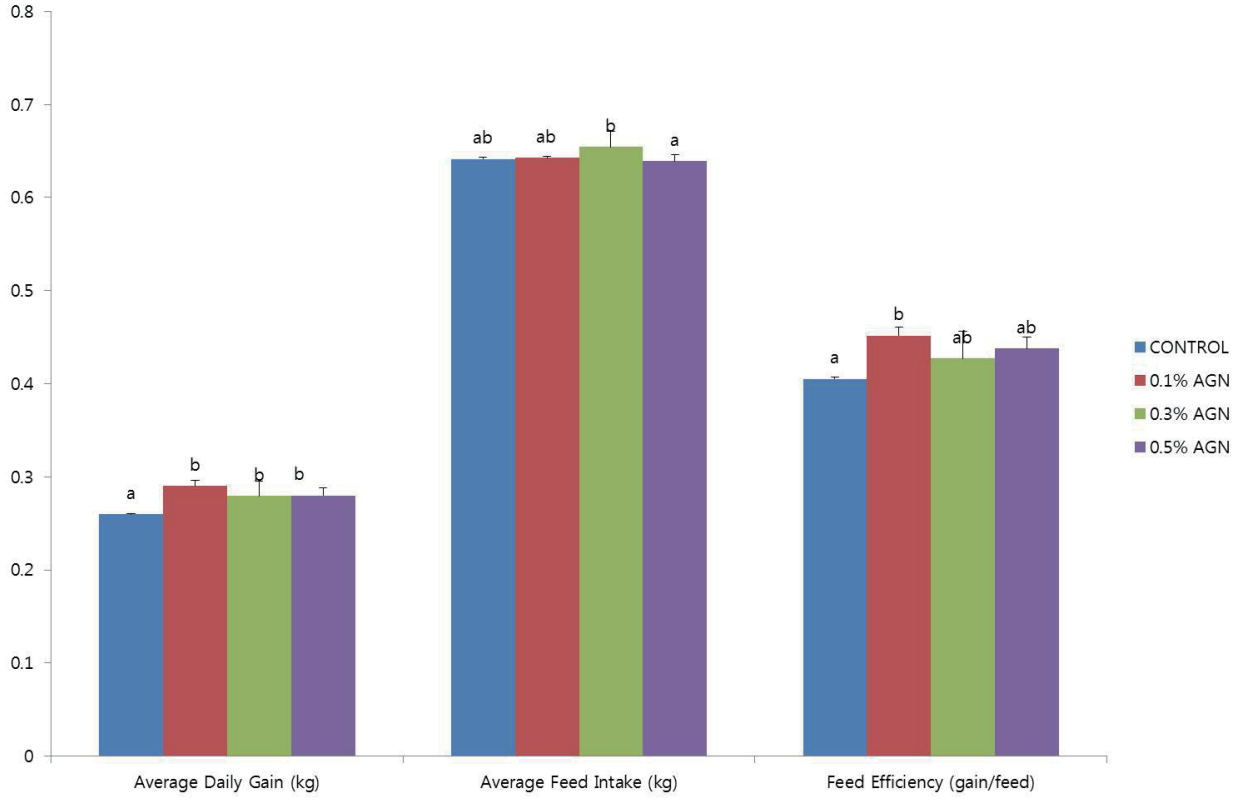

Figure 1. Effects of AGN powder supplementation on the growing performance of broiler chickens grown on normal environmental temperature.

The results are expressed as mean± standard deviation. Bars with different superscript are significantly different ($p<0.05$).

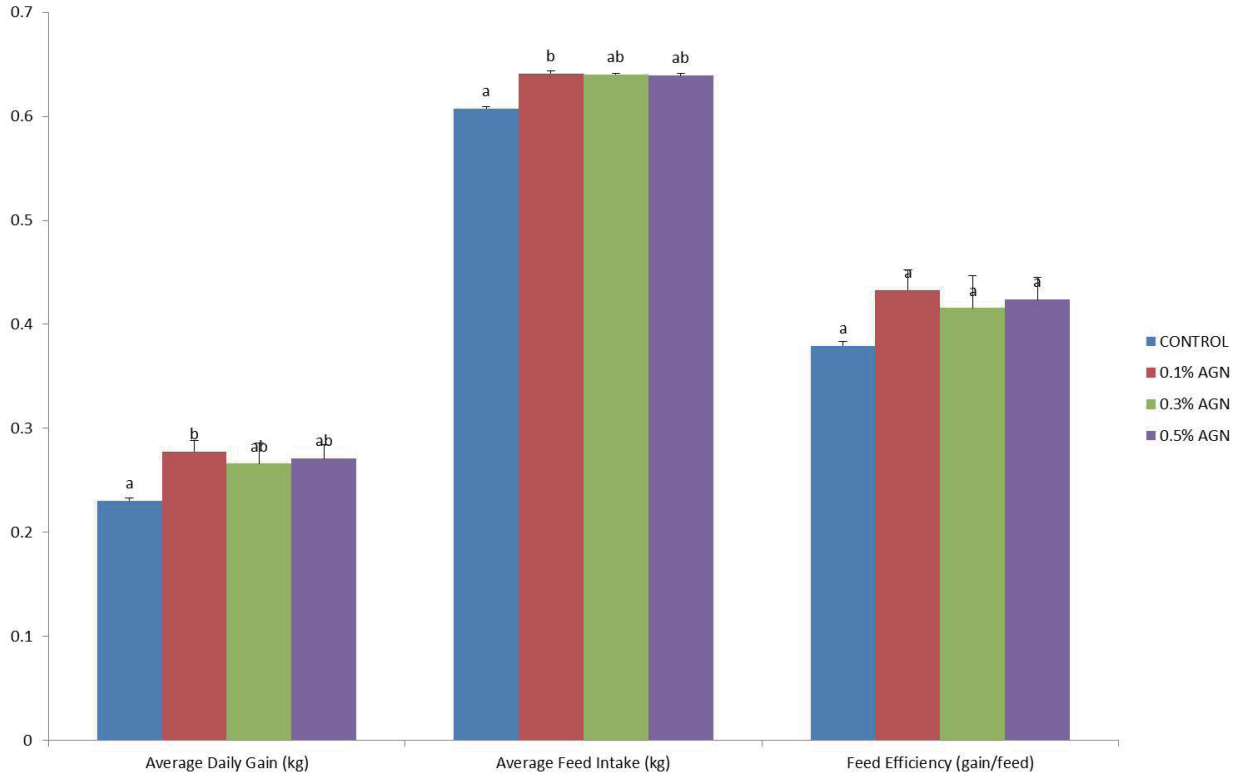

Figure 2. Effects of AGN powder supplementation on the growing performance of broiler chickens grown on increased environmental temperature.

The results are expressed as mean± standard deviation. Bars with different superscript are significantly different ($p<0.05$).

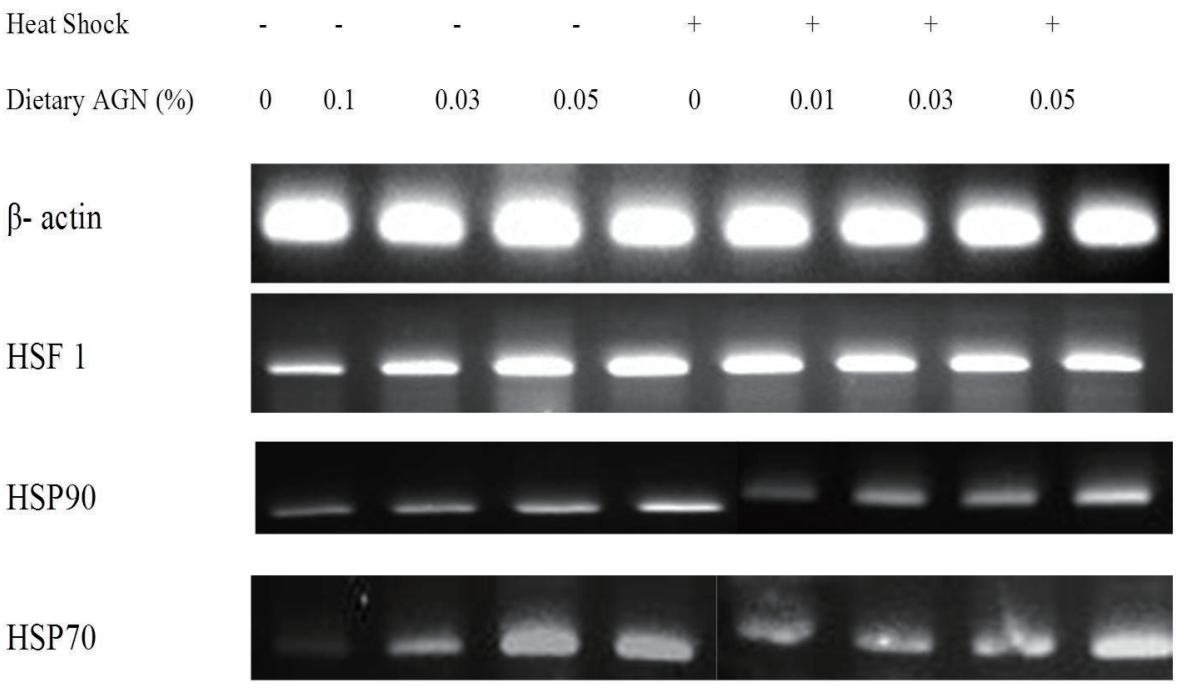

Figure 3. mRNA expressions of splenic heat shock related genes of control and AGN supplemented Ross broiler chickens grown on normal and increased environmental temperature.

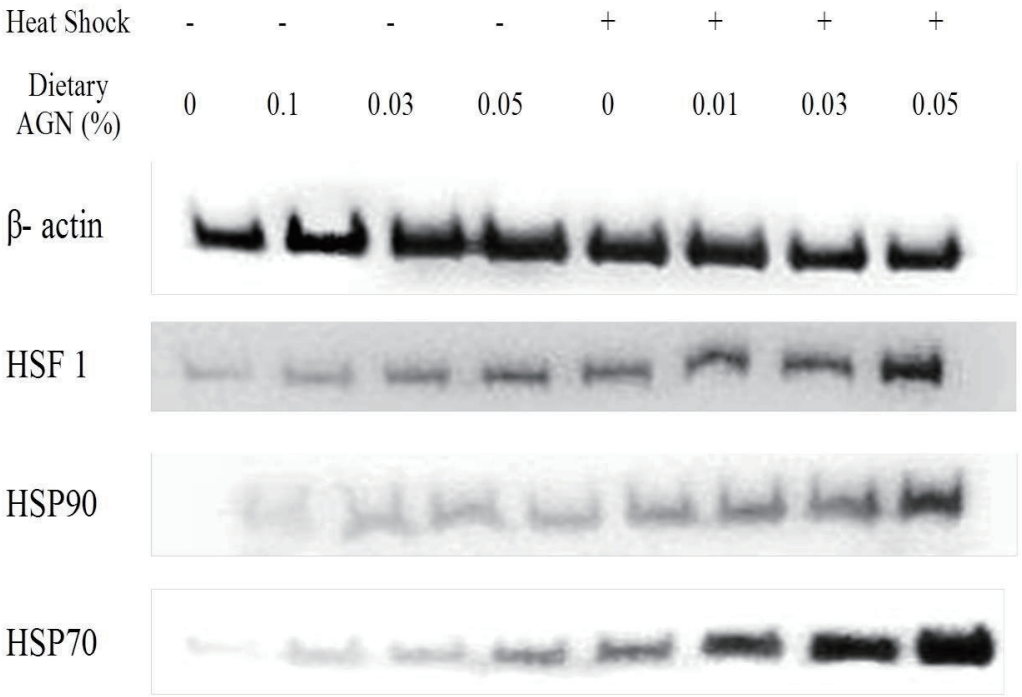

Figure 4. Protein expressions of splenic heat shock related genes of control and AGN supplemented Ross broiler chickens grown on normal and increased environmental temperature.

Table 1. Plasma biochemical analysis of normal and heat stressed broiler chickens supplemented with varying amounts of AGN.

	NON HEAT STRESS				HEAT STRESS			
Dietary AGN (%)	**0** (n=4)	**0.1** (n=4)	**0.3** (n=4)	**0.5** (n=4)	**0** (n=4)	**0.1** (n=4)	**0.3** (n=4)	**0.5** (n=4)
Glucose (mg/dl)	248.5±10.34^{a}	224.0±15.58^{a}	218.5±18.05^{ab}	241.7±13.45^{b}	229.5±17.94^{a}	223.0±14.07^{a}	238.75±16.5^{a}	223.5±11.47^{a}
Total Cholesterol (mg/dl)	145.25±20.84^{a}	143.25±11.24^{a}	132.25±10.81^{a}	152.25±17.23^{a}	161.75±9.29^{a}	145.5±12.26^{a}	161.0±24.10^{a}	139.75±11.35^{a}
Total Bilirubin (mg/dl)	0.15±0.10^{a}	0.15±0.10^{a}	0.2±0.08^{a}	0.175±0.10^{a}	0.13±0.05^{a}	0.225±0.19^{a}	0.275±0.17^{a}	0.125±0.05^{a}
GOT (IU/L)	186.50±12.26^{a}	188.25±11.76^{a}	178.25±17.00^{a}	180.5±15.07^{a}	187.0±12.68^{a}	171.5±11.73^{a}	176.0±24.29^{a}	166.5±13.43^{a}
Insulin (μIU/ml)	21.23±2.60^{a}	17.85±3.54^{ab}	16.62±3.78^{ab}	17.04±4.57^{a}	20.26±1.11^{a}	19.66±2.07^{b}	19.56±0.7^{b}	19.77±2.57^{c}
Triiodothyronine (T3) (ng/ml)	3.53±0.27^{a}	3.58±0.14^{a}	3.69±0.18^{ab}	3.31±0.23^{b}	3.81±0.16^{a}	3.714±0.06^{b}	3.84±0.15^{b}	3.63±0.89^{b}
Thyroxine (T4) (ng/ml)	221.39±30.36^{a}	203.09±69.29^{ab}	212.86±27.68^{ab}	204.32±28.39^{b}	255.71±22.97^{a}	256.39±49.23^{a}	283.12±18.27^{a}	269.10±22.06^{a}
Cortisol (ng/ml)	186.50±12.26^{a}	188.25±11.76^{b}	178.25±17.00^{b}	180.5±15.07^{c}	133.47±43.59^{a}	97.58±15.30^{b}	83.63±18.72^{b}	62.09±24.12^{b}

1Glucose: 180-400 mg/dL; Total cholesterol: 129-297 mg/dL;; Total Bilirubin: 0-0.1 mg/dL; GOT: 10-400 IU/L. Normal values for the plasma biochemistry parameters used in the study (The Merck Veterinary Manual, 10th ed, 2010).
All data are presented as mean ± SD. Results with different superscripts are significantly different at $P<0.05$.

REFERENCES

Abdul-Careem MF, Hunter DB, Lamboume MD, Barta J and Sharif S. Ontogeny of cytokhle gene expression in the chicken spleen. Poultry Science 86: 1351-1355. 2007.

Bensaude,O.,S. Bellier, M.F. Dubois, F. Giannoni, and VT. Nguyen. 1996. Heat-shock induced protein modifications and modulation of enzyme activities. Stress-Inducible Cellula rResponses EXS,77:199-219.

Jeurissen SH. Structure and fUnction of the chicken spleen. Research in 1mmunology l42:352-355.1991.

Khajavi M, Rahimi S, Hassan ZM, Kamali MA and Mousavi T. Effect of feed restriction early in life on humoral and cellular immunity of two comm ercial broiler strains under heat stress conditions. British Poultry Science 44: 490-497. 2003.

Sonna, L.A., J. Fujita,S.L. Gaffin, and C.M.Lilly 2002. Invited Review: Effects of heat and cold stress on mammalian gene expression. J.Appl.Physio l.92: 1725-1742.

O-07-7

Effect of Dried Banana (Musa sapientum L.) in Diet on Growth Performance and Intestinal Morphology of Nursery Pigs

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INTRODUCTION

The main cost of pig production industry is related to the provision of a sustainable energy source. Feed cost account for 60 to 80% of livestock production costs and the energy component of feed accounts for 40 to 60 % (Ademosun, 1976). Banana is an alternative feedstuff can be used as an energy source for animal feed because banana are a rich of carbohydrate and high contents of sugars, mainly sucrose, glucose and fructose (Imam and Akter, 2011; Tsen *et al.*, 2004). Moreover, Renaudeau *et al.* (2014) reported that grower pigs that were fed up to 60% banana in place of corn had no effect on final body weight, average daily gain, and feed conversion ratio.

In addition, banana also contains certain amounts of fructooligosaccharide (FOS). FOS are oligosaccharide which are not hydrolysed by digestive enzymes, and may act as growth substrate for the intestinal microflora (Monsan and Paul, 1995, Tsen *et al.*, 2004). They are considered as prebiotics. They have been shown to have beneficial effects on the intestinal microflora by stimulating the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* and inhibit pathogenic bacteria such as *E. coli* and *Salmonella* (Bailey *et al*., 1991; Xu *et al.,* 2003). For example, Xu *et al.* (2005) report that supplementation with FOS improved feed conversion ratio (FCR) and increasing the villi height in jejunum and ileum of small intestine in piglets. Therefore, this study focused on the effect of banana (*Musa sapientum L*.) in diet on growth performance and intestinal morphology of nursery pigs.

MATERIALS AND METHODS

This study was conducted at the Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok and Thailand. Experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Animals and managements

Sixty male commercial crossbred piglets (Duroc×Large White×Landrace; 8.00±0.27kg body weight) were used in this trial. The pigs were randomly divided into four treatments and each treatment consisted of five replications (three pigs/pens) of three pigs each. The average body weight of each replication were homogenized and balanced. During six weeks experimental periods, an evaporative cooling system was used to control air ventilation and temperature. Feed were offered as *ad libitum* and water were provided by water nipples. During the feeding trial, the house was cleaned weekly, while the faces of piglets were removed every day.

Experimental diets

Four experimental diets were used a control diet without the banana and diets containing 2.5%, 5.0% and 7.5% of dried banana (*Musa sapientum L*.); respectively. Chemical composition of banana is given in Table 1. The experimental diets were formulated to provide the same amount of nutrients and met the requirement as NRC (1998) without an antimicrobial agent.

Measurements

Growth performances. The body weight of each pigs was recorded and at the end of feeding trial (6 weeks) the body weight, body weight gain and feed intake was measured weekly. The feed conversion ratio (FCR) was calculated from body weight gain and feed intake data.

Morphology of small intestine. At the end of the trial, one pig from each replication were put down. Tissue samples were collected from the duodenum, jejunum and ileum and were immediately fixed in 10% neutral buffer formalin. For each specimen, at least 10 sections of 7 μm thicknesses were prepared. Tissues were then stained with haematoxylin-eosin for histological evaluation. Histology of the duodenum, jejunum and ileum tissue was assessed by light microscope in accordance with Nunez *et al*. (1996). The morphology of the small intestines in this study included villous height, crypt depth and the villous height to crypt depth ratio were conducted by a computer-assisted for image-analysis system (Biowizard, Thaitec, Thailand). The microscopic of histological sections were randomized and assessed the height of 10 villous and the depth of 10 crypts in each sample.

Statistical analysis

The data collected were subjected to one way analysis of variance (ANOVA) of SAS. (SAS Institute, 2000) in a completely randomized design arrangement. Duncan's Multiple Range Test was used to compare measured values obtained from the four independent groups on growth performance and intestinal morphology (Khattree and Naik, 2000).

RESULTS AND DISCUSSION

Growth performance

The effects of banana (*Musa sapientum L.*) in diet on growth performance are presented in Table 2. There was no significant effect of banana on final body weight, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) throughout the experimental period.

Banana (*Musa sapientum L.*) was used as a substituted for corn to supply 2.5%, 5.0% and 7.5% of the total diet for nursery pigs. Base on the chemical composition of banana in table 1, the experimental diet was formulated to provide the same amount of nutrients and met the requirement as NRC (1998). Increasing the level of incorporation of banana did not have a negative effect on body weight gain, ADG, ADFI and FCR. Similarly, growth performance remained constant in post-weaning pigs fed by diet with 15% and 30% of green banana meal (Restrepo and Gonzalo, 1973). In a study of Renaudeau *et al.* (2014) found that banana meal did not have a negative effect on ADG and FCR in grower pigs fed by diet with 20%, 40% and 60% of banana meal.

In a previous study, banana (*Musa sapientum L.*) contains 0.54 percent of fructooligosaccharide (FOS). The experimental diet was calculated to contain an inulin type fructan at 0.0%, 0.01%, 0.03% and 0.04%; respectively. In the present study, there were not any significant effects of banana on growth performance. This may be due to the FOS level is not enough in the diet. However, there many reported an improved ADG and FCR as a consequence of FOS inclusion in piglet diets (Fukuyasu *et al.*, 1987; Xu *et al.*, 2005). Other authors, however, reported no or slightly negative effects of FOS on growth performance in piglets (Kornegay *et al.,* 1992; Farnworth *et al*., 1992).

Intestinal morphology

Effects of banana (*Musa sapientum L.*) in the diet on intestinal morphology are shown in Table 3. There was no significant effect of banana on villous height and crypt depth of duodenum, jejunum and ileum ($P > 0.05$). Consequently, there were not any significant effects of banana on the ration of villous height: crypt depth in each segment of the small intestine.

Fructooligosaccharide (FOS) are oligosaccharide which are not hydrolysed by digestive enzymes, but FOS was fermented by *Bifidobacterium* and *Lactobacillus* induced short-chain fatty acids (SCFA) production in the large intestine (Hartemink *et al.,* 1997). SCFA, butyrate in particular, support the major function of epithelial cells, such as water, mineral and nutrient absorption. SCFA production is related to the development of intestinal villous and crypts (Scheppach et al., 1995). The increase in the height of the villous increases the surface area for nutrient absorption (Buket and Ebru, 2015).

In this study, used as a substituted for corn to supply 0%, 2.5%, 5.0% and 7.5% in the diet by banana (*Musa sapientum L.*). The banana contains 0.54 percent of fructooligosaccharide (Megazyme Fructan HK Assay kit; Mccleary *et al*., 2000). The experimental diet was calculated to contain an inulin type fructan at 0%, 0.01%, 0.03% and 0.04%; respectively. In the present study, there were not any significant effects of banana on the ration of Villous height: Crypt depth in each segment of the small intestine ($P>0.05$). This may be due to the FOS level is not enough in the diet. However, Xu *et al.* (2005) reported that supplementation with 0.4% FOS improved feed conversion ratio (FCR) and increase the villous height in jejunum and ileum in piglets. Because, Feeding FOS to young piglets may have other effects. A number of complex sugars have been shown to alter the morphology of the intestinal lining, presumably through increased production of SCFA (Tellez *et al*., 1993; Howard *et al*., 1995).

CONCLUSION

Banana (*Musa sapientum L.*) can be used as an energy source in diets. Since, banana has not had a negative effect on growth performance and intestinal morphology. In conclusion, banana can be used to incorporate up to 7.5% in nursery pig diets.

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Thailand for the suggestion, guidance and support throughout this trial.

KEYWORD：Banana, Growth Performance, Intestinal Morphology, Nursery Pigs

Table 1. Chemical composition of dried banana (*Musa sapientum L.*)

Item	**Banana**
GE (Gross energy) kcal/kg	3638.52
Moisture %	5.60
Crude Protein %	3.02
Fiber %	1.35
Ash %	4.54
Calcium %	0.14
Total Phosphorus %	0.06
Reducing Sugar %	8.63
Total Sugar %	13.89
*Inulin (Fructan) %	0.54

*Inulin type fructan (Megazyme Fructan HK Assay Kit; McCleary et al. 2000).

Table 2. Effect of banana (*Musa sapientum L.*) in diet on growth performance of nursery pigs

Item	Control	Level of banana (%)			P-value	SEM
		2.5	5.0	7.5		
Initial body weight (kg)	8.00	8.00	8.00	7.99	0.99	0.05
Final body weight (kg)	32.31	31.82	31.91	31.99	0.98	0.38
Body weight gain (kg)	24.31	23.82	23.91	24.00	0.97	0.37
ADFI (kg/day)	0.89	0.85	0.86	0.88	0.87	0.02
ADG (kg/day)	0.58	0.57	0.57	0.57	0.97	0.01
FCR	1.54	1.50	1.51	1.53	0.62	0.01

Table 3. Effect of banana (*Musa sapientum L.*) in diet on intestinal morphology of nursery pigs

Item	Control	Level of banana (%)			P-value	SEM
		2.5	5.0	7.5		
Villous height (μm)						
Duodenum	629.00	631.18	616.74	670.24	0.91	24.01
Jejunum	643.93	617.24	568.35	577.49	0.50	19.11
Ileum	490.62	539.10	501.65	572.01	0.49	19.90
Crypt depth (μm)						
Duodenum	302.72	306.13	294.63	312.02	0.93	8.67
Jejunum	296.15	275.53	276.78	302.38	0.53	7.65
Ileum	202.07	220.06	222.68	239.42	0.83	13.25
Villous height:crypt depth ratio						
Duodenum	2.09	2.11	2.10	2.12	0.99	0.09
Jejunum	2.21	2.25	2.08	1.91	0.38	0.07
Ileum	2.55	2.55	2.49	2.48	0.99	0.15

REFERENCES

Ademosun, A. (1976). Livestock Production in Nigeria: Our Commissions and Omissions, Inaugural Lecture Series 17 delivered at the University of Ife on February 29 1976: Ife: University of Ife Press.

Bailey, J., L. Blankenship and N. Cox. 1991. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poult. Sci. 70 (12): 2433-2438.

Farnworth, E., H. Modler, J. Jones, N. Cave, H. Yamazaki and A. Rao. 1992. Feeding Jerusalem artichoke flour rich in fructooligosaccharides to weanling pigs. Can. J. Anim. Sci. 72 (4): 977-980.

Fukuyasu, T., T. Oshida and K. Ashida. 1987. Effect of oligosaccharides on growth of piglets and on bacterial flora, putrefactive substances and volatile fatty acids in their feces. Bulletin of Animal Hygiene. 26: 15-22.

Howard, M. D., D. T. Gordon, L. W. Pace, K. A. Garleb and M. S. Kerley. 1995. Effects of dietary supplementation with fructooligosaccharides on colonic microbiota populations and epithelial cell proliferation in neonatal pigs. J. Pediatr. Gastroenterol. Nutr. 21 (3): 297-303.

Imam, M. Z. and S. Akter. 2011. Musa paradisiaca L. and Musa sapientum L.: a phytochemical and pharmacological review. J. App. Pharm. Sci. 1: 14-20.

Khattree, R. and D. N. Naik. 2000. Applied multivariate statistics with SAS software. SAS Institute.

Kitt, S. J., P. S. Miller and A. Lewis. 2001. Factors affecting small intestine development in weanling pigs. Nebraska Swine Reports: 99.

Kornegay, E., C. Wood and L. Eng. 1992. Effectiveness and safety of fructooligosaccharides for pigs. J. Anim. Sci. 70 (Suppl 1): 19.

Mccleary, B. V., A. Murphy and D. C. Mugford. 2000. Measurement of total fructan in foods by enzymatic/ spectrophotometric method: collaborative study. J. AOAC. Int. 83: 356-364.

Monsan, P. F. and F. Paul. 1995. Enzymatic synthesis of oligosaccharide. FEMS. Microbiol. Revs. 16: 233-245.

National Research Council. 1998. Nutrient requirements of swine. 10th ed. National Academy Press, Washington, DC.

Nunez M. C., J. D. Bueno, M. V. Ayudarte, A. Almendros, A. Rios, M. D. Suarez and A.Gil. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursery piglets. Nutr. 126: 933-944.

Pluske, J. R., D. J. Hampson and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock production science. 51 (1): 215-236.

Pluske, J., I. Williams and F. Aherne. 1995. Nutrition of the neonatal pig. The neonatal pig: development and survival: 187-235.

Renaudeau, D., J. Brochain, M. Giorgi, B. Bocage, M. Hery, E. Crantor, C. Marie-Magdeleine and H. Archimède. 2014. Banana meal for feeding pigs: digestive utilization, growth performance and feeding behavior. Int. J. Anim. Biosci. 8: 565-571.

Restrepo, L. G. A. 1973. Digestibilidad, valor nutritivo y energtíco del pltàno, Musa paradisiaca L., en cerdos. Thesis, Universidad Nacional de Colombia. Facultad de Agronomá.

SAS. 1988. SAS User's Guide, Statistics. SAS Institute, Cary, North Carolina.

Scheppach, W., H. Bartram and F. Richter. 1995. Role of short-chain fatty acids in the prevention of colorectal cancer. Eur. J. Cancer. 31 (7): 1077-1080.

Tellez, G., C. Dean, D. Corrier, J. Deloach, L. Jaeger and B. Hargis. 1993. Effect of dietary lactose on cecal morphology, pH, organic acids, and Salmonella enteritidis organ invasion in Leghorn chicks. Poult. Sci. 72 (4): 636-642.

Tsen, J.-H., Y.-P. Lin and V. a.-E. King. 2004. Fermentation of banana media by using κ -carrageenan immobilized Lactobacillus acidophilus. Int. J. Food. Microbiol. 91 (2): 215-220.

Xu, C., X. Chen, C. Ji, Q. Ma and K. Hao. 2005. Study of the Application of Fructooligosaccharides in Piglets. Asian-Aust. J. Anim. Sci. 18 (7): 1011-1016.

Xu, Z., C. Hu, M. Xia, X. Zhan and M. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82 (6): 1030-1036.

O-07-8

Effect of Dietary Fish Oil in Combination with Tomato Powder on Egg Polyunsaturated Fatty Acids Profile of Native Laying Hens

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INTRODUCTION

Recently, omega-3 polyunsaturated fatty acids (n-3 PUFA) are known as an important substance to support human health. Consumption of n-3 PUFA could give some physiological effects and associated health benefits including cardioprotective effect, antihypertensive, antithrombotic, anticancer, inhibit tumor growth, and also can support visual and cognitive development (Klek, 2016). However, although possess interesting health benefits, most of the people worldwide still does not meet daily minimum intake of this substance (Sioen et al. 2009).

In order to find an alternative of n-3 PUFA source, development of n-3 PUFA enriched egg become an attracting research topic. This idea is turn up because of egg consumption is increasing year by year (Windhorst, 2011) and PUFA profile of yolk can be easily modified through nutritional strategies (Fraeye et al. 2012). Production of n-3 PUFA enriched-egg can be done by adding some dietary supplements rich in n-3 PUFA (e.g. fish oil, flaxseed, or microalgae) in laying hens diet. Moreover, fish oil was shown to be best sources due to its higher efficiency to accumulate n-3 PUFA to the egg yolk. Lemahieu et al. (2015) reported that n-3 long chain PUFA accumulation to the egg yolk by dietary fish oil supplementation was at the rate of 55%, compared with dietary flaxseed and microalgae in the rate of 6% and 30%, respectively. However, dietary fish oil supplementation in laying hens diet is very susceptible to oxidation, and as a consequence, it should be supplemented in combination with dietaryantioxidant.

Tomato is a major source of lycopene, one of well-known substance which has strong antioxidant activity, in the amount of 0.9-55.5 mg/100g (Chauhan et al 2011). Other antioxidant substances which also found in tomato are vitamin A, vitamin C, vitamin E, and folic acid (Borguini and Torres 2009, Kotíková et al 2011). These substances make tomato as one of the potential antioxidant sources. Furthermore, the aim of this experiment was to investigate the effect of dietary fish oil in combination with tomato powder on egg polyunsaturated fatty acids profile of native laying hens.

MATERIALS AND METHODS

Birds and dietary treatments

Ninety 30-weeks-old female native laying hens (*Gallus turcicus*) were randomly distributed in a completely randomized design with 3 treatments and 6 repetitions (5 birds of each repetition). Birds were fed one of three dietary treatments, either containing 2.5% palm oil (control), 2.5% fish oil (FO), or 2.5% fish oil + 0.5% tomato powder (FOTP) during 6 weeks. During experimental periods dietary treatments was supplied twice daily in the amount of 100 g/bird (50 g/bird was fed in the morning at 08.00 am and another 50 g/bird was given in the afternoon at 02.00 pm), while drinking water was supplied *ad libitum*. All of the dietary treatments were formulated to be *isocaloric* (2700 Kcal/kg of metabolizable energy) and *isonitrogenous* (17.30% of crude protein) and met Indonesian National Standard (2006) for layer nutrition. Feedstuff composition and calculated nutrient content of each dietary treatments are shown in Table 1. PUFA profile of palm oil and fish oil used in this experiments are shown in Table 2.

Fatty acid analysis

At the last day of the experiment, 54 egg (3 egg from each repetition) were randomly collected and directly transported to Centre for Agro-Based Industry, Bogor-16122, West Java, for PUFA analysis (n-3 and n-6 content).

Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) and significant treatment was further tested by Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

Effect of dietary fish oil and its combination with tomato powder supplementation on egg PUFA profile of native laying hens are presented in Table 3. Results showed that dietary fish oil (FO) and its combination with tomato powder (FOTP) had higher ($P<0.05$) egg n-3 contents as compared to that of control. Egg n-3 contents of control, FO, and FOTP groups were 159.25, 326.10, and 393.70 mg/egg, respectively. This finding is in agreement with previous studies which also found that fish oil supplementation could increase n-3 contents of egg (Carrillo-Domínguez et al. 2012, Pita et al. 2011). Fish oil contains high amount of n-3 PUFA (Table 2) which led to increasing n-3 PUFA content in the feed, and give higher intake of n-3 PUFA, then will allow higher deposition of this substance to the egg.

Table 3 showed that dietary control, FO, and FOTP had similar trends ($P>0.05$) on egg n-6 contents with averages of 631.75, 627.11, and 673.56 mg/egg, respectively. In contrast, Carrillo-Domínguez et al. (2012) reported that fish oil supplementation could decrease egg n-6 content. This different result may be affected by different basal oil used in the experiment. In this current experiment, basal oil used is palm oil which had lower n-6 content (12.10%) than that of soybean oil (60.24%) which used by Carrillo-Domínguez et al. (2012).

Supplementation of fish oil, either as a single component (FO) or in combination with tomato powder (FOTP) had lower ($P<0.05$) egg n-6/n-3 ratio with the amount of 1.96 and 1.71, respectively, as compared to that of control group (4.10). This finding is in accordance with previous study which also noted that fish oil and its combination with antioxidant supplementation could decrease egg n-6/n-3 ratio (Carrillo-Domínguez et al. 2012, Carillo et al. 2008).

CONCLUSION

The conclusion of this research is that dietary fish oil and its combination with tomato powder supplementation could increase egg n-3 contents and decrease egg n-6/n-3 ratio in native laying hens.

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KEYWORD : Antioxidant, Functional Food, Omega-3, Omega-6, Poultry

Tabel 1. Feedstuff composition (%) and nutrient content of dietary treatments

Feedstuff	Control	FO	FOTP
Corn	40.00	39.30	39.90
Rice polishing	29.50	25.80	24.70
Soybean meal	16.50	22.10	22.00
Corn gluten meal	4.50	0.80	0.90
Palm oil	2.50	-	-
Fish oil	-	2.50	2.50
Tomato powder	-	-	0.50
DL-methionine	0.10	0.10	0.10
Salt	0.10	0.10	0.10
Limestone	7.80	7.80	7.80
Vitamin-mineral mix	1.50	1.50	1.50
Total	100.00	100.00	100.00
Calculated nutrient content			
Metabolizable energy, Kcal/kg	2,700.00	2,700.00	2,700.00
Crude protein, %	17.30	17.30	17.30
Ether extract, %	6.14	7.96	7.84
Crude fiber, %	2.34	2.31	2.41
Calcium, %	3.31	3.31	3.32
Phosphorus, %	0.32	0.32	0.32
Lysine, %	0.80	0.93	0.92
Methionine, %	0.42	0.4	0.39
Methionine + Cysteine, %	0.64	0.61	0.61

Table 2. Polyunsaturated fatty acid profile of palm oil and fish oil (expressed as % of total fatty acid)

Oil source	n-3	n-6	n-6/n-3
Palm oil	0.30	12.10	40.33
Fish oil	26.03	2.03	0.08

Table 3. Effect of dietary fish oil in combination with tomato powder on egg polyunsaturated fatty acids profile of native laying hens (expressed as mg/egg)

Treatments	n-3	n-6	n-6/n-3
Control	159.25±49.80^{a}	631.75±125.15	4.10±0.58^{b}
FO	326.10±79.68^{b}	627.11±104.84	1.96±0.17^{a}
FOTP	393.70±22.85^{b}	673.56±38.57	1.71±0.01^{a}

Notes: Control: feed containing 2.5% palm oil, FO: feed containing 2.5% fish oil, FOTP: feed containing 2.5% fish oil + 0.5% tomato powder

^{ab} means in the same column followed by different letters are significantly different at $P<0.05$

REFERENCES

Borguini RG. & Torres EAFDS. 2009 Tomatoes and tomato products as dietary sources of antioxidants. Food Review International, 25: 313–325.

Carillo S., López E., Casas MM., Avila E., Castillo RM., Carranco ME., Calvo C., & Pérez-Gil F. 2008. Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs. Journal of Applied Phycology, 20: 721-728.

Carrillo-Domínguez S., Avila GE., Vásquez PC., Fuente B., Calvo CC., Carranco JME., & Pérez-Gil Romo F. 2012. Effects of adding vitamin E to diets supplemented with sardine oil on the production of laying hens and fatty-egg acid composition. African Journal of Food Science, 6(1): 12-19.

Chauhan K., Sharma S., Agarwal N., & Chauhan B. 2011. Lycopene of tomato fame: its role in health and disease. International Journal of Pharmaceutical Sciences Review and Research, 10: 99-115.

Fraeye I., Bruneel C., Lemahieu C., Buyse J., Muylaert K., & Foubert I. 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Food Research International, 48: 961–969.

Indonesian National Standard (SNI). 2006. Layer nutrition (in Bahasa Indonesia). National Standardization Agency of Indonesia. Jakarta.

Klek, S. 2016. Omega-3 fatty acids in modern parenteral nutrition: a review of the current evidence. Journal of Clinical Medicine, 5 (34): 1-16.

Kotíková Z., Lachman J., Hejtmánková A., & Hejtmánková K. 2011. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. LWT - Food Science and Technology, 44: 1703-1710.

Lemahieu C., Bruneel C., Ryckebosch E., Muylaert K., Buyse J., & Foubert I. 2015. Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. Journal of Functional Foods, 19: 821-827.

Pita MCG., de Carvalho PR., Neto EP., & de Mendonça Júnior CX. 2011. Modulation of plasma levels and percentages of incorporation of w-3 PUFAs in egg yolk under the influence of supplementation sources rich in omega 3 to diet of laying hens. Pakistan Journal of Nutrition, 10(8): 735-754.

Sioen I., De Henauw S., Van Camp J., Volatier J-L., & Leblanc J-C. 2009 Comparison of the nutritional-toxicological conflict related to seafood consumption in different regions worldwide. Regulatory Toxicology and Pharmacology: RTP, 55: 219–228.

Windhorst, HW. 2011. Special Economic Report: The Role of the Egg in the Global Poultry Industry. Accessed https://www.internationalegg.com/corporate/downloads/details.asp?id=1493

O-07-9

Effect of α -Galactosidase Supplementation in Diet on Egg Production and Egg Quality of Laying Hens

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INTRODUCTION

Soybean meal (SBM) used as a high-quality protein source in most swine producing and poultry producing countries. There was well-balanced amino acid pattern but energy utilization was poor (Odetallah *et al.*, 2002). SBM consisted of 48% protein, 35-40% carbohydrates, 7-10% water, 5-6% minerals and less than 1% fat (3-4% of acid hydrolyzed fat) (USDA, 2009). The carbohydrates in soybean consist of approximately 10% oligosaccharides (5% sucrose, 4% stachyose and 1% raffinose) and between 20-30% NSP, in which approximately 8% are cellulose and the remaining are pectic polysaccharides (Choct, 1997). All of those oligosaccharides were potential anti-nutritional factors (Coon *et al*., 1990). The problems related with the α -galactosidic oligosaccharides cannot be broken down in the small intestine of monogastric animal because of the absence of endogenous α -1,6 galactosidase (Gitzelmann and Auricchio, 1965). That may increase digesta passage rate, decrease digestion and absorption of dietary nutrients (Coon *et al*., 1990). The objectives of this experimental were to evaluate effect of alpha-galactosidase supplementation in diet on egg production and egg quality of laying hens.

There were so many techniques to eliminate those oligosaccharides such as Coon *et al*. (1990) reported that removal of raffinose and stachyose in SBM by ethanol extraction greaty increased the TMEn of SBM and reduced digesta passage and feeding with exogenous α -1,6 galactosidase that increased release of reducing sugar from SBM because galato-oligosaccharides were hydrolysis by enzyme (Ao *et al*., 2010). Addition with exogenous α -1,6 galactosidase could be a simply way to digest α -galactosidic bond and short time consuming.

The ability of α -galactosidase could increase efficiency of digest α -galactosidic oligosaccharides from α -1,6 galactooligosaccharide to monosaccharides (glucose, galactose, fructose), reduce passage and viscosity of digesta in gastrointestinal tract, improves nutrient availability and Metabolisable energy (ME) of soybean meal. All of those reasons could improve growth performance and egg production in poultry.

In the previous research, Scheideler and Webber (2003) studied the role of α -galactosidase that improved egg production and the ME of the diet in laying hens but egg quality had no different. Ao *et al.* (2009) shown that supplementation with α -galactosidase could increase AMEn of corn-soybean diet. Which is in agreement the results by Knap *et al*. (1996) reported that addition of α -galactosidase improved feed conversion ratio of broiler. Wang *et al.* (2005) reported that α -galactosidase supplementation linearly improved body weight, feed intake and average daily gain. On the other hand, feeding with α -galactosidase was not significant difference on growth performance but the enzyme could degrade raffinose and stachyose (Graham *et al.,* 2002). Therefore, the objectives of this experimental were to evaluate effect of alpha-galactosidase supplementation in diet on egg production and egg quality of laying hens.

MATERIALS AND METHODES

Animal and Management

A total of 576 Lohmann Brown-Classic hens were used. At 27 weeks of age, the hens were divided into 24 units and each group consists of 24 hens. At 28 weeks of age, all 24 units would be randomly divided into 3 groups, each group consisted with 8 replications. During 12 weeks experiment, an evaporative cooling system was used to control air ventilation and temperature. The hens were being housed in wire cages with 4 birds per cage. Therefore, 4 adjacent cages were used as the replicate. The lighting program was set 16 hours. Feed were offered 2 times daily as ad libitum and water were provided by water nipples.

Experimental diets

The exogenous enzyme used in this study wa α -galactosidase (activity 176 IU/kg of product). The experiment diets were 1) positive control diets were formulated to meet nutrient standards for metabolizable energy and amino acid in a leading recommended guidelines 2) positive control diet decreased 88 ME kcal/kg with supplemental α -galactosidase 0.022% and 3) positive control diet decreased 88 ME kcal/kg without supplemental α -galactosidase 0.022% (negative control). Inert filler (corn starch) was added as need to adjust for differences in quantity of added enzyme. Compositions of diet are shown in Table 1 and 2.

Parameters record

Egg production

At 2 weeks interval, The egg production were accomplished as following egg production (%), mean egg weight, feed consumption, egg mass (g/hen/d), feed conversion ratio (FCR) (kg feed/kg egg mass) and mortality (%).

Egg quality

At the end of the 2 weeks interval, all eggs from each experimental unit were weighted, and 4 eggs from each replication that have weight close to the replication's mean were chosen to analyze egg qualities such as specific gravity, shell thickness (mm), albumen (mm), albumen (%), yolk (%), eggshell (%), albumen : yolk ratio, Haugh unit and yolk color.

Statistical analyses

Data will be evaluated with ANOVA in a completely randomized design. Differences means among treatments will be tested for significance by using the Duncan's multiple range tests at 5% significance level (Duncan, 1955).

RESULTS AND DISCUSSIONS

Egg production

The effects of α -galactosidase supplementation on egg production fed experimental diets were shown in Table 3. Feeding with α -galactosidase diet were significantly improved egg production ($p<0.05$), egg mass ($p<0.05$) and FCR ($p<0.01$) than those fed with negative control diet. There was no significant effect of egg weight, feed intake and mortality. The galacto-oligosaccharides in SBM were not digested by monogastric animals. This α -Galactosidase could afford digestible nutrients and animal energy.

Knudsen (1997) reported that there were 60 g/kg of oligosaccharide that could not digest but supplemental with α -galactosidase could broke down these oligosaccharide and maybe gave approximately 240 ME kcal/kg. Waldroup et al. (2006) report that diets were formulated in which the ME of SBM was increased by 10% in anticipation of improvements from the addition of α -Galactosidase enzyme but the performance of chick were not different.

Scheideler and Weber (2003) who found that egg production were enhanced ($p<0.05$) by feeding with α -galactosidase. Hens fed the negative control diet had lower egg production compared to the positive control. This is in agreement with Wang *et al*. (2005) research. Their study linearly increased BW, ADFI and ADG at day 21 of feeding period. In accordance with Ao *et al*. (2009) who found that supplementing α -Galactosidase in diet was significantly increased body weight gain and feed intake during the overall 21 days period and SBM-based diet that supplemental with α -Galactosidase has been verified significant improvement on energy bioavailability, feed conversion ratio and weight gain of broilers (Knap *et al.*, 1996). In the other hand, feeding with α -galactosidase was not significant difference on growth performance but the enzyme could degrade raffinose and stachyose (Graham *et al*., 2002). As same as Waldroup *et al.* (2006) reported that FCR were not different effected by α -Galactosidase supplementation.

Egg weight, feed intake and mortality were not significantly affected by α -Galactosidase treatments. In one study, Kidd *et al.* (2001) reported reduced mortality during heat stress in birds fed an enzyme with α -Galactosidase activity.

Egg Quality

The effect of α -galactosidase supplementation on egg quality fed experimental diets were shown in Table 4. Supplementation with α -galactosidase diet were significantly improved yolk percentage ($p<0.05$) and specific gravity ($p<0.01$) than those fed with negative control diet. The other parameters were not significant ($p>0.05$). On the other hand, Scheideler and Weber (2003) who found that egg quality were not significant affected by feeding with α -galactosidase.

This α -Galactosidase enzyme could hydrolysis that raffinose to glucose and galactose and released energy for birds to maintain their life. Also decreased oligosaccharides concentration would potentially reduce viscosity of digesta, leading to slower digesta passage rate, greater access of digestive enzymes to substrates and more rapid diffusion of absorbable nutrients to intestinal mucosa (Graham *et al.,* 2002). In addition, Zhang *et al***.** (2010) found that feeding with α -Galactosidase significantly reduced the chyme viscosity of ileum. From the nutritional viewpoint, the protein or amino acids liberation induces by that enzyme would have a sparing effect on supplemented levels of proteins and crystalline amino acid, which would decrease the cost of poultry diets (Wang *et al.*, 2005)

CONCLUSION

This research supported the hypothesis that supplementation with α -Galactosidase improved egg production and egg quality from soybean meal. Cost saving could be substantial with addition of α -Galactosidase enzyme and equal performance to positive control.

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KEYWORD：-Galactosidase, Egg production, Egg quality, Laying hen

Table 1 Feed ingredient of experimental diets.

Ingredient name	Control	α-Galactosidase	Negative Control
Corn	57.20	59.25	59.25
Rice bran oil	2.21	0.53	0.53
Rice solvent bran	4.00	4.00	4.00
Soybean Meal 48%	24.36	23.99	23.99
DL-Methionine	0.12	0.12	0.12
Monocalciumphosphate 22%	1.55	1.54	1.54
Calcium carbonate	8.91	8.92	8.92
Salt	0.25	0.25	0.25
Premix*	0.50	0.50	0.50
Choline Chloride 60%	0.08	0.08	0.08
Corn starch	0.02	-	0.02
α-galactosidase	-	0.02	-
Sodium bicarbonate	0.80	0.80	0.80
Total	100.00	100.00	100.00

*Premix: consist of vitamin A 5.0 MIU; D3 MIU; E 4,000 IU; K3 0.6 g; B1 0.8 g; B6 1.2 g; B12 0.0025 g; nichotinic acid 5.00 g; pentotinic acid 3.76 g; folic acid 0.2 g; biotin 0.036 g; Mn 24.00 g; Zn 20.00 g; Fe 16.00 g; Cu 4.00 g; Iodine 0.8 g; Co 0.08 g; Se 0.04 g and carrier added to 1.00 kg premix

Table 2 Chemical composition of experimental diets

Ingredient name	Control	α-Galactosidase	Negative Control
ME for poultry (Kcal/Kg)	2750.00	2750.00**	2662.00
Crude Protein (%)	17.00	17.00	17.00
Crude Fat (%)	4.53	2.94	2.94
Crude Fiber (%)	3.38	3.41	3.41
Calcium (%)	3.73	3.73	3.73
Available Phosphorus (%)	0.38	0.38	0.38
Salt (%)	0.25	0.25	0.25
Lysine (%)	0.87	0.87	0.87
Methionine + Cystine (%)	0.68	0.68	0.68
Methionine (%)	0.39	0.39	0.39
Threonine (%)	0.63	0.62	0.62
Tryptophan (%)	0.19	0.19	0.19

**α-Galactosidase 0.02g gave 88 Kcal/Kg of ME

Table3 Effect of α-galactosidase supplementation in diet on egg production of laying hens

Item	Control	α-Galactosidase	Negative control	SEM	P-value
Egg production (%)	92.36 ± 1.64[a]	92.29 ± 1.54[a]	89.68 ± 2.89[b]	0.49	0.03
Egg weight (g)	61.28 ± 1.10	61.53 ± 0.86	61.14 ± 1.07	0.20	0.74
Egg mass	56.61 ± 1.55[a]	56.80 ± 1.38[a]	54.82 ± 1.58[b]	0.35	0.03
Feed intake (g)	115.32 ± 3.70	117.03 ± 2.58	116.17 ± 2.02	0.58	0.50
FCR	2.04 ± 0.05[b]	2.06 ± 0.03[b]	2.12 ± 0.07[a]	0.01	0.01
Mortality	0.29 ± 0.44	0.65 ± 1.01	0.13 ± 0.24	0.13	0.28

*a,b Treatment means with different superscripts in the same row are significantly different (P<0.05). Values reported represent the mean ± SD.

Table4 Effect of α-galactosidase supplementation in diet on egg quality of laying hens

Item	Control	α-Galactosidase	Negative control	SEM	P-value
Specific gravity	1.094 ± 0.00[ab]	1.095 ± 0.00[a]	1.093 ± 0.00[b]	0.00	0.01
Shell Thickness (mm)	0.411 ± 0.03	0.405 ± 0.01	0.390 ± 0.02	0.00	0.17
Albumen (mm)	8.03 ± 0.46	8.43 ± 0.38	8.42 ± 0.50	0.10	0.15
Albumen (%)	64.98 ± 0.96	65.66 ± 0.42	65.66 ± 0.71	0.16	0.12
Yolk (%)	25.22 ± 0.91[a]	24.40 ± 0.55[b]	24.49 ± 0.58[b]	0.16	0.05
Eggshell (%)	9.81 ± 0.28	9.92 ± 0.24	9.85 ± 0.30	0.05	0.70
Albumen:Yolk ratio	2.59 ± 0.13	2.70 ± 0.07	2.69 ± 0.09	0.02	0.07
Haugh unit	89.09 ± 2.55	91.19 ± 2.11	91.21 ± 2.73	0.53	0.17
Yolk color	5.73 ± 0.48	5.96 ± 0.19	5.68 ± 0.36	0.08	0.30

*a,b Treatment means with different superscripts in the same row are significantly different (P<0.05). Values reported represent the mean ± SD.

O-08-2

PHENOTYPIC RELATIONSHIPS AMONG SOMATIC CELL COUNT AND UDDER TRAITS IN DAIRY GOATS

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Objectives

The aim of present study was to evaluate the phenotypic relationships among the lactation average of somatic cell count with eight udder traits in Saanen goats.

Methodology

Information from 3686 records of Saanen goats, of 123 herds belonging to the American Dairy Goat Association (ADGA) from the United Stated of America was used. All animals were classified based on fifteen conformation, or type traits, and from them, eight udder traits (UT) which were: fore udder attachment (FUA), rear udder height (RUH), rear udder arch (RUA), medial suspensory ligament (MSL), udder depth (UDE), teat placement (TPL), teat diameter (TED) and rear udder side view (RSV). Also was evaluated final score (FIS), a variable which consider all conformation appraisal in the animals. A scale of 50 to 99 points was used for FIS trait, while for all other type traits a scale of 1 to 50 points was used, according to the linear appraisal system from the ADGA (ADGA, 1993).

Milk samples were collected as part of the official national milk recording system used by ADGA. Individual data on milk yield were obtained monthly during the complete lactation by using equipment built into the milking system. Milk analysis included somatic cell count (SCC) using Somacount equipment's, which uses laser based on flow cytometry, and were calibrated with cow milk. From monthly determinations of SCC was obtained for each animal an average SCC for the whole lactation (ASC). Goats were classified in two groups of ASC (binary trait): 0=with low ASC ($\leq 700x10^3$), and 1=with high ASC ($\geq 701x10^3$).

Goat scores outside the normal range were eliminated, using as criterion two standard deviations above average; the total number of deleted records was <1% of the total. Descriptive statistics of the studied traits were also obtained. In order to evaluate the effect of real milk production level on ASC, first lactation milk yield unadjusted to mature equivalent and 305 d, was used.

All udder traits were classified in three groups: 1=low score, 2=ideal score and 3=high score (table 1).

Other variables that could affect to ASC as herd, days in milk (DIM), lactation number (LN) (two groups: first and ≥ 2 lactations) and milk production (MP) (three levels: 1 ≤ 660, 2=661 to 1647 and 3 ≥ 1648 kg) were used.

Prior analyses Chi square test was used to evaluate dependence between udder traits and ASC and variables which resulted significant ($P<0.05$) were RUA, RSV, UDE, MSL, MP and LN. A stepwise logistic regression model was used for the analysis of ASC (as dependent variable), and the complete model included herd, LN, MP, FUA, RUH, RUA, MSL, UDE, TPL, TED, RSV and FIS, as independent variables. All statistical analyses were conducting with the Statistical Analysis System program (SAS, 1995).

Results

The general means and standard deviations for ASC, MP, LN and DIM of present study were 475.4 ± 132.9 $x10^3$ cells/ml, 1155 ± 496.3 kg, 2.21 ± 1.4 lactations and 298 ± 78 days, respectively. No references on this topic and with this methodology were found worldwide. The results of odds ratio, probability and confidence intervals for odds are in table 2. From the completed model the variables than resulted significant were LN, MP, RSV and RUH.

Goats with ≥ 2 lactations were 1.3 times more possibilities to get high ASC scores than goats of first lactation ($P<0.01$). For the case of MP this results indicate than goats with lower milk yields (≤ 660 kg) were 3.1 times more probable to have high averages of ASC than goats with high milk production level (≥ 1648 kg). These results agree with those obtained by Barrón-Bravo et al., (2013) founding that group of goats with lower milk production (0.2-2.5 kg/day) registered a higher somatic cell score (SCS) (5.28 in a lineal scale), and those animals with higher milk yield (≥ 4.1 kg/day) showed lower SCS (4.72) these differences being highly significant ($P < 0.01$). Goats classified with 10 to 27 points for rear udder side view were at increasing risk of presenting high ASC, compared

with goats with ideal or high scores for RSV. Goats classified with ideal score in RUH (40 to 45 points), were 2.48 times more probable to have high averages of ASC than goats with higher RUH scores (≥ 46 points); also goats with low score in RUH (7 to 39 points) were 1.8 time more probable to have high averages of ASC than goats with higher RUH scores.

Rupp et al. (2011) founded that lactation somatic cell score was genetically correlated with udder floor position (-0.24 and -0.19 in the Alpine and Saanen breeds, respectively), and, in Saanen, teat length, teat width, and teat form (0.29, 0.34 and -0.27, respectively).

Linear appraisal system from ADGA suggests the selection of animals with better overall conformation, for example goats with high final scores, and recommending ideal scores for each trait as well. However the results of this study are against the recommendation of ideal scores for rear udder height so that must be reviewed because of high SCC on these goats.

The improvement of some type traits can contribute to reduce the productions costs through diminishing of illness as mastitis, and in the probable increasing of longevity of goats (Castañeda-Bustos et al., 2014).

Conclusions

The highest phenotypic relationships founded in this study between somatic cell count and udder traits were with rear udder side view and rear udder height. These relationships are phenotypic and thereby include environmental effects that can influence the two traits simultaneously. Somatic cell count in milk should continue being used in goats as an indirect predictor of udder health status. Producers must not overestimate the importance of secondary type traits, regarding the primary production traits as milk, fat or protein. Ideal scores should be reviewed by ADGA.

Acknowledgements

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KEYWORD : somatic cell score, type traits, goats

Table 1. Udder score means and minimum and maximum by category (low, ideal and high scores) in Saanen goats (n=3686).

Udder trait	Mean	Low score	Ideal point ranges*	High scores
Fore udder attachment	34.9	10-34	35-42	43-48
Rear udder height	35.5	7-39	40-45	46-50
Rear udder arch	29.3	9-31	32-40	41-48
Medial suspensory ligament	29.0	14-21	22-28	29-35
Udder depth	29.6	6-21	22-27	28-50
Teat placement	20.8	7-17	18-28	29-50
Teat diameter	25.1	8-24	25-30	31-45
Rear udder side view	26.8	10-27	28-32	33-47

*According to the linear appraisal system from the ADGA.

Table 2. Results of the stepwise logistic regression model for somatic cell count in Saanen goats (n=3686).

Variable	Estimate	Probability	Estimated odds ratio	Lower Limit*	Upper Limit*
Constant	-9.347	0.0000			
LN=2	0.264	0.0000	1.30	1.18	1.43
MP=1	1.132	0.0004	3.10	1.65	5.81
MP=2	0.347	0.1091	1.41	0.92	2.16
RSV=1	0.462	0.0495	1.58	1.00	2.51
RSV=2	-0.087	0.5855	0.91	0.66	1.25
RUH=1	0.593	0.1674	1.81	0.77	4.20
RUH=2	0.909	0.0422	2.48	1.03	5.96

Group 3 was the comparison base for the other groups; *=95.0% confidence intervals for odds ratios; LN=lactation number; MP=milk production; rear udder side view (RSV); rear udder height (RUH).

REFERENCES

ADGA (1993). American Dairy Goat Association. Linear appraisal system for dairy goats. ADGA, Spindale, NC 28160, USA, p18.

Barrón-Bravo, O.G, Gutiérrez-Chávez, A.J., ángel-Sahagún, C.A., Montaldo, H.H., Shepard, L., Valencia-Posadas, M. 2013. Losses in milk yield, fat and protein contents according to different levels of somatic cell count in dairy goats. Small Rum Res 113(2): 421-431.

Castañeda-Bustos, V.J., Montaldo, H.H., Torres-Hernández, G., Pérez-Elizalde, S., Valencia-Posadas, M., Hernández-Mendo, O. and Shepard, L. (2014). Estimation of genetic parameters for productive life, reproduction, and milk-production traits in US dairy goats. J. Dairy Sci. 97(4): 2462-2473.

Rupp, R., Clément, V., Piacere, A., Robert-Granié, C. and Manfredi, E. (2011) Genetic parameters for milk somatic cell score and relationship with production and udder type traits in dairy Alpine and Saanen primiparous goats. J. Dairy Sci. 94: 3629-3634.

SAS, (1995). Statistical Analysis System. User's guide. Cary, North Carolina. SAS Institute Inc. US. 422 pp.

O-08-4

BALI CATTLE OUTPUT INSTALLATION OF POPULATION IN THE AREA POTENTIAL AS A SOURCE OF BALI CATTLE BREEDING

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Introduction

Bali cattle reproductive ability of a known height will not be able to increase the cattle population and the potential of young Bali cattle candidate, where the population is not known a certain age group. The number of cattle belonging to the age of productive and nonproductive is essential in preparing the beef cattle breeding program. Further to support the implementation of breeding programs necessary to support the energy intake from feeding is good and rational.

To increase the population of Bali cattle management and handling of livestock required good, especially in livestock the expenditure control with regard to the value of natural increase (natural increase of), mortality, livestock replacement (replacement stock), the number of cattle were knocked out, the influx of live cattle and magnitude of the potential ability of young Bali cattle candidate.

Efforts to prevent the expenditure of Bali cattle continuously from the area of young Bali cattle candidate on the island of Bali cattle, in particular, must be immediately addressed by the pattern of correct and consistent breeding. Among the inventory of calf crop, annual population growth, the structure of population by age group and the fertility status of Bali cattle in the area of the base population as a potential supplier of seeds to the test station performance. In addition, to guard against the depletion of the population in the area of seed sources should be no restrictions on spending livestock or livestock expenditure according to the tolerance limit its output value. Basic data of the above is technical coefficients to be prepared in advance to prepare for the cows to be selected into Breeding stock center Bali Cattle (BPTU), and cows will be prepared again for breeding.

Bali cattle are selected specifically prepared to increase the population in BPTU Pulukan, the technical coefficients of each individual must be equipped with data growth is not enough, but it should be equipped with vital statistics and the data size of the genetic potential of their ancestors. Factors, as mentioned above should be observed in order to complete the selection criteria in order to get of young Bali cattle candidate production potential of Bali cattle BPTU truly superior.

Materials and Methods

The material used in this study primary data in the IPD (Population Basic Installation). Installation of Basic Population date taken from four areas, namely unit Jembrana, Tabanan, Karangasem unit, and the unit Bangli) of 200 farmers (assuming ownership of livestock 2-3 head / farmer) is identical to the 500 tail as directed, Roscoe (1982) in Research Methods for Business is cited Sugiyono (2009) determination of sample size can be approximated by considering the total population, 20 percent level of precision, and standard error of 10 percent. The method used in this study is a case study is carried out intensive research, detailed, and thorough examination of a particular symptom to get data (Arikunto, 2002). Sampling was conducted in areas that are used purposive sampling is based on the determination of material of potential seed sources Bali cattle as potential suppliers of beef cattle BPTU seed in Bali.

Techniques of data collection at random (Random Sampling) with the amount to be taken following the instructions Yamane (1979)

To know the structure of the population, population growth (Natural Increase), and the output of livestock using the formulation as follows below:

Natural increase of = percent of births - percent mortality (Harjosubroto, 1988)Out Put = residual replacement -% Target Population increase (Harjosubroto, 1994) or by using a simulated population structure by age group was found in a field of cow.

Results and Discussion

In determining the size of the Natural Increase the availability of data required a number of adult females, the birth rate and mortality of a population. The Natural increase the value will be more meaningful if the high birth

rate is offset by low levels of mortality, and the counting is done every year. If the high value of Natural Increase is the idea that in the area concerned there are a number of adult females are productive as well as handling and good management. The Natural increase of the first year of observations obtained can be used as an evaluation of the successful management of the parent material in the coming years. Components to calculate the Natural Increase of Bali cattle in the area of the base population as in Table 1.

Tables. 1 Components of Natural Increase of reproduction to calculate the Cow

From Table 1 above shows that with 61.39 percent of adult females are available, the birth of the calf acquired 29.72 percent of the population, but the number of deaths reached 2.32 percent increase of the value of natural seed sources in the region in 2011 amounting to 27.40 percent. Bali cattle population growth naturally can result in calf crop at 48.41 percent, although this result is still 3 percent lower than the results of research Pane (1989) this difference is likely a result of the decline of adult stem handling and refineries so that energy intake for less production of basic needs. However, since five years earlier breeding in the region are relatively constant source of seed IPD, it can be observed from the results of research Supriyantono, A (2006) in Tabanan IPD in 2004, the percentage of births 41.78% (average over last 5 years 28, 37% ± 13.88%) 110.69% calf crop percentage (83.27 ± 37.13); percentage of mortality 0.19 (1.29 ± 0.76); Natural increase of 41.59% (27, 09 ± 13.93). Reproductive performance of Bali cattle generally showed stable as the result of research conducted in North Central Timor (TTU), East Nusa Tenggara Province (NTT demonstrated the efficiency of reproduction (ER) 83.60%, 21.72% Natural Increase (Tonbesi, 2008).

Supply of young cattle at the age of 2 years (26.77%) made up 10.94% and 15.83% young males young females, compared to the needs of the cow seeds 2 years instead of (11.48%) consists of 1, 25% for males and 10.23% for females, indicating a Net Reproduction Rate is high, so the percentage of these two components can be used as a measure of the sample areas are those areas of potential as a source of seed.

In determining the output of Bali cattle, basically, want to know the management of productive adult cows in the nursery. Management of adult females is meant the extent to which the composition of the population that existed at the time of observation that the number of cows that have a high reproductive efficiency to the end of the productive age group (age group 7-8 years), resulting in offspring as a replacement and keep the breeding population in the region. In connection with this then Gurnadi (1988) states to increase calf birth rate is optimal handling and management is required, the appropriate substitute for short yearling (Replacement) and productive cow.

Table. 2. Coefficient calculation of Bali cattle output in 2011

Table 3. Cattle composition (%) by age group

Table 4. Calculation of Out Puts cattle in the region IPD
Natural Increase (%)
27,40
Male calf
11,20
Female calf
16,20
The percentage of calves at the age of 2 years (with basic calf mortality 0.26)
Young males
10,94
Young Females
15,83
Cattle needs replacement At Age 2 Years
Male (%)
1,25
Female (%)
10,23

the rest of the young cattle
Male (%)
9,69
Female (%)
5,60
Number
15,29
Culling
Male (%)
1,24
Female (%)
10,18
Number
11,42
Percentage and composition Out Put
Young cattle
15,29
Olds cattle
11,29
Number
26,58
The increase in population
4,5
Out Put
22,08

The composition of the invention based on the age of the animals could explain that the rest of the young cattle and cattle 15.29% 11.29% eliminated an output value of livestock from the seed source. If the increase in cattle population of Bali every year 4.5% (secondary data BPTU 2011) the population of Bali cattle in the population base will not be interrupted in case of cows spending up to 22.08%. However, this value will change in the years to come, and at least, this result can be used for evaluation of ground improvement and reproductive ability in the following years.

Conclusion

From the results of the research output in the region of Bali cattle population Installation Basic in 2011 concluded: Bali cattle population structure in four regions showed that IPDadult cattle population of Bali is quite available in the amount of 63.88 percent, with 61.39% of whom were adult females productive. The percentage of young Bali cow 6.70 % and calf 29.72%. The percentage of the overall male Bali cattle are 15.48% and 84.52% females.

2. Maintenance of particular patterns of Bali cattle handling and management of Bali cattle in the area of adult female IPD, the general said in terms of both reproduction and breeding performance a: S/C (IB) 1.13; CR 78.20%; Calving Interval (CI) 13.50 months; calf crop 48.41%, so we get:
Reproductive efficiency (IB) 76.39%
b. Fertility index (IB) 70.50%
c. Natural Increase 27.40% 3. Based on limit values and the maintenance of natural increase of Bali cattle age is obtained: Six age groups of females and 2 males age groups as follows: age of Bali cattle was first used in the nursery for a male and a female aged 1.9 years. The oldest age used in the nursery for 4-year-old males and for females was 7.35 years. The spending limit value out of Bali cattle breeding area (output) without interfering with seed source population of 22.08%.

Suggestion

The pattern suggested the handling and management of productive females still need to be improved.
To support the implementation of breeding programs in the region of the source of seeds and seedlings to tighten

BPTU candidate selection, it is recommended each IPD implement recording system adapts to the operational patterns of use of software SREPSI 4.0 (Hakim.L, 2011)Crosses by using frozen semen are expected to be enhanced and transmitted more evenly throughout the IPD, and to avoid the use of males belonged to the people that have not been selected.Bali cattle good seed expenditures for supplies to BPTU and exit the area does not exceed the output (22.08%)Bali cattle IPD region has a higher Net Replacement Rate, so the area has potential as a source of seed.

KEYWORD : Bali Cattle; Installation of Population, Natural Increase; Output

Tables. 1 Components of Natural Increase of reproduction to calculate the Cow

	Component	Values
1	Percentage of Adult Females (%)	61,39
2	Birt of calf An Adult Females (%)	48,41
	Of the population (%)	29,72
3	Total Livestock Mortality (%)	2,32
4	Natural Increase (%)	27,40

Table. 2. Coefficient calculation of Bali cattle output in 2011

Natural Increase		27,40
Adult Cattle	Male (%)	2,49
	Female (%)	61,39
Use in breeding	Male (year)	1,9
	Female (year)	1,9
Oldest age used	Male (year)	4,0
	Female (year)	7,35
Long breeding	Male (year)	2
	Female (year)	6

Table 3. Cattle composition (%) by age group

1	Age (years)	(2-3)	(3-4)	(4- 5)	(5-6)	(6-7)	(7-8)	Number
2	% Male	1,25	1,24					2,49
3	% Female	10,23	10,22	10,21	10,2	10,19	10,18	61,39
Male and Female ratio				41/59				

REFERENCES

Arikunto, S. 2002. *Prosedur Penelitian*. Rineka Cipta. Jakarta.

Gurnadi, E. 1988. *Teknik Penanganan dan Pengelolaan Ternak Ruminansia Besar*. Teknik Laboratorium ilmu Pemuliaan Ternak dan Genetika Ternak. Bogor.

Hakim, L. 2011. Manajemen Rekording Ternak Menggunakan Software untuk Program Seleksi Sapi Bali. Dewan Riset Nasional Kementerian Negara Riset dan Teknologi kerjasama dengan Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Brawijaya. Malang.

Hardjosubroto, W. 1988. Produktivitas Sapi Ongole dan Persilangannya. Seminar Export Ternak Potong. Jakarta

Hardjosubroto, W. 1994. Aplikasi Pemulibiakaan Ternak di Lapangan. Jakarta. PT Gramedia Widiasarana Indonesia.

Mattheij, J.A.M, T. Vanderlender, A. Osinga, Alih Bahasa oleh Winantea. 1982.*Reproduksi dan Dasar-dasar Endokrinologi Pada Hewan-hewan Ternak* Nuffic-Universitas Brawijaya. Malang.

Pane, I. 1989. Pelaksanaan Perbaikan Mutu Genetik Sapi Bali. Denpasar, Bali.

Sugiyono. 2009. Metode Penelitian kuantitatif,kualitatif dan R&D. Sitasi Research Methods for Business oleh Roscoe , 1982:253. Penerbit Alfabeta Bandung

Supriyantono,A., 2006. Estimasi Parametrer genetic Kinerja Produksi dan Reproduksi sebagai dasar penyusunan program pemuliaan pada sapi Bali. Disertasi. Prog Pasca sarjana Fakultas Pertanian Universitas Brawijaya. Malang

Tonbesi, Trimeldus Tulak. 2008. Estimasi potensi dan kinerja sapi Bali di Kabupaten Timor Tengah Utara Propinsi Nusa Tenggara Timur. Penerbit Universitas Gadjah Mada Yogyakarta

Yamane, 1979. *Mathematic for Economic and Elementary Survey*. Englewood Cloots. New York

O-08-5

POPULATION STRUCTURE AND PERFORMANCE OF PO CATTLE, CASE STUDY IN CITY OF PROBOLINGGO, EAST JAVA, INDONESIA

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Abstract

Decreasing the number of population in PO (Fillial Ongole) beef cattle has been an important issue in East Java, Indonesia. This study has aimed to evaluate the population structure and performance of PO beef cattle as basic data for improvement and conservation of PO beef cattle in East Java, especially in Probolinggo. Survey on population structure and direct observation on vital statistics of PO cattle were conducted in Kedopok district, city of Probolinggo, East Java, Indonesia. Result showed that the proportion of PO was only 35% and crossbred occupied the rest (65%). The population number of PO in Kedopok district was decreased significantly, from 2,235 in 2011 to be 182 in 2015. Population structure showed that young females as replacement stock was 21.43%, birth rate was 0.55%. Reproductive performance of PO showed service per conception (S/C) was 1.23; anestrus post partum (APP) was 3 – 4 months. Performance of vital statistics was categorized good. Chest girth (143.55 cm) and body height (119.70 cm) of female at 1.5 – 2 years old and chest girth (128.38 cm) of male at 2 – 3 years old fulfill the requirement of the first class of Indonesian National Standard for superior PO. Reproductive performance of PO was good, but the population structure was lack of replacement stock for females. Breeding program of PO cattle in Probolinggo should be focused on grading up and regulation on semen distribution, especially promotion of artificial insemination using PO semen.

Introduction

Filial Ongole (PO) beef cattle are one of the local genetic resources of Indonesia originated from crossing between Ongole and local Javanese breed. The specific characteristics are white-gray colour, stumpy horns and well-developed hump in male. PO cattle have high adaptability to differences environmental conditions, strong power and efficient reproductive performance (short anoestrus post partum) (Affandhy et al., 2009).

City of Probolinggo is one of centre for PO breeding area in East Java. The problem in the development of PO is low productivity caused by the genetic quality and the limited availability of superior male. There are indications that population growth of PO decreased which caused by high numbers of slaughtered productive female and negative selection in which good performance of PO was sold or slaughtered. On the other hand, in East Java have been found crossbred of PO with exotic breed, such as Limousin. Most farmers prefer natural mating for breeding system of PO. The application of natural mating without a good recording will increase the chances of inbreeding. Those phenomenons will lead to extinction of PO cattle in Indonesia. In order to maintain the existance of PO cattle as Indonesia genetic resources, study on population structure and performance of PO cattle in the city of Probolinggo was conducted.

Material and Methode

Research was done in the Kedopok District, Probolinggo during June to December 2015. Observation was used in this study. Materials used were PO cattle and the PO cattle farmer. Data on population structure and performance of PO cattle consisted of vital statistics (chest girth, body height and body length) and reproductive traits (service per conception and anestrus post partum) were observed and measured. Data were analysed by software GENSTAT 16 edition (Anonymous, 2015).

Result and Discussion

Population Structure

The total number of PO cattle in Kedopok. Probolinggo was 182 with the ratio of male to female was 42 males and 140 females. This number decreased significantly when compared to 2011 in which the population number of PO was 2,235 (Anonymous, 2012). The decline in population number was likely caused by the high farmer interest in crossing PO females with semen of Limousine. The high preference in crossbred was caused by the high price of crossbred calves. (Table 1)

The proportion of male and female are 23.08% and 76.92%, respectively. The highest proportion is occupied by adult female cow that is equal to 54.95% and the lowest proportion was female calf which only 0.55%. Adult female cow dominates more than 50% of total cattle population, while the proportion of young cows was much smaller. This condition is less favorable for the development of PO cattle in the Kedopok district because of the number of young female cattle less than 50% of adult cows. This illustrates that the population was lack of young female cow as replacement stock.

The improvement of PO cattle is largely determined by the structure of the population. in which the replacement stock should be at least 70% of the ideal number of adult cattle. However, it can be seen that the largest proportion of adult female cows occupied by cows aged 2 to 4 years (62.5%). This high proportion can be utilized to increase the population number through improved reproductive efficiency and accurate control of reproduction.

Reproductive Performance of Female PO Cattle

Most farmers (86.5%) apply the technology of artificial insemination (AI), while the rest are still using natural mating. (Table 2)

Reproduction performance of PO cattle is good with the average service per conception is 1.23. It means that the maximum number of insemination for pregnancy was two. Maximum weaning age is 4 months. In these conditions make it possible for a cow to give birth every year.
Anestrus post partum (APP) was classified too long, in which 77% of the population have APP of 4 months after birth, while the rest (35%) reached 6 months after birth. Postpartum cows should be mated back at the time of the estrus cycle after emerging signs of estrus postpartum (66 days after birth). At this time, cow was ready for having calf again.

Performance of Female PO cattle

The average chest girth of a young female PO (1.5 to 2 years of age) was 143.55 ± 11.74 cm. This value was significantly greater than previous studies done by Prihandini, Hakim and Nurgiartiningsih (2011) which reported chest girth of 1 year age PO cattle was 117.60 ± 10.91 cm. Chest girth of female PO was increased from 160.25 ± 4.79 to 165.72 ± 8.33 cm for age of 2.5 and 4 – 6 years old, respectively. Decline in performance (1.38%) occured when cow was more than 7 years of age or cow's giving birth more than 6 times. (Table 3)

Chest girth of 1.5 – 2 years old female PO cattle was higher than Indonesian National Standard for first class of female PO cattle, which is 143 cm (NSAI, 2008). Body length was increased from the average of 120.85 cm in the age group of 1.5 – 2 years to 140.39 cm in the age group of 4 – 6 years. Body length of female PO cattle at aged of 1.5 – 2 years was higher than those reported by Prihandini et al. (2012), which was 103.70 cm.

Performance of Male PO cattle

Mean of chest girth of male PO cattle in Kedopok, Probolinggo was categorized in first class of Indonesian National standard for PO cattle. (Table 4)

Body length of male PO cattle at aged of 2-3 years was in third class of Indonesian national standard for male PO, which is 130 cm. It is estimated that there are 1.6% of the bulls in the population that were in first or second-class.

Conclusion

Population structure of PO cattle in Kedopok, Probolinggo was lack of replacement stock for female as candidat dam. The population number was decreased significantly during three years. PO cattle's Performance of female at 1.5-2 years old and male at 2-3 years was categorized in first class of Indonesian National standard for PO cattle. Reproductive performance of female PO cattle was good with low service per conception and short anestrus post partum. Breeding program of PO cattle in Probolinggo should be focused on grading up and regulation on semen distribution, especially promotion of artificial insemination using PO semen.

KEYWORD : Crossbred, Indonesian National Standard, Reproductive performance, vital statistic

Table 1. Population structure according to sex and age group in Kedopok, Probolinggo

	Male			Female			
	Calf	Young	Adult	Calf	Young	Adult	Total
Total	13	20	9	1	39	100	182
Percentage (%)	7.14	10.99	4.95	0.55	21.43	54.95	100

Table 2. Reproductive Performance of PO cattle in Kedopok, Probolinggo

Paramater		**Observation**
Mating System	AI	86.5 %
	Natural Mating	13.5 %
Service epr conception (S/C)		1.23
Anoestrus Post Partum	3-4 month	77.1 %
(APP)	5-6 month	22.9 %

Table 3. Means and standard deviations of chest girth, body length and body height in Female PO cattle in Kedopok, Probolinggo

Age (year)	N	Chest Girth	Body Length	Body Height
1.5 - 2	20	143.55±11.74	120.85±9.58	119.7±4.17
2.5	3	160.25±4.79	132.25±6.4	122.5±2.89
4 – 6	19	165.72±8.33	140.39±11.6	129.72±3.77
7 - 10	19	163.42±10.05	135.00±8.34	123.95±7.27

Table 4. Means and standard deviations of chest girth, body length and body height in male PO cattle in Kedopok, Probolinggo

Age (year)	N	Chest Girth	Body Lenght	Body Height
0.5 - 1	8	128.38±8.47	106.75±13.01	105.38±8.00
1 – 1.5	9	144.89±14.78	124.11±10.75	118.78±7.39
2 - 3	9	168.78±13.93	130.44±12.96	133.78±11.11

REFERENCES

Affandhy, L., W.C. Pratiwi, D. Ratnawati. 2009. Implementation of sexing semen in liquid straw in PO cattle in Pasuruan, East Java.

Anonymous, 2012. Annual Report of Agriculture Department City of Probolinggo 2011.

Anonymous, 2015. Annual Report of Agriculture Department City of Probolinggo 2014.

Anonymous, 2015. GENSTAT Release 16. VSN International Ltd.

NSAI-National Standardization Agency of Indonesia, 2008. Beef Cattle – PO Cattle. SNI 7356:2008

Prihandini, P.W., L. Hakim dan V.M.A. Nurgiartiningsih. 2011. Seleksi pejantan sapi Peranakan Ongole (PO) di loka penelitian sapi potong Grati – Pasuruan berdasarkan nilai pemuliaan. Jurnal Ternak Tropika, Vol. 12, No. 2.

O-08-6

Improvement of Bali Cattle Performance by Additional Concentrate in Sumbawa Island, Indonesia

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Abstract

Study on performance of Bali cattle was conducted in station of Bali cattle breeding and forage in Serading, Sumbawa, West Nusa Tenggara, Indonesia. Two groups consisted of group A (43 Bali cattle) received additional concentrate and group B (44 Bali cattle) received standard feed without additional concentrate were used in this study. Body weight and vital statistics of Bali cattle were collected before and after treatment. Data were analysed using Genstat software (version 16). Result showed that body weight of group A before and after treatment were 202.60 ± 32.84 kg and 202.42 ± 32.37 kg, respectively. Body weight of group B before and after two months were 199.07 ± 28.87 kg and 195.30 ± 29.16 kg, respectively. Group of cattle after received additional concentrate showed higher vital statistics than before. It could be concluded that additional concentrate has effect on increasing body weight and vital statistic of Bali cattle. Without additional concentrate, performance of Bali cattle decreased due to lack of energy consumption.

KEYWORD：Body weight, vital statistic, additional concentrate

Introduction

Bali cattle are one of the important beef cattle breeds contributing to the development of livestock in Indonesia, and are the most predominant genotype within Sumbawa Island, Indonesia. Taxonomical names of Bali cattle are *Bos sondaicus*; *Bos javanicus*; *bos/Bibos banteng*. This indigenous beef cattle breed plays an important role in increasing beef meat production to meet the national need. Bali cattle have superiorities in high fertility, high genetic resistance to tropical environment and high carcass quality (low fat content). Population of beef cattle in Indonesia in 2013 was 14.2 million, in which Bali cattle occupied the highest proportion (32.31%) in number of 4.8 million. The second place was followed by 4.4 million of crossbred between exotic breed with local cattle (30.14%), 4.3 million of Ongole (28.88%), and 1.3 million of Madura cattle (8.67%) (BPS, 2013).

Most of Bali cattle are in Bali and Nusa Tenggara Islands, which occupied 43.75% of total number of Bali cattle in Indonesia. The important role of Bali cattle in Nusa Tenggara is as source of household incomes; increase working labour and regional income (Bamualim and Wirdahayati 2003). Most farmers in those areas are smallholder traditional farmers, in which most of cattle are kept in semi extensive system. Only small portion of farmer were applying cut and carry system (Dahlanuddin et al 2011). Source of forage for cattle feed in Sumbawa were grass in pasture, field, garden, a long the street and agriculture waste product, such as rice straw and corn straw, which is available abundantly. Feed additive or feed supplement were given rarely due to limited income and knowledge.

Population of Bali cattle in Bali - Nusa Tenggara decreased from 2.36 million in 2011 to be 2.13 million in 2013 (Anonymous 2011 and Anonymous 2013). Limited availability of forage due to extreme long dry season and lack of good management in breeding and feeding affected the productivity of Bali cattle in the area. Recently, artificial insemination using exotic breed have been executed in West Nusa Tenggara Island. Reduced population and increased application of crossbreeding has been considered might lead to the extinction of Bali cattle. Effort has been made to increase population and also genetic quality of Bali cattle. Adapted breeding and feeding strategy for Bali cattle in Sumbawa should be applied to improve the genetic potency in the existing condition and finally increase genetic potency and number of population of Bali cattle. Exploration and identification of genetic potency and performance of Bali cattle as indigenous genetic resources of Indonesia should be conducted as an important basis for good breeding strategy. This research was conducted to evaluate the population structure and analysed the effect of additional feeding on performance of Bali cattle in station of Bali cattle breeding and forage in Serading, Sumbawa, West Nusa Tenggara Island, Indonesia.

Materials and Methods

Research was conducted in station of Bali cattle breeding and forage in Serading, Sumbawa West Nusa Tenggara. Effect of additional concentrate was studied using two groups of cattle; each group consisted of 50 Bali cattle. Group A was given additional feed, which was 0.5 kg/cattle/day of concentrate and group B was given standard feed, which usually given in the station. Each group was housed in open housing system 15x25 m^2. Experiment was started with preliminary treatment of 15 days for adaptation and followed by 30 days for experiment. Body weight and vital statistic before and after treatment of additional concentrate were collected and were analysed using student's t test applying software of Genstat version 16 (Anonymous 2014). Statistical model to evaluate effect of treatment on body weight and vital statistics was as follows: , in which: body weight and vital statistics of j individual; population mean; fixed effect of treatment i; Eij = random error.

Results and Discussion

Initial body weight for A group (without treatment of additional concentrate) and B group (with treatment of additional concentrate) were shown in the following Table:

Mean of cattle's age in group A with additional feeding (standard feed + 1 kg of rice brand) was 5.15 year old and mean of initial weight was 190.07 kg; on the other hand, in group B (standard feed), mean of cattle's age was 4.95 year old and mean of initial body weight was 187.96 kg. Coefficient of variation of both groups was assumed to be equal, which was less than 20%.
The research result on body weight and vital statistics before and after treatment were presented in Table 1 and 2.

Body weight after treatment in A and B was tended to be decreased 0.09% and 1.89%, respectively. The more decrease in group B (4 kg) was might be due to the less energy required for maintenance which affects on using of fat reserve for maintenance. Rice brand was usually used for feed because it contents enough energy and protein, easy to be digested and can increase feed efficiency.
Statistic vital of cattle that given additional feed increased significantly, 8.24%, 4.08%, and 1,15% for chest girth, body length and body height, respectively. Cattles in group B were tended to be increase in smaller size compared to group A. Chest girth, body length and body height in group B were increased 1.38%, -2.28%, and 1.12%, respectively. In term of body condition, body conformation score of cattle received additional feeding was good with the value of 5-6 (the highest is 8 and the lowest is 1). This condition occurred because the treatment was done in hot season (September-October 2014) in which the temperature was very hot caused the low quality of rice straw and forage in the farm.

Conclusions

Additional feeding has effect on increasing of body weight and vital statistic of Bali cattle Considering availability of feed resources in West Nusa Tenggara and high adaptability of Bali cattle in Sumbawa Island, there is high potency of improving genetics and performance of Bali cattle as indigenous genetic resources of Indonesia.

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KEYWORD：Body weight, Vital statistic, Additional concentrate

Table 1. Mean and coefficient of variation of samples

Group	n	Age (year)	Coefficient of variation (%)	Birth weight (kg)	Coefficient of variation (%)
A	43	5.15±2.51	48.74	190.07±31.84	16.75
B	44	4.95±2.41	48.69	187.96±29.98	15.95

n = number of offspring

Table 2. Body weight (BW) and vital statistics (chest girth/ CG, body length/ BL, body height/ BH) of group A

	Before treatment (n = 43)				After treatment (n = 43)			
Variable	BW (kg)	CG (cm)	BL (cm)	BH (cm)	BB (kg)	CG (cm)	BL (cm)	BH (cm)
Mean	202.60	137.35	110.02	109.60	202.42	148.67	114.51	110.86
SD	32.84	13.88	6.01	4.12	32.37	8.66	7.00	3.77
CV (%)	16.21	10.11	5.46	3.76	15.99	5.82	6.11	3.40

Table 3. Body weight (BW) and vital statistics (chest girth/ CG, body length/ BL, body height/ BH) of group B

	Before treatment (n = 44)				After treatment (n = 44)			
Variable	BW (kg)	CG (cm)	BL (cm)	BH (cm)	BW (kg)	CG (cm)	BL (cm)	BH (cm)
Mean	199.07	141.86	112.80	109.57	195.30	143.82	110.23	110.80
SD	28.87	7.02	5.93	3.98	29.16	9.54	4.83	5.79
CV (%)	14.50	4.95	5.26	3.63	14.93	6.63	4.38	5.23

REFERENCES

Anonymous, 2000. Sambutan Gubernur Bali Pada Acara Pembukaan Seminar. Prosiding Seminar Nasional Peranan Balai Inseminasi Buatan Singosari Dalam Menghadapi Swasembada Daging Tahun 2005 Melalui Uji Keturunan Sapi Bali dan KSO Semen Beku. Universitas Brawijaya. Malang.

Anonymous 2014 GENSTAT Release 16 VSN International Ltd.

BPS 2013 Berita Resmi Statistik. Badan Pusat Statistik. No.62/09/Th.XVI. 2 September 2013. Jakarta.

Budiarto A, Hakim L, Maylinda S, Nurgiartiningsih V M A and Ciptadi G 2011 *Output* of Bali Cattle in Base Population as Genetic Resources. Research Report. Faculty of Animal Husbnadry. Universitas og Brawijaya. Malang.

Gary L A 2014 Diagnosing Reproductive Problems in Beef Cattle Herds. Cattle Research and Education Center.

Martoyo H 2003 Indigenous Bali Cattle: The Best Suited Cattle Breed for Sustainable Small Farms in Indonesia. http://www.angrin.tlri.gov.tw/.../ chapter2cattle_1-2. pdf.

Mastika I M 2003 Feeding Strategies to Improve the Production Performance and Meat Quality of Bali Cattle (*Bos sondaicus*). In: Strategies to Improve Bali Cattle in Eastern Indonesia. Aciar Proceedings No. 110. Ed. K. Entwistle and D.R. Lindsay.

PSPK. 2011. Rilis Hasil Akhir Pendataan Sapi Potong. Sapi Perah. dan Kerbau (PSPK) 2011. Kementerian Pertanian RI - Badan Pusat Statistik.Jakarta.

Supriyanto A 2006 Estimation of genetic parameter of productivity and reproductivity as dasar penyusunan program pemuliaan pada sapi bali, studi kasus di P3Bali. Disertasi. PS Ilmu Pertanian minat Imu Ternak. Universitas Brawijaya.

O-08-7

Growth and Milk Production of Thai Milking Zebu Cattle

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Abstract

In 1989, The Department of Livestock Development (DLD) of Thailand established a dairy breeding project to develop the Thai Milking Zebu cattle (TMZ), which contain specifically 75% Holstein - 25% Other breeds, for reducing the number of dairy cattle importation. The TMZ has been improving for medium milk production and low cost of health care. Since then, the TMZ have been produced and distributed to small dairy holders across Thailand. Pedigree, birth weight (BW), weaning weight (WW), yearling weight (YW), 18 months weight (18MW), and ADG from yearly to 18months of 2,271 TMZ cattle, and accumulated 305-day milk yield (MY) of 898 TMZ cattle were analysed using linear models and used to statistically describe their growth and milk production. In general, the TMZ cattle was 28.4 ± 5.1 kg for BW, 90.3 ± 13.1 kg for WW, 188.0 ± 37.9 kg for YW, 243.4 ± 51.8 kg for 18MW, and 338.3 ± 152.6 g/d for ADG. Male TMZ had higher weights ($P < 0.01$) than female for BW, WW and YW, had similar ADG. The TMZ had first calving at 34.0 ± 7.6 months old. On average, the TMZ cows produced 2,863.5 ± 212.4 kg of MY, with 239.0 ± 87.2 days in milk. Range of MY was from 2,451.5 ± 36.7 kg (the 1st lactation) to 3,059.8 ± 64.5 kg (the 5th lactation). The partial regression coefficient indicated an increment of MY for 81.8 ± 24.4 kg per lactation ($P < 0.05$). These results implied that TMZ could be an alternative cattle for small holders under tropical environmental conditions.

INTRODUCTION

Thailand is a tropical country in Southeast Asia, with a total area of approximately 513,000 km^2 (198,000 sq mi), located in between latitudes 5° 37¢ North to 20° 27¢ North and longitudes 97° 22¢ East to 105° 37¢ East. Countrywide, temperatures are generally high (24.0 ℃ to 30.2 ℃) with high humidity (66% to 81%) and rainfall (1,050 mm to 2,742 mm.). The boundaries of Thailand connect to 4 countries including Myanmar, Laos, Cambodia, and Malaysia (Thai Meteorological Department, 2015). Climate in Thailand has influence of tropical Northeast and Southwest monsoons. Changing of season weather and climate, especially the increasing of ambient temperature are the important effects for dairy cattle stress, low quality and quantity of roughage, and the distributions of tropical diseases and insects (Sae-tiao et al., 2015).

In order to have tropical adapted dairy cattle, a project to develop the Thai Milking Zebu (TMZ) cattle was established in 1989 by the Department of Livestock Development (DLD), the Ministry of Agriculture and cooperatives. The TMZ contains specifically 75% Holstein (H) and 25% Other breeds (e.g., Brahman, Sahiwal, and Thai Native) and they had been optimized for suitable body size, feed intake, milk production, fertility, tolerating to heat and tropical diseases than Zebu, especially for small holder production. To produced TMZ, upgrading the crossbred to 75%H cows, and Inter Se mating among 75% H cattle for five generations were practiced before spreading the TMZ to dairy producers.

The TMZ had been distributed to dairy smallholders across Thailand for improving profitability and sustainability of their operations. Growth and production performance of dairy cattle are economically important traits. Cattle with fast growing and has high production will have more chance to earn more profit in the dairy business. Phenotypic difference among male and female (growth), and among lactations (milk production) would give benefit information for the improvement programs.
Thus, the objective of this study was to characterize growth and milk production of Thai Milking Zebu cattle raised under Thai tropical conditions.

MATERIALS AND METHODS

The dataset included 1) pedigree, 2) growth performances (i.e., birth weight, BW; weaning weight, WW; yearling

weight, YW; 18 months weight, 18MW; average daily gain from yearly to 18months, ADG) of 2,271 TMZ cattle (874 males and 1,397 females), and 3) accumulated 305-day milk yield (MY) of 898 TMZ cows in particular lactation (from 1 to 7). All of them were TMZ, (75% H and 25% Others from one or more of the following breeds: Brahman, Sahiwal and Thai Native). These cattle were raised under management and environmental condition in the Lumprayaklang Livestock Breeding and Research Center, Lopburi province in the period of 1992 to 2015.

Seasons were classified as winter (November to February; cool (14.5℃ to 31.6℃) and dry (65% RH, precipitation 50 mm/season), summer (March to June; hot (20.8℃ to 34.2℃) and dry (72% RH, precipitation 239 mm/season) and rainy (July to October; hot (23.2 ℃ to 31.8 ℃) and humid (77% RH, precipitation 624 mm/season). All TMZ cattle were kept in open barns (total size 44×40 m^2) and received the same management and health care. Feeding was based on fresh roughage, e.g., Guinea (*Panicum maximum*), Ruzi (*Brachiaria ruziziensis*), Napier (*Pennisetum purpureum*), and Para (*Brachiaria mutica*) and the concentrate contained of 16% to 18% CP and of 70% to 75% TDN (DM basis). Ingredients used in the concentrate were protein sources (e.g., palm meal, soybean meal, cotton seed meal, leucaena), energy sources (e.g., cassava, rice bran, broken rice, fat from animals and plant, molasses), and mineral and vitamin sources (e.g., di-calcium and premixes). In the dry season (November to March), when fresh grass was limited, TMZ cattle were fed by Guinea grass and Ruzi grass hay, and silage produced by the DLD. In addition, all TMZ cattle had free access to water source and mineral supplement throughout the year and vaccinated for Foot and Mouth Disease (FMD), Tuberculosis (TB) and de-worming twice a year (every six months).

Growth performances (BW, WW, YW, 18MW, and ADG) between 874 males and 1,397 females TMZ cattle were analyzed through general linear model (SAS, 2004) that considered contemporary group (year-season) and sex of cattle as fixed effects. Least squares means (LSM) of each sex subclasses were estimated for particular traits, and were compared using t-test ($\alpha = 0.05$). Accumulated 305-day milk yield were gathered from 898 cows, which had complete milk production from first to seventh lactation in the period of 1992 to 2015. In this dataset, cows were the progenies of 115 sires and 664 dams. All cows had complete information of birth date, calving date, and drying off date. The analysis of MY was done using general linear model (SAS, 2004) that contained contemporary group (year-season) and lactation number as fixed effects. The LSM for MY were estimated for each lactation. Regression coefficient of LSM for MY on lactations was calculated to assess phenotypic trend across lactations.

RESULTS AND DISCUSSION

On the average, the TMZ in this population had 28.4 ± 5.1 kg for BW, 90.3 ± 13.1 kg for WW, 188.0 ± 37.9 kg for YW, 243.4 ± 51.8 kg for 18MW, and 338.3 ± 152.6 g/d for ADG. Growth performances of TMZ were close to other Thai H crossbred cattle population (22.5 to 33.3 kg for BW, 153.6 to 217.2 kg for YW, 192.0 to 269.6 kg for 18MW; Jeanmas et al., 2008). However, they were lower than H cattle raised in Canada (44.8 to 49.2 kg for BW; Forrest, 1980) and Korea (562 to 568 kg for 18MW; 0.66 to 0.68 kg/d for ADG; Choi et al., 1997). Male TMZ had higher ($P < 0.01$) weights than female for BW (29.3 ± 0.2 kg *vs* female: 27.7 ± 0.2 kg), WW (97.8 ± 1.3 kg *vs* 91.1 ± 0.5 kg) and YW (193.8 ± 3.9 kg *vs* 185.9 ± 1.2 kg), but there were not significantly different ($P > 0.05$) between male and female for 18MW (227.9 ± 6.4 kg *vs* 238.7 ± 1.9 kg) and ADG (301.8 ± 32.9 g/d *vs* 338.2 ± 6.24 g/d; Table 1).

Table 1 Growth performances of TMZ male and female cattle

Traits[1/]	Male	Female	P-value
BW (kg)	29.3 ± 0.2[a]	27.7 ± 0.2[b]	<0.01
WW (kg)	97.8 ± 1.3[a]	91.1 ± 0.5[b]	<0.01
YW (kg)	193.8 ± 3.9[a]	185.9 ± 1.2[b]	0.04
18MW (kg)	227.9 ± 6.4	238.7 ± 1.9	0.09
ADG (g/d)	301.8 ± 32.9	338.2 ± 6.24	0.12

[1/] BW= birth weight, WW= weaning weight, YW= yearling weight, 18MW = 18-month weight, and ADG = ADG from yearly to 18 months

[a, b] Least squares means within the same row with different superscripts differ ($P < 0.05$).

The TMZ cows had first calving at 34.0 (SD =7.6) months of age, and produced 2,863.5 (SD = 212.4) kg of MY in 239.0 (SD = 87.2) days. These results confirmed production performance of the TMZ cows (35.5 ± 5.4 months for age at first calving, 2,682.8 ± 710.1 kg for MY, and 244.1 ± 58.7 days for days in milk) reported by Jindatajak and Sanghureyphria (2004).

Table 2 Least squares means and standard errors for milk production of TMZ dairy cows

Lactation number	Number of records	Milk yield (kg)
1	389	2,451.5 ± 36.7[c]
2	315	2,738.5 ± 40.8[b]
3	253	2,834.5 ± 45.5[b]
4	193	3,001.5 ± 52.1[a]
5	126	3,059.8 ± 64.5[a]
6	86	2,980.0 ± 78.0[a]
7	35	2,978.8 ± 122.3[a]

[a, b, c] Least squares means within the same column with different superscripts differ (P < 0.01)

Table 2 shows the LSM for milk production of TMZ dairy cattle from first to seventh lactations. The LSM for MY across lactation ranged from 2,451.5 ± 36.7 kg (first lactation) to 3,059.8 ± 64.5 kg (fifth lactation). Milk production performance trended to increase as lactation increased (regression coefficient = 81.8 ± 24.4 kg/ lactation; P < 0.01). Lactation fifth, fourth, sixth, and seventh had the highest MY (2,978.8 to 3,059.8 kg), followed by second and third lactations (2,738.5 to 2,834.5 kg), and the lowest one were from first lactation (2,451.5 kg) respectively. Pattern of MY across lactation found in this TMZ population was similar to, but had higher production performance than those reported for dairy cattle in a multibreed dairy population in Ethiopia (Gebreyohannes, et al., 2013).

Considering growth and production performance, TMZ could be one of choices for small holder dairy farmers, especially who have limited afford for providing high quality and quantity of feed and cares. The weather in Thailand generally has high temperature and high humidity. The average temperature-humidity index (THI) was 81.8 and the standard deviation was 4.2 (Sae-tiao et al., 2015). With these high average and high variation of THI, cows that kept in opened-barns would have heat stress (THI > 72; Armstrong, 1994). Under Thai tropical condition, cows with higher H fraction tended to have higher levels of heat stress (Boonkum et al., 2011) and they would need more intensive management and care, which increase production costs, than cows with lower H fractions (Koonawootrittriron et al., 2009; Boonkum et al., 2011; Jattawa et al., 2012). Thus, TMZ would fit well under these conditions, especially with small dairy holders.

CONCLUSION

The TMZ cattle had growth and production performance in ranges of dairy cattle raised in Thailand. Male TMZ grew faster than female TMZ from birth to one year old. Milk production of TMZ increased from first to third and then consistently highest from fourth to seventh lactations. Increment of MY for 81.8 ± 24.4 kg per lactation was found in this study.

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KEYWORD : dairy, breeding, tropics, growth, milk

REFERENCES

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77:2044-2050.

Boonkum W., Misztal I., Duangjinda M., Pattarajinda V., Tumwasorn S., & Sanpote J. 2011. Genetic effects of heat stress on milk yield of Thai Holstein crossbreds. J Dairy Sci. 94:487-492.

Choi, Y. J., I. K. Han, J. H. Woo, H. J. Lee, K. Jang, K. H. Myung and Y. S. Kim. 1997. Compensatory Growth in Dairy

Heifers: The Effect of a Compensatory Growth Pattern on Growth Rate and Lactation Performance. J. Dairy Sci. 80:519-524.

Forrest, R. J. 1980. A comparison of growth and carcass characteristics between Holstein-Friesian stccrs and Simmental × Holstein (F1) crossbreds. Can. J. Anim. Sci. 60: 591-598.

Gebreyohannes, G., S. Koonawootrittriron, M. A. Elzo, and T. Suwanasopee. 2015. Genotype by environment interaction effect on lactation pattern and milk production traits in an Ethiopian dairy cattle population. Kasetsart J. (Natural Science).48: 38-51.

Jattawa, D.,S. Koonawootrittriron, M. A. Elzo, T. Suwanasopee. 2012.Somatic cells count and its genetic association with milk yield in dairy cattle raised under Thai tropical environmental conditions.Asian-Aust. J. Anim. Sci. 25: 1216-122

Jeanmas, A., P. Sopannarath, S. Koonawootrittriron, and S. Tedprasit. 2008. Estimation of growth curve in crossbred heifer between Holstein Friesian and Red Sindhi. Pages 205 - 214. In: Proc. 46th Kasetsart University Conference, January 29- February 1, 2008, Bangkok.

Jindatajak, Y. and N. Sanghureyphria. 2004. Milk yield and fertility of crossbred holstein friesian 75% (TMZ) at Lampayaklang Livestock Breeding and Research Cente. 2004 Anbual Research Report Dairy Cattle. Animal Husbandry Division, Department of Livestock Development, Thailand, pp. 1-9.

Koonawootrittriron S., Elzo MA., & Thongprapi T. 2009. Genetic trends in a Holstein × other breeds multibreed dairy population in Central Thailand. Livest Sci. 122:186-192.

Sae-tiao, T., S. Koonawootrittriron, T. Suwanasopee , and M. A. Elzo. 2015. Changes in temperature-humidity index and number of hot days related to heat stress of dairy cattle in Thailand. ADSA- ASAS Joint Annual Meeting, Orlando, FL, July 12-16, 2015.

SAS. 2004. SAS OnlineDoc, Version 9.0. SAS Institute Inc., Cary, NC, USA.

Thai Meteorological Department (TMD). 2015. Thailand weather. Thai Meteorological Department. Ministry of Information and Communication Technology, Thailand.

O-08-8

Genetic Polymorphism at Growth Hormone Receptor and Prolactin locus in Friesian Holstein Crossbred Cows

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Abstract

The aim of the research were to (1) to analyze degree of polymorphism of GHR gene and Prolactin gene in the smallholder dairy farm at East Java. (2) to analyze the potential of candidate gene GHR and Prolactin as a marker in selection. 76 blood sample of lactating cows (1^{st} - 3^{rd} period) were collected for DNA analyzing. Variable measured were milk yield per day (corrected to Energy Corrected Milk), fat and protein content. Method used was PCR-RFLP, using primer F = 5' - TGCGTGCACAGCAGCTCAACC - 3' and R = 5' - AGCAACCCCACTGCTGGGCAT - 3' for GHR gene to amplify 836 bp fragment, and F = 5'-CGAGTCCTTATGAGCTTGATTCTT-3' and R = 5' -GCCTTCCAGAAGTCGTTTGTTTTC-3'for Prolactin gene to amplify 156 bp fragment. PCR product of GHR gene was digested with Alu1 restriction enzyme, and Rsa1 to digest Prolactin gene fragment. Polymorphism degree was calculated using formula $PICi = 1 - \Sigma\ tp^2$

and tested using Chi Square for Hardy Weinberg equilibrium. *One WayAnova* was used to analyze the influence of genotype. Result showed that degree of polymorphism of GHR gene is high (49.98 %), but polymorphism degree of Prolactin gene is 0 (monomorphic). GHR gene is very polymorphic but not in Hardy Weinberg equilibrium. GHR genotype has no influence on milk production and fat content, but strongly influence on protein content ($P < 0.01$). CD genotype affect a higher protein content than both CC and DD genotype. It was concluded that (a) GHR locus is very polymorphic (49.98 %), but Prolaction locus is monomorphic. (b) GHR gene is potential candidate gene compare to Prolactin gene. (c) CD genotype has a positive effect on protein content than CC and DD genotype.

Introduction

In dairy cows, several candidate genes that were reported by several investigators. Some genes controlling hormone in the milk production process shown to be associated with milk production and milk quality. Growth hormone gene polymorphism (GH) and growth hormone receptor gene (GHR) has been shown to have an influence on milk production in Local dairy cows and imports (from New Zealand) (Maylinda 2007). In addition, Prolactin gene known as coding generates Prolactin hormone that plays a role in the production of milk, is also expected as a candidate gene that has a positive effect on milk production and milk fat (Buske, Gengler and Soyeurt, 2011). In order to increase the effectiveness of selection based on genetic markers, the other candidate genes related to milk production and quality need to be implemented.

In dairy cows, several candidate genes that were reported by several investigators. Some genes controlling hormone in the milk production process shown to be associated with milk production and milk quality.

The objective of the research :

(1) to analyze degree of polymorphism of GHR gene and Prolactin gene in the smallholder dairy farm at East Java.
(2) to analyze the potential of candidate gene GHR and Prolactin as a marker in selection.

Methodology

Research material is blood samples that taken from 76 being lactating cows. Cows was in lactation period I - III and month of lactation 1-3. Dairy cattle used in this study is the local dairy cows that are kept by smallholder dairy farms in the district of Jabung, in Malang, East Java province.

Field research

Collection data in the field is done using direct measurement of milk production, fat and protein content. Fat and protein content were measured using *lactoscan* equipment on 200 cc milk sample. In the same time, blood sample is collected from the cows at jugular vein using venoject. The next process were isolation of DNA from white blood cells, then PCR and RFLP.

Laboratory research

Research in the laboratory includes a series of procedures for DNA analysis. This research was conducted at Biotech Laboratory, Department of Agronomy, Faculty of Agriculture, University of Brawijaya in many stages . Description of each stage:

Isolation of DNA to separate the DNA from white blood cells. At the beginning of the study, blood sampling was done from each cow in 5 cc with venoject in the jugular vein in the neck and kept in a vacutainer tube that has been given EDTA. Isolation of DNA is done according to standard protocols isolation from blood samples (Roe 2002).PCR (Polymerase Chain Reaction)that is to amplify the GHR gene fragment with 836 bp (Aggrey, Yao, Zadworny, Hayes and Kuhnlein. 1998).PCR to amplify / duplicate gene fragment of 156 bp Prolactin (Alipanah, Kalashnikova, Rodionov, 2007). Program of PCR in GHR and Prolactin fragment can be seen in Table 1.

RFLP (Restricted Fragment Lenngth Polymorphism) In this case the above PCR products was digested with restriction enzymes Alu1 (for the GHR gene) and Rsa1 (for Prolactin gene). The detailed guidelines about the enzyme, as well as cutting results as presented in Tabel 2.

Genotype and allelic frequency

Genotype frequency is the proportion of GHR genotype (CC, CD and DD) or Prolactin genotype (AA, AB and BB) in the population. Allelic frequency is the proportion of GHR alleles (C and D) or Prolactin alleles (A and B) in the population. The genotype and allelic frequency were calculated using equation according to McClean (1998) as below :

The allele frequency for dominant (or M) is p, and for recessive allele (or N) is q. So, p=f(MM) + ½ f(MN) and q=f(NN) + ½ f(MN), where f(MM) is frequency for MM genotype, f(NN) is frequency for NN genotype and f(Nn) is frequency for MN genotype.

Genetic Polymorphism

Genetic polymorphism expressed as PIC (Polymorphic Information Content), which can be defined as the degree of polymorphism in the population (%). The calculation was below :

$PIC_i = 1 - \sum p^2_{ij}$ (Budak et al 2003)

Where PIC_i is degree of polymoprhism at i-locus, p_{ij} is frequency of j-allele at i-locus. Locus is the location of gene at particular location.

Data analysis

To analysis the effect of genotype on milk production, fat and protein content used One Way Anova using Minitab software version 14 (Minitab 2003), with model as below :

$Y_{ij} = \mu + G_i + e_{ij}$

Whereas Y_{ij} is measurements (milk yield, fat and protein content), μis the overall mean, G_i is the effect of the i^{th} GHR genotypes or Prolactin genotypes (i = 3, CC, CD and DD for GHR or AA, AB and BB for Prolactin), e_{ij} is the random residual error.

Result and Discussion

Genetic polymorphism After digestion of PCR and subsequently performed on PCR products either on the locus of GHR and Prolactin, then obtained the GHR locus genetic polimrofisme high (see Table 4, Figure 1 and 2). However, it turns on genes Prolactin results are monomorphic. This is different with Kaplan and Boztepe (2006) result, where in Brown Swiss cow at Konya Province they found that the population is polymorphic (0.82 for A allele and 0.18 for B allele)

Hardy Weinberg equilibrium in GHR and Prolactin genotypes

GHR gene polymorphisms in this population is quite high, nevertheless remains to be examined whether the circumstances under Hardy Weinberg equilibrium. Table 5 shows the Chi Square analysis to test the balance. For Prolaction gene, the chi square test is not done because the condition is monomorphic. Table 5 presented the result of chi square test to analyse Hardy Weinber equilibrium of alleles and genotype frequency.

The chi square test show that genetic equilibrium at GHR locus was not in balance. The cause is the size of the studied population is small so it does not fulfill the Law of Hardy Weinberg.

Influence of GHR and Prolactin genotypes on Milk Production and Milk Quality

The statistical analysis showed that both of the GHR gene and Prolactin genes have no effect on milk production

and milk fat content. However, it turns out GHR gene polymorphism associated with milk protein content and highly significant (P <0.01) (see Table 6). For Prolactin gene is impossible to analyze besuase is is monomorphic. This is different with Kaplan and Boztepe (2006) result, where in Brown Swiss cow at Konya Province they found that the population is polymorphic (0.82 for A allele and 0.18 for B allele). Random genetic drift could be one factor that led to the loss of one allele in a population of dairy cows at the sites (Holsinger, 2001). The size of the farms in local locations in the area known small.

Based on the analysis of data, it turns out the average milk production and milk fat content is almost the same in all genotypes. However, protein content showed a significant influence (P <0.01). From Table 6 seems that genotype CD showed the best effect compared to genotype CC and DD.

Conclusions

Genetic polymorphisms in the GHR locus in the study area is quite high (49.98%) while the locus Prolactin is 0 (monomorphic) because there is only one kind of genotype is AA.Milk production, milk fat and protein content of milk in the study area is quite high but with a higher level of diversity.Application of marker-based selection GHR gene on milk production and milk fat content still needs to be studied again with more samples, but for the protein content of milk can be a potential marker is genotype CD.In Prolactin gene still need to be reexamined because the population of research location is monomorphic.

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KEYWORD : candidate gene, genetic marker, polymorphism, genotypic equilibrium, genetic marker, polymorphism, genetic equilibrium

Table 1: Program and primer of PCR of GHR and Prolactin fragments

Gene	Primer	PCR Program
GHR	F = 5' – TGCGTGCACAGCAGCTCAACC - 3' R = 5' – AGCAACCCCACTGCTGGGCAT – 3'	Pre-denaturation at 94o C for 5, denaturation at 92 o C for 1 min, annealing at 66 o C for 80detik, and elongation at 72 o C for 2 minutes, repeated 35 cycles and extension at 4 o C during tatime (Aggrey et al. 1998).
Prolactin	F = 5′-CGAGTCCTTATGAGCTTGATTCTT-3′ R = 5′-GCCTTCCAGAAGTCGTTTGTTTTC-3′	Predenaturasi at 94ºC for 5 minutes, followed by 30 cycles consisting of denaturation at 94ºC for 30 seconds, annealing at 59ºC for 40 seconds, and extension at 72ºC for 20 seconds, and a final extension at 72ºC for 3 minutes (Alipanah et al. 2007)

Table 2.The detailed about the enzyme cutting result and the alleles				
Gene	Restriction enzyme	Size	Result of digestion	Allele
GHR	Alu1	836 bp	Truncated: 602, 145, 75 and 14 bp (normal allele) truncated: 747, 75 and 14 bp (mutant allele)	C D
Prolactin	Rsa1	156 bp	Not truncated : 156 bp (normal allele) truncated: 82 bp and 74 bp (mutant allele)	A B

Table 3. Frequency of genes and genetic polymorphisms each locus				
Gene	Genotype	Number of cows	Allele frequency *)	Polymorphism level **)
GHR	CC	1	p = 0.49 q = 0.51	PIC = 49.98 %
	CD	62		
	DD	13		
Prolactin	AA	76	p = 1 q = 0	PIC = 0 %
	AB	0		
	BB	0		

*) p is frequency of dominant allele, q is frequency of recessive allele. PIC = Polymorphic Information Content according to Budak et al (2003)

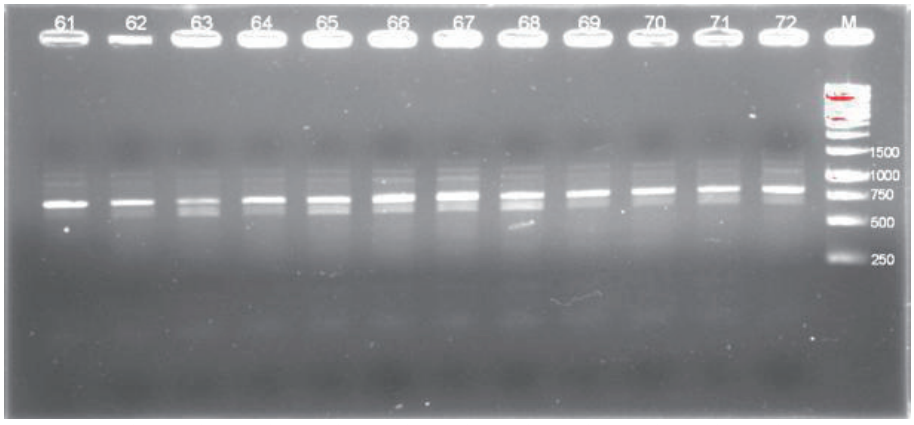
Table 4. GHR genotype balance according to Hardy Weinberg					
Gene	Genotype	Genotype frequency	Number of individu (observed)	Number of individu (expected)	χ^2
GHR	CC	0.2401	1	18	33.66**
	CD	0.4998	62	38	
	DD	0.2601	13	20	

χ^2 is highly significant

Table 5. Mean Production of Milk, Milk Fat Content and Milk Protein in Various GHR and Prolactin Genotypes

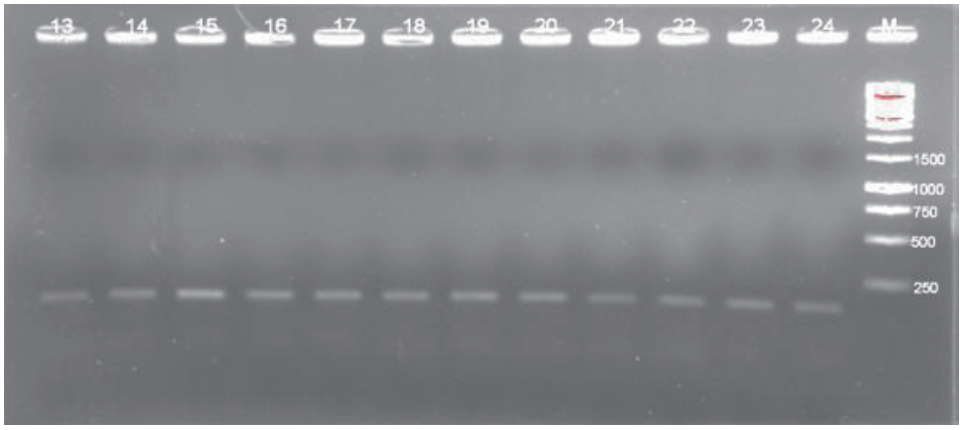
Variables	Genotypes	Mean	Significance
Milk yield (l)			
GHR :			
CC		10.31	Ns
CD		11.89)	Ns
DD		12.89 + 3.67 (59)	Ns
Prolactin :			
AA		12.51 + 3.73 (52)	Ns
Fat content (%)			
GHR :			
CC		4.39 + 0.01 (2)	Ns
CD		4.26+ 0.88 (19)	Ns
DD		4.75 + 0.91 (48)	Ns
Prolactin :			
AA		4.61 + 0.92 (69)	
Protein content (%)			
GHR :			
CC		2.82+ 0.05 (2) a	**)
CD		2.95 + 0.27 (10) b	**)
DD		2.72 + 0.18 (48) a	**)
Prolactin :			
AA		2.79 + 0.23 (69)	Ns

Note : Ns = non significant, **) = sigficantly difference ($P < 0.01$), different superscript in the same column means significantly different.



DD CD CD CD CD CD CD CD CD CD CD CD

Figure 1. Result of 2 % gel electrophoresis of PCR-RFLP product in GHR Locus



AA AA AA AA AA AA AA AA AA AA AA AA

Figure 2. Result of 2% gel electrophoresis of PCR-RFLP products at the locus Prolactin

REFERENCE

Aggrey SE, Yao J, Zadworny D, Hayes JF and Kuhnlein 1998 Synergism between genetic markers in the growth hormone and growth hormone receptor genes in influencing milk related traits in Holsteins. In: 6th World Congress on Genetics Applied to Livestock Production. Armidale NSW 2351, Australia. 26 : 281-284

Alipanah, M, L. Kalashnikova, G. Rodionov. 2007. Association of prolactin gene variants with milk production traits in Russian Red Pied cattle. Iranian J. of Biotechnology, Vol. 5, No. 3, July : 158-161

Budak H., F. Pedraza,P.B. Cregan,P.S. Baenziger,I. Dweikat 2003 Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of Pearl Millet germplasm. *Crop Sci. of America.* 43: 2284-2290

Holsinger KA. 2001. Lecture Notes in Population Genetics. Department of Ecology & Evolutionary Biology, U-3043. University of Connecticut Storrs, CT 06269-3043.

http://creativecommons.org/licenses/by-sa/3.0/

Kaplan S and Boztepe S. 2006. The Determination of Prolactin Gene Polymorphism Using PCR-RFLP Method within Indigenous Anatolian Water Buffalo and Brown Swiss.

http://eprints.ibu.edu.ba/362/1/issd2010_science_book_p168-p173.pdf

Maylinda, S. 2007. Genetic marker for milk production in Imported and Local Dairy cattle at Grati Pasuruan, Provinsion of East Java. http://repository.ipb.ac.id/handle/123456789/46734

Maylinda S. 2011. Genetic polymorphism of growth hormone locus and its association with bodyweight in Grati dairy cows. http://www.academicjournals.org/IJBMBR. International Journal for Biotechnology and Molecular Biology Research Vol. 2(7), pp. 117-120

McClean P 1998 Deriving Genotypic and Allelic Frequencies. www.ndsu.edu/pubweb/~mcclean/plsc431/popgen/popgen2.htm

Roe BA 2002. Methods for DNA Isolation. Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, Oklahoma 73019 broe@ou.edu

O-08-9

PHENOTYPE AND GENOTYPE CHARACTERISTIC OF FILIAL ETAWAH DAIRY GOAT

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INTRODUCTION

Dual purpose Filial Etawah dairy goats are now increasingly kept by small farmers. More recently the local Governments have given priority to development of poor villages by promoting goat keeping schemes with emphasis on the landless farmers. While the goats were expected to produce milk for human consumption few of them are milked regularly. So the selection of milk goats for commercial dairy herds and breeding herds must be accomplished in term of keeping and maintaining good record an becoming familiar the different strengths that dairy goats possess.

Prediction of the future performance of goats is the most rational point in animal breeding and animals of superior traits and the phenotype should be selected to hasten genetic improvement. The use of polymorphic genes as genetic molecular markers is a promising surrogate to the current methods of selection once these genes are proven to be associated with traits of interest in animal.

Many studies were performed to investigated the effect of β-LG genotypes on milk production traits, milk composition and quality. Moioli et al. (2007) suggest that β-LG is the candidate genes that affect the production of sheep\'s milk and dairy goats. Intensive examination of how large the contribution variant genotype of β-LG in affecting the production and quality of milk in dairy goats has been carried out by Kumar et al., (2006) in 1098 samples from 8 different Indian goats breed, the result were some genes β-LG AA genotype had higher milk production of the β-LG gene AB genotype and said that PCR-RFLP is a good analysis that can be used to identify genes β-LG in goat milk. Elmaci et al. (2009) on Turkey concluded that polymorphism goat milk proteins and variability of β-LG locus could potentially be used as a genetic marker in dairy goats. Susilorini (2013), β-LG gene polymorphisms had affect on milk production of *Peranakan Etawah* dairy goat, which AA and AB genotypes were higher milk production than BB genotype.

The aim of this study was to determine the main polymorphisms in β-LG genes in Filial Etawah goats and to design a selection method based on the presence the genetic marker.

Materials and Methods

*Samples and DNA isolation*A total of 66 blood samples were collected from goat in keep by small farmer in Ampelgading East Java. Blood samples from the goats were placed into an EDTA tubes for DNA isolation. Genomic DNA was isolated using Genomic DNA Isolation kit (NORGEN) following the manufacturer's protocol. The quality of DNA was checked on 1.0% agarose gels and stained with ethidium bromide.

*DNA Amplification and Genotyping*Genotyping for β-LG-*Sac*II polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) as proposed by Kumar and Kumar (2006) .The sequences of the forward and reverse primers for the amplification of the β-LG gene (accession numberZ33881.1) were: 5'-CGG GAG CCT TGG CCC TCT GG-3'; reverse 5'-CCT TTG TCG AGT TTG GGT GT-3'. PCR for the β-LG gene was performed PCR Master mix Solution, (iNtRON) : in a 5 μl reaction mixture containing 2 μl ddH2O; 1 μl Primer F 1 μl Primer R; 5 μl master mix and 1μl of genomic DNA template.

Thermal cycling conditions included: an initial denaturation step at 95℃ for 5 min followed by 35 cycles of 95℃ for 30 s, 65℃ for 30 s, 72℃ for 30 s and a final extension at 72℃ for 10 min. The PCR products were digested with 0.2 μl of *Sac*II restriction endonuclease (Fermentas) at 37℃ for at least 60 min. PCR products and restriction fragments were electrophoresed on 2% agarose gels respectively and stained with ethidium bromide.

Statistical Analysis Direct counting was used to estimate genotype and allele frequencies of β-LG gene *Sac*II genetic variants. Chi-square statistic (χ^2) was used to check whether the populations were Hardy-Weinberg equilibrium.

RESULT AND DISCUSION

Performance of Filial Etawah goat

The results of performance Filial Etawah goats, milk production (935.8 ± 171.5 mL/day); milk protein (2.66 ±

0.24 %); milk fat (6.92 ± 1.07 %); birth weight (3.75 ± 0.23 kg) the litter size was 1.6 at first parity and increased up to 2.4 at fourth parity. The average kidding interval ranged from 7 to 9 months with pregnancy duration of 5.2 month. The kids were naturally weaned when they were 3.5months old ranging from 2 to 5 month and the weaning weight (19.39 ± 1.30 kg). The result statistical analysis proves that the litter size was highly significant ($P < 0.01$) towards the production of milk that is higher litter size, the higher the milk production.

Identification of polymorphisms in the goat B-LG gene

The β -LG gene (exon 7 to the 3'flanking region) of Filial Etawah goat was investigated by PCR-RFLP method. A fragment of 426 bp was successfully amplified and digested with *Sac*II restriction enzymes to detect the presence of A or B variants . As a result of amplification product with *Sac*II digestion, two alleles, A and B, were observed. Restriction digestion of 426bp PCR products with *Sac*II enzymes revealed two genotypes *(Fig. 1)* of AA (426 bp), AB (426bp and 349 bp).

Fig. 1. Electrophoresis of RFLP of Filial Etawah goats β -LG gene after digestion by *Sac*II of animals with AA (426bp), AB (426 bp and 349bp)

The allelic and genotypic frequencies of the β -LG gene polymorphism for Filial Etawah goats were given in *Table 1*.

Table 1. Allele and genotype frequencies of B-LG gene of Filial Etawah goats

The results of Chi-square statistic reflected that breeds were in Hardy-Weinberg equilibrium.

Results of statistical analysis showed that genotype significantly affect milk production. Goats with genotype AA had higher milk production than those with AB genotype. Therefore polymorphisms of β-LG gene had effect on milk production. Chen et al (2005) also prove the influence of β-LG gene to milk production in Saanen goats, while Kumar et al (2006) showed on the native goats in India. Pena et al (2000) and El-Hanafi et al (2010) reported that the AA genotype superior to milk production.

CONCLUSION

In conclusion, the result of the present study indicated significant association between AA genotype of β -LG gene and higher milk production. A selection based on markers not only minimizes problems but also they are more reliable, and animal can be selected at an early age for breeding program.

KEYWORD : Filial Etawah dairy goat, β-Lactoglobulin gene, birth weight, weaning weight

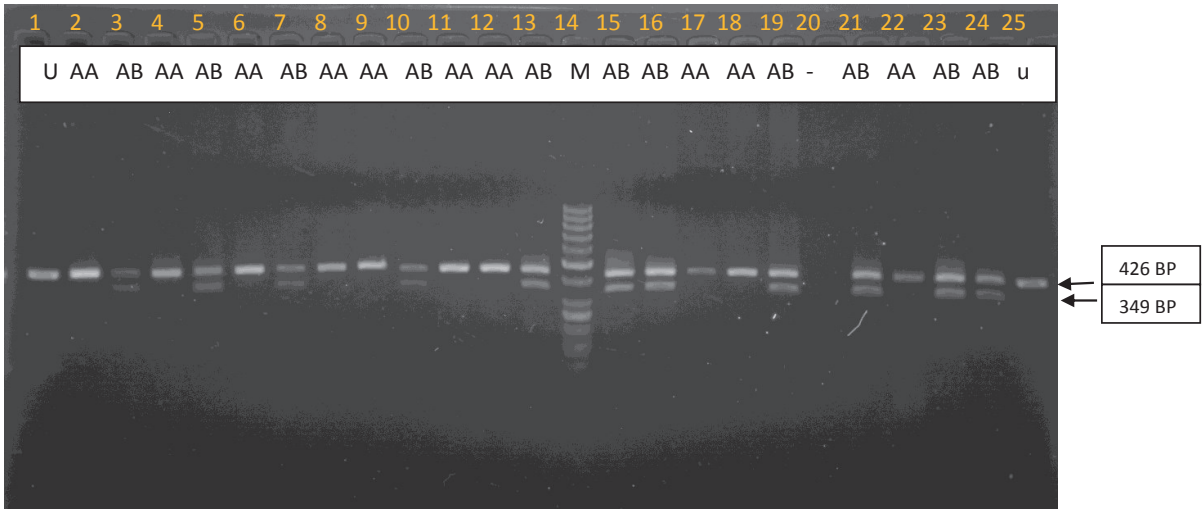

Fig. 1. Electrophoresis of RFLP of Filial Etawah goats β-LG gene after digestion by *Sac*II of animals with AA (426bp), AB (426 bp and 349bp)

Table 1. Allele and genotype frequencies of B-LG gene of Filial Etawah goats

Genotype	n	Frequencies genotype	Allelic	Frequencies Allelic	X^2 test	Milk production (mL/day)
AA	35	0,54	A	0,77	0,09	961.38 74.35
AB	31	0,46	B	0,23		826.55 94.39
Total	66					

REFERENCE

Chen, H. Lan XY, Li RB. Lei CZ, Sun WB, Zhang RF, Zheng YL, and Zhu BC. 2005. The Effect of CSN1 S2 CSN3 and Beta-LG Genes on Milk Performance in Xinong Saanen Dairy Goat. http://www.ncbi.nlm.nih.gov/pubmed/1623174/Abstract

Elmaci, C; Y Oner and M. Koyuncu. 2009. Allelic Frequencies of a *SacII* at Exon 7 of the β-lactoglobulin gene in Turkish Hair Goat Breed. Asian J. Of Anim. and Vet. Advances 4(3): 130-133. http://www.scialert.net

El-Hanafy, A.A., M.A. El-Saadani, M.Eissa, G.M. Maharem, Z.A. Khalifa. 2010. Polymorphism of β -lactoglobulin Gene in Barki and Damascus and Their Cross Bred Goats in Relation to Milk Yield. Biotecnology in animal Husbandry 26 (1-2), p 1-12, 2010. Publisher: Institute for Animal Husbandry, Belgrade-zemum. http://www.doiserbia.nb.rs/img/doi

Kumar, A., P. Kumar Rout, R. Roy. 2006. Polymorphism of β -Lactoglobulin gene in Indian Goats and Its Effect on Milk Yield. J. Appl Gene 47(1),2006. http://jay.up.poznan.pl/JAG/pdfy/2006_Volume_47/2006_Volume_47_1-49-53.pdf

Moioli, B., M.D'Andrea and F. Pilla. 2007. Candidate Genes Affecting Sheep and Goat Milk Quality. http://www.smallruminantresearch.com/article/

Susilorini, T.E. 2013. Polymorphism of β -Lactoglobulin Gene in Peranakan Etawa (PE) Dairy Goats in Relation on Milk Yield. Proceeding The Second APIS (Animal Production International Seminar, Malang Indonesia Proceeding

O-08-10 ROLE OF ARTIFICIAL INSEMINATION ON THE PROPAGATION OF IMPROVED BREEDS OF GOAT IN NORTHERN LUZON, PHILIPPINES

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INTRODUCTION

Goat raising in the Philippines is regarded as an integral component of farming system especially for small raisers. Majority of the goat raisers in the country are located in the backyard-level, and they only keep 5 to 25 does. The small herd size on these farms is due to the frequent selling of stocks especially in times of need. Due to limited capital investment, Philippine Native goat are being raised. This breed of goat is characterized by it small, stocky, and low set appearance with hair color of red, white or black, or combination of these colors; and weighs only 20kg on the average at mature stage. With this kind of production performance, their profit is low. Breeding is one important activity in livestock production, as the raiser has the opportunity to increase herd size and improve the production performance. To attain this, introduction of exotic breed is needed to improve the native. The Philippine government started to import Boer and Anglo-nubian breeds since early 2000 to start the cross breeding. Qualified raisers were allowed to avail buck loan program. However, because many raisers cannot meet the requirements, they were not able to avail the loan. The use of artificial insemination (AI) was introduced in 2009 to help spread the genetic materials of the superior buck in the countryside. In the country, the use of AI to improve the performance of large ruminant and swine is already established, but for goat, the technology is not well utilized.

OBJECTIVE

The main objective of the paper is to present to role of AI in the propagation of improved breeds of goat in Northern Luzon. Specifically, the paper will present production performance of the different breed group within five-year period.

METHODOLOGY

The semen from pure breed Boer (B) and Anglo-nubian (AN) bucks were collected and stored at the Cagayan Valley Small Ruminants Research Center. Frozen goat semen were used in insemination activities. The doe were identified by putting ear tags to facilitate efficient recording. Prior to breeding activities, a breeding program was first developed for implementation. The goal is to produce stocks with heavier weights from birth to slaughter (8 month), which will ensure higher carcass yield and better body conformation. The first cross is between Anglo-nubian (AN) and the Philippine native doe (N). The stocks produced from this cross is expected to have an improved milking ability to support the kids they will be producing. The next cross used is Boer (B) and the 50%Anglo-nubian, 50%Native doe. The stocks produced in this cross will have an improve growth performance and better meat quality. The stocks produced (50%B, 25%AN,25%N) is crossed with Boer to sustain the improvement of the meat quality.

The record of the stocks produced in backyard farms were retrieve from 2010 to 2015 and was analyzed to determine the improvement of the breed groups per year in terms of birth and slaughter (8month). The record of the stocks qualifies to the breed group expected was also used to established baseline information. A total of 500 stocks with record was used in the analysis. The difference on the growth performance of each breed group per year was analyzed using Analysis of Variance (ANOVA) and the Least Significance Difference (LSD) to determine the difference between mean.

RESULT

There were four (4) different blood groups produced from the breeding activities. These are 50%AN,50%N; 50%B,25%AN,25%N, 75%B,12.5%AN,12.5%N; and 87.5%B,6.25%,6.25%. The performance of these blood groups were analyzed to determine the improvement over the year.

Using available record from on-station and on-farm, the average weight per growth stage of 88 goats with 50%AN, 50%N from 2010 to 2015 was analyzed. It was noted that birth weight (BW) alone, there was an improvement

from 0.8kg to 1.4kg in 2015. On the other hand, and improvement was also noted for the animal's slaughter weight from 2010 at 15.45kg to 24kg in 2015 or an improvement of 8.55kg over five-year period. The ANOVA of the recorded BW and SW form 2010 to 2015 revealed that there is a high significance difference between the performances of the stocks produced, an indication of improved production management employed in the farm.

On the recorded growth performance of the stocks with blood composition of 50%B,25%AN, 25%N it was noted that the highest recorded BW, was 1.9kg in 2015, wherein improvement was observed at 1.14kg baseline in 2010. Moreover, SW also constantly improved from 17kg in 2010 to 20.65kg in 2015. The performance can be attributed to management system applied in the farm and other environment related factors. The result of ANOVA reveals that the weaning weight of stocks with 50%B,25%AN,25%N blood composition is significantly different at 5% level of significance from 2010 to 2015 recorded growth performance.

For the animals with blood composition of 75%B, 12.5%AN, 12.5%N, a steady improvement was observed on BW and SW record from 2010 to 2015. On the average, BW improved from 1.7kg in 2010 to 2.1kg in 2015; while in SW, improvement is noted from 20.8kg in 2010 to 25kg in 2015, or an improvement of more than 5.8kg over 5 years. The improvement is an indication of improve farm production management. It can also be noted that since the appearance of the stocks raised are already improved, different management is given to the stocks. The result of ANOVA for BW from 2010 to 2015 was found to be not significant; however, the difference of the recorded SW is highly significant from 2010 to 2015.

On the growth performance record of the stocks produced with blood composition of 87.5%B, 6.25%AN, 6.25%N, the birth weight improved from 2.7kg in 2010 to 3.13kg in 2015. The recorded slaughter weight was more than 30kg in 2015. The improvement in growth performance is already attained based on the goal set prior to breeding plan execution. Generally, the improvement is a positive attribution to the improvement of blood composition of the animal as well as in the production management. The analysis of variance (ANOVA) revealed that there is no significant difference on the BW recorded from 2010 to 2015. However, the difference per year of the recorded SW is highly significant. (See Figure 1, 2)

Based form the results presented, the improvement of goat's breed composition is attainable following a breeding plan (Ogola et al, 2010). Thru the use of artificial insemination, breeding can be facilitated faster even at backyard-level of production. On the other hand, the improvement on the growth performance per year is an indication that there has been an improvement in the production management including proper nutrition and health management which are vital in the expression of the good traits inherited that the raisers employed. It is also important that the breeding initiative implemented in the farms, it should be coupled with raisers' proper knowledge and understanding of the practices as these have an interrelationship with each other (Kumba, 2003; Gutierrez, 2010; Landon and Powell, 1996). It has been documented earlier that introduction of exotic breed of goat improves the growth performance at barangay level (Orden et al, 2007); however, it should be sustained in order to attain the goal of improving the profits from goat production (Simon, 2013) and to have an increase of meat supply in the countryside (Peacock, 1996).

CONCLUSION

The application of artificial insemination as breeding tool is effective in improving of genetic composition of the animal. A breeding plan must be followed in order to ensure the stocks produced are within the breeding target. The improvement of the animal may take place over time, and production management must also improve as the animal with different breed groups may have different requirement. The traits inherited by animals may only be expressed under proper production management with emphasis on feeding and nutrition. Moreover, as the advantages of breeding the stocks may only be realized by the weight improvement and the quality of meat produced, it is important the raiser has adequate knowledge in production.

KEYWORD : goat, breeding, artificial insemination, production, performance evaluation

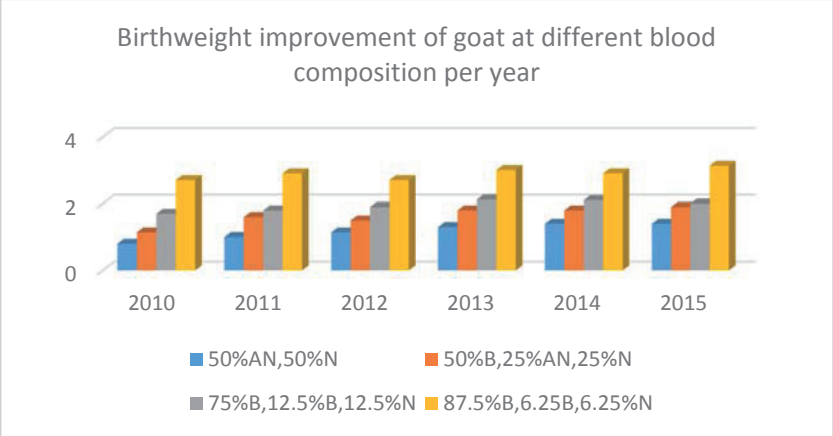

Figure 1. Birth weight of goat belonging to different breed group from 2010 to 2015

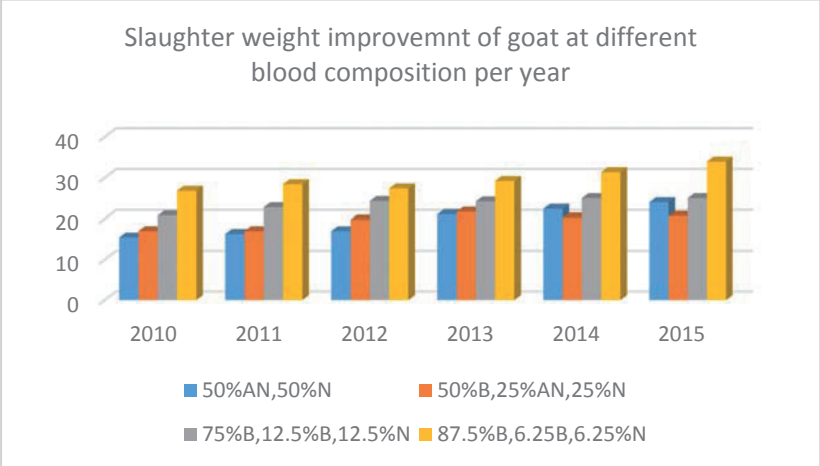

Figure 2. Slaughter weight of goat belonging to different breed group from 2010 to 2015

Table 1 Effect of roselle extract on *Staphylococcus aureus* reduction in ground pork stored at 4 °C for 10 days

Storage time (days)	Number of *Staphylococcus aureus* (log CFU/g)		
	Control	Roselle extract (50 mg/ml)	Log reduction
0	$3.26 \pm 0.08^{a,A}$	$3.20 \pm 0.11^{a,A}$	0.06
2	$4.16 \pm 0.37^{a,B}$	$3.35 \pm 0.05^{b,A}$	0.81
4	$4.53 \pm 0.18^{a,C}$	$3.64 \pm 0.20^{b,AB}$	0.89
6	$4.86 \pm 0.03^{a,D}$	$3.73 \pm 0.23^{b,AB}$	1.13
8	$5.07 \pm 0.16^{a,D}$	$4.17 \pm 0.74^{b,B}$	0.90
10	$6.42 \pm 0.06^{a,E}$	$4.43 \pm 0.65^{b,B}$	1.99

[a-b]The means having different superscripts in a row are significantly different ($P < 0.05$)
[A-E] The means having different superscripts in a column are significantly different ($P < 0.05$)

Table 2 Effect of roselle extract on *Salmonella* Typhimurium reduction in ground pork stored at 4 °C for 10 days

Storage time (days)	Number of *Salmonella* Typhimurium (log CFU/g)		
	Control	Roselle extract (50 mg/ml)	Log reduction
0	$3.12 \pm 0.17^{a,A}$	$3.07 \pm 0.08^{a,A}$	0.05
2	$4.14 \pm 0.11^{a,B}$	$4.25 \pm 0.25^{a,B}$	Increased
4	$5.20 \pm 0.10^{a,C}$	$5.13 \pm 0.01^{a,C}$	0.07
6	$5.64 \pm 0.06^{a,C}$	$5.59 \pm 0.01^{a,C}$	0.05
8	$6.98 \pm 0.38^{a,D}$	$6.38 \pm 0.57^{a,D}$	0.60
10	$7.41 \pm 0.68^{a,D}$	$7.17 \pm 0.57^{a,E}$	0.24

[a] The means having different superscripts in a row are significantly different ($P < 0.05$)
[A-E] The means having different superscripts in a column are significantly different ($P < 0.05$)

REFERENCES

Kumba, FF. 2003. Farmer Participation in Agricultural Research and Extension Service in Namibia. Journal of International Agricultural and Extension Education.

Gutierrez, N. 2010. Economic constraints on sheep and goat production in developing countries. Small Ruminant Production in Developing Countries.

Landon, LC and AP Powell. 1996. Participatory Rural Appraisal concepts applied to agricultural extension: a case study in Sumatra. Quarterly Bulletin of IAALD, 41 (1),100-103.

Ogola, TDO, WK Nguyo, IS Kosley. 2010, Dairy goat production practices in Kenya: Implications for a breeding program. Livestock Research for Rural Development 22(1), 2010.

Orden, EO, EM Cruz, MEM Orden, AS Galamgam and PQS delos Reyes. 2007. Barangay goat breeder farms in selected areas in Nueva Ecija and Pangasinan. Philippine Journal of Veterinary and Animal Sciences. Volume 33, Number 1.

Peacock, C. 1996. Improving Goat Production in the Tropics. Oxfam (U.K and Ireland)

PCARRD, Invest in Goat Farming. 2007. Los Banos, Laguna.

PCARRD, Goat Production in Asia. 1985. Los Banos, Laguna.

PCARRD, Philippine Recommends for Goat Farming. 2006. Los Banos,Laguna.

Simon, Marion. 2013. Marketing Meat Goats, the Basic System. Small Ruminant Production in Developing Countries. Published by the America's Research- based Learning Network.

O-09-1

FACTORS INFLUENCE ON SMALLHOLDER DAIRY FARMING INCOME (Case Study in Malang, Indonesia)

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INTRODUCTION

Milk consumption in Indonesia reaches about 3.3 million ton in year 2013 where it was supplied by both domestic and imported from some countries. Majority fresh milk are produced by smallholder dairy farming which characterised with small size ownership, simple technology, low milk productivity and its quality, lack of market orientation, and quite insensitive with the environment alteration. Dairy farming is commonly used as the second earning sources, while the primary rural livelihood comes from agriculture sector.

Farmers usually made the partnership system with milk industry of Greenfields Indonesia co. which utilised milk co-operative in offering facilities along the dairy agribusiness sector. They provide concentrate feed, medicine, and vitamin in the up-stream sub-sector, services in rearing management for on-farm, and marketing milk in down-stream sub-sector. Similarly, the Greenfields Indonesia co. will get more milk that supplied by farmer as the payment of the concentrate feed, medicine and vitamin. The mutually benefit relationships might be useful to sustain the existence of dairy farming. Moreover, this area has a suitable environment and high potential in developing dairy farming. Therefore, the objectives of the research were to determine the income of smallholder dairy farmers, and examine the factors differentiating on the dairy farming profit.

Hypothesis

Productions costs play an important role in determine the dairy farming product such as fresh milk production. Variable cost has a high proportion in the dairy farming expenses compared to fixed costs. According to theory, feed cost can obtain up to 80% and it therefore stated as:

Hypothesis 1: “It is predicted that dairy feed cost has a negative influence on smallholder farming income”.

Hypothesis 2: “It is predicted that milk production has a positive effect on smallholder dairy farming profit”.

METHODOLOGY

Case Study was carried out at small scale dairy farming at Ngajum Sub-District, Malang Regency. Multistage sampling method was applied to obtain 45 dairy farmers with criteria owning at least one lactating cow, and experiencing four years and more in raising dairy farming. Respondents were divided into three scales, scale -1 (owning 1 - 2.7 AU, n = 21), scale-2 (controlling 2.71 - 4.41 ST, n = 17), and scale-3 (raising 4.42 - 6.12 AU, n = 7). Data were collected from 1st April 2014 to 1st May 2014. Survey method with a structured questionnaire was applied to obtain primary data, for instance production cost, revenue, consumer characteristics, dairy farming profile, and internal resources. Secondary data were provided by milk co-operative and the related institutions.

Data were analysed by descriptive analysis to delineate dairy farming income which derived from the subtraction between dairy revenue and its production costs. Regression analysis is employed to explore the determinants influencing on dairy farming profit. The income in dairy farming is a function of the internal and external variables of the model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + e_i$$

where:

Y : income in smallholder dairy farming per AU (IDR /year),

X1 : farmer’s age (year),

X2 : farmer’s education (year),

X3 : farmer’s experience in raising dairy farming (year),

X4 : Number of household members (person),

X5 : variable cost of dairy feed per AU (IDR / year),

X6 : number of lactating cow (head),

X7 : land possession (hectare),

X8 : the number of milk production (litre),

X9 : farming scale (0= scale 1, 1=scale 2, and 2 = scale 3)
β_0 : constant
β_1..... β_9 : estimation parameter
e: error

RESULTS

Dairy farmers' characteristics

Majority farmers who have 3-4 family members aged between 32-57 years old with low education and having about 4-10 years' experience in rearing dairy farming. They have poor knowledge in rearing management of dairy farming, for instance low feed quality and lack ability in improving milk productivity. These circumstances have an impact on the slow and even stagnant in dairy farming development. Fortunately, farmers included in productive period that capable to decide more efficient and effective in improving dairy farming since they become easier to adopt the technology development. Moreover, the longer experience implied in the ability in correcting their weakness in producing milk. In addition, as the number of household members increase, a greater amount of time may be devoted in dairy farming to acquiring more income from dairy farming.

The income statement of smallholder dairy farming

Income statement aimed to investigate the financial performance of the dairy farming in obtaining either loss or profit at the time of them running this enterprise. It is composed by total revenue, production costs, and profit. Dairy farming in scale-1 achieved the best revenue of IDR 7,058 in comparison with IDR 6,211 of scale-2 and IDR 5,440 of scale-3 in each litre of fresh milk production. Overall, the highest revenue was coming from selling milk across the three scales of dairy farming ranging 63.75 - 82.72%. Scale-3 has generated a great number (82.72%) of fresh milk revenue than those of scale-1 (63.75%) and scale-2 (72.46%). The average fresh milk production of scale-3 was higher (15.98 litre/day) compared to scale-1 (12.27 litre/day) and scale-2 (9.66 litre/day). Non-milk earning of scale-1 (36.25%) however, exceeded those of scale-2 (27.48%) and scale-3 (17.28%). Selling culled dairy cow is account for approximately 23.35% of scale-1 than 17.44% and 10.09% for scale-2 and scale-3, respectively. Similarly, selling calves achieved about 12.90% in scale-1, whereas it was only 10.02% of scale-2 and 7.19% of scale-3.

Production costs per litre milk were efficiently (IDR 3,854) observed for scale-3 than those of scale-2 (IDR 4,943) and scale-1 (IDR 5,541). Fixed costs structured dairy production cost with a little proportion and it is account for about 2.22%, 2.42%, and 2.05% for scale-1, scale-2, and scale-3 in order. The expenses were mostly composed by variable costs among the three scales of dairy farming namely scale-1 (97.78%), scale-2 (97.58%), and scale-3 (97.95%). with 97.78% of variable costs and 2.22% of fixed costs of scale-3. Generally, concentrate feed has dominated in the expenditure among the three scales of dairy farming. It is about 48, 60%, 41.98%, and 39.99% of concentrate feed costs for dairy farming scale-3, scale-2, and scale-1, respectively. Likewise, forage feed of scale-3 has generated a great number of 31.79%, whereas it is account for approximately 29.21% of scale 2 and 25, 31% of scale-1. This discovery differed with the study of Utami and Seruni (2014) which concentrate feed composed 59.80% of dairy production costs. Previous research found that feed cost structured about 84.9% of the expenses in dairy farming (Vaida, 2013) that consisted of 60.10% in concentrate feed cost and 25.50% distributing in forage cost (Maharani, 2014).

On the contrary, dairy farming scale-1 has required more expenses in breeding stock (23.38%), transportation cost (7.14%), Artificial Insemination (1.7%), and water cost (0.31%). Dairy farming scale-2 and scale 3 exhibited the less expenditure about 19.30% and 12.75% of breeding stock, 5.52% and 3.75% of transportation cost, 1.31% and 0.89% of Artificial Insemination, 0.26% and 0.18% of water cost, respectively.

Scale-3 appeared the excellence of profit (IDR 1,586) compared to those of scale-1 (IDR 1,517) and scake-2 (IDR 1,268) in producing one litre of milk. The assessment of profit utilised in evaluating financial position on the farm which consist of monitoring cost, ensuring high labour efficiency, measuring the production levels and determining the appropriate herd size (Field, 2012).

Factors influencing on smallholder dairy farming income

The regression analysis was employed to explain the different in smallholder dairy farming income according to farmer characteristics, internal resources, production cost, and the number of milk production. The results of regression analysis showed that coefficient determination (R^2 Adjusted) were about 0.80 (Table 1). It reveals about 80% of the annual income of dairy farming per animal units (AU) were explained by farmer's age (X_1), farmer's education (X_2), number of household members (X_4), feed costs (X_5), land possession (X_7), and the number of milk

production (X8).

F-calculation t (30.378) was higher than F-table (1.94). It implied that eight predictor variables namely farmer's age (X_1), farmer's education (X_2), number of household members (X_4), feed costs (X_5), land possession (X_7), and the number of milk production (X8) together influenced on smallholder dairy farming income. Partial analysis however depicted that only the expense in dairy feed and the number of milk production were negatively (P <0.000) and positively (P<0.000) associated with the income in smallholder dairy farming, respectively.

Table 1. Determinants influencing in smallholder dairy farming income

Explanation	Regression coefficient
Constant	-4.893
Farmer\'s education	0.026
Experience in rearing dairy cattle	0.036
Number of household members	0.067
Feed cost	-0.876***)
Land possession	0.056
Number of milk production	1.341***)
R square (R^2)	0.827
Adusted R square (Adj.R^2)	0.800
F	30.378
n	44

Note : ***) : P < 0.000

Dairy feed cost

This variable had a significant (P< 0.000) and negative associated with the income of smallholder dairy farming. The finding accepted hypothesis 1 in that: "It is predicted that dairy feed cost has a negative influence on smallholder dairy farming income". It means that a 10% improvement of the dairy feed expenses would have a corresponding 8.76 % decrease in the profit of smallholder dairy farming, if other factors are held constant. The concentrate feed and forage expenses revealed the reverse trend with the accruing dairy farming income. This invention indicated that the total feed costs dominated in production costs as having impact on reduction on the profit of dairy farming. Likewise, smallholder farmer in Thailand have to used 100% commercial feed (15.4%), 100% homemade concentrate (30%) and mixing concentrate (54.6%) (Lucila, 2005).

Number of milk production

This variable were both significantly (P<0.001)) and positively influenced on smallholder dairy farming income. The discovery received hypothesis 2: "It is predicted that milk production has a positive effect on smallholder dairy farming profit". It can be interpreted, as when controlling all other variables, a 10% increase of the number of milk production, would have a corresponding 13.41% improvement in the smallholder dairy farming income. The study demonstrated that the accruing household income can influence the income in dairy farming.

The strong relationship between those variables indicated that the more dairy cattle owned by farmers, the more lactating cows were present on farms. This is consistent with the expectation that dairy farmers with higher dairy cattle numbers should have more lactating cows. Similarly, more lactating cow can reflect the higher total milk production and result in the improvement in dairy farming income. Farmers required sharing of experiences and support strategies among organizations to speed up improvements in dairy production efficiency(Koonawootrittriron et al., 2012), and government might develop basic infrastructure and facilitate the access to yield increasing technology (Uddin et al. 2010) which addressed to reduce costs, improve on productivity and farm profit.

CONCLUSIONS

Dairy farmers who grouped into three farm scales (scale-1 with 1.96 AU, n=21; scale-2 with 3.37 AU, n=17; and scale-3 with 5.25 AU, n=7) were concluded that:

Farmers who raised 5.25 AU were more profitable with IDR 3,854 of production cost, IDR 5,440 revenue, and IDR 1,586 of income in producing one litre of milk. Dairy farming profit will improve along with the increase of milk production. However, dairy income will decrease since the feed cost increase.

KEYWORD : Production cost, Revenue, Income, Profitable, Milk

REFERENCES

Field, G. R., 2012. **Scientific Farm Animal Production: An Introduction to animal science**. Tenth edition. United States of America: Pearson Education Inc.

Koonawootrittriron, S., Elzo, M.A., Yeamkong, S. and Suwanasopee, T., 2012. A comparative study on dairy production and revenue of the dairy farms supported by a dairy cooperative with those supported by a private organization in Central Thailand.

Livestock Research for Rural Development. Vol. 24 (4).

Lucila A., Lapar, Alexis G., Satit A., and Patcharee S. 2005. Measuring Cost Efficiency of Small Holder Dairy : Imperial Evidence from Northeast Thailand. **American**

Agricultural Economics Association Annual Meeting, Providence, Rhode Island, July 24-27, 2005.

Maharani, B.R.D., 2014. Factors influencing on dairy farming income at Banjarejo village,

Ngantang Sub-District, Malang Regency. **Under Graduate Thesis**. Faculty of Animal Husbandry, University of Brawijaya. Malang, Indonesia

Utami, H.D. and Seruni, A.P. (2014). Financial performance of small scale dairy farming in East Java Indonesia. Livestock Research for Rural **Development**. Vol. 26 (11).

Uddin, M.M., Sultana, M.N., Ndambi, O.A., Hemme, T. and Peters, K.J.,2010.A farm economic analysis in different dairy production systems in Bangladesh. **Livestock**

Research for Rural Development. Vol. 22 (7).

Vaida,N. (2013): Economic Analysis of Small Scale Dairy Farmers At “KUB Sami Mandiri” : Study t Kasembon District, Malang Regency. **Under Graduate Thesis**. Faculty of Animal Husbandry, University of Brawijaya. Malang, Indonesia.

O-09-3

THE DETERMINATION OF BUSINESS SCALE OF COW FARMERS USING BREAK EVEN POINT ANALYSIS IN KEBAR DISTRICT OF WEST PAPUA PROVINCE

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INTRODUCTION

Kebar is one area in Papua, Indonesia that declared as a Village Breeding Center of bali cattle. But based on results of the study on poverty and food security of Kebar people showed that 67% of people are still live under the poverty line (Supriyantono *et al*., 2012). The production cycle of beef cattle is very long, which is about 3 years (Bandini, 2003). This will be difficult for people who are still live under the poverty line to make beef cattle as its main livelihood. Therefore, there needs to be support from local government to first consider the economic strengthening of the beef cattle farmer community through the implementation of multi farming business. Through this program, farmers are not only retain ownership of cattle up to the age of the harvest, but also given assistance to implement the system quickly crop farming (under one year harvesting). The main problem faced by farmers in business is difficult area to reach the market. Kebar is located at 500 kilometers from the city center, and it has a topography that are difficult to reach public transportation so that the cost for marketing is very expensive.

The important thing that support the business is availability of land to develop livestock and agriculture. Therefore, by looking at the opportunities and weaknesses of the area development, it needs to be done right business scale calculations in order to obtain adequate profit from the farmer's business.

Material and Methods

Data were obtained from 20 household farmers who are member of Sejahtera cooperative in Kebar District. Parameters measured consisted of fixed costs (FC), variable costs (VC), land area, and the price of sale of agricultural and livestock products. Data were processed as descriptive using break event point calculation using formula as below:

The break-even point (BEP) in Unit Sales (X):

It can be directly computed in terms of Total Revenue (TR) and Total Costs (TC) as:

$$TR = TC$$
$$P \times X = TFC + (V \times X)$$
$$(P \times X) - (V \times X) = TFC$$
$$(P - V) \times X = TFC$$
$$X = \frac{TFC}{P - V}$$

where: TFC is Total Fixed Cost, P is Unit Sale Price, and V is Unit Variable Cost.

The break-even point (BEP) in currency unit

To calculate the break-even point in currency unit is multiplied the above calculation by Price (Garrison *et al* , 2006).

RESULTS AND DISCUSSION

In order to achieve Village Breeding Center of Bali Cattle, so that one of the absolute requirement is to achieve increased income household members of farmers being targeted. In the previous study, Supriyantono *et al*. (2012) revealed that most of farmer with conditions that still have problem of poverty is very difficult to be participated in development program of beef cattle.

Cattle production cycle that takes years make this commodity is difficult to be maintained until the age of sale. It is very necessary to look at the overall program, especially to study readiness economic of community to support the development of cattle by the government. The results of the study of food security in previous study showed that 73% of the group farmer is still in a vulnerable situation of food. It was found that farmers who have multiple income in the family has high food security. The purpose of applying multi farming business was to get a variety of livestock and agriculture business. Hopefully, through this business system, beef cattle farmers are able to

maintain operation of cattle business up to the age specified (cow up to 5 times the birth, the bulls kept until the age of 2 years).

In the management of multi farming business needs to know the number of products and minimal sales in a business or Break Even Point (BEP). As we know that the profits of the business is highly depend on the level of production, the level of sales and the total cost. The relationship of these three factors is a reciprocal relationship of mutual influence. Break even point is the point when or where the production capacity or the volume of production is in the circumstances no profit or no loss (Garrison *et al*., 2006).

The calculation of the break even point for the multi farming business in Kebar District intended to determine the amount of production that gives value the break even to cost. Hopefully, through the calculation of the BEP will facilitate the management of production cooperatives in providing instruction to its members to produce above the break even value of farmers so that farmers can benefit. Based on Alnasser *et al.* (2014) study that using BEP analysis give significant advantages in planning, controlling, and decision making in business.

Some of these efforts will be done in Kebar are chicken and peanuts the business. The BEP calculation of those business are presented in Table 1.

Increasing production value can be done through an increasing in number of livestock ownership, disease prevention in order to reduce mortality and use of local input production, especially feed.

By knowing the value of the break even production will facilitate the calculation of the amount of production of each commodity that should be pursued, which must be greater than the value of BEP. BEP value can also be used to calculate the area of land needed to plant crops. Based on the above calculation of BEP and productivity per commodity (Table 1), it can be calculated minimal land as presented in Table 2.

To achieve BEP of peanut business, then the amount of peanuts produced is 413.35 kg or sales value of IDR. 4,133,516.77. In order peanuts business can provide benefits, then farmers in Kebar District should be able to produce peanuts above 413.35 kg. The land productivity of West Papua to produce peanuts is 1.5 ton/ha. This value breakeven can be obtained on planting peanuts on the land area of 0.3307 ha. Therefore, to obtain advantage of business peanut, planting peanut should be done on the land above 0.3307 ha.

To achieve BEP of corn, cabbage, cucumbers, tomatoes, broccoli business, then each vegetable should be produced as much as 1006.82, 1.080.67, 640, 1.671.36 and 460.50 kg, respectively. Breakeven value can be obtained at each planting vegetables on an area of 0.5888, 0.3188, 0.2126, 0.3184, 01.633 ha.

The average of native chicken ownership of cooperative members is 5 head of hen and 1 cock. Base on study by Resnawati and Bintang (2005) showed that egg production of native chicken which is raised intensively varied from 105 to 112 per year. It can be estimated that the production of native chicken on a scale ownership of 5 females and 1 cock for a period of one year is 450 pullet and 60 eggs. The result of the calculation of Break Even Point production of chicken in Kebar District (Table 1) is 28.62 head, which is equivalent to IDR 4,292,606. In order to chicken business in Kebar District can provide benefits, then the farmer should produce chicken over 28.62 head.

It is needed a commitment of local government to provide access to production inputs such as seeds, medicines, agricultural equipment. The government through the cooperative may allocate local government budgets for the routine procurement of breeding stock, crops and medicines that are needed for agricultural and livestock developments or soft loans for members of the Kebar cooperative and the most important is to create a condition for farmers to have the certainty of marketing either the transportation or marketing area.

CONCLUTION

The break even point of peanuts, corn, mustard, cucumbers, tomatoes, broccoli and native chicken business as much as 1.25, 3, 25, 30, 20, 15 tons and 28.62 head, it must be applied on each land area of 0.3307, 0.5888, 0.3188, 0.2126, 3184 and 0.003 ha. In order to household farmers can get benefit then they have to produce over the break event point.

KEYWORD : Break even point, the scale of business, agriculture, animal husbandry

Table1. The Calculation of BEP each commodity by Farmers in Kebar District

Commodity	Fixed cost	Variable cost	Variable cost/ unit	Selling price/unit	BEP Unit	BEP IDR
Peanuts	3,154,700	2,960,000	2,368	10,000	413.35 kg	4.133.516,77
Corn	3,154,700	2,600,000	866,67	4 ,000	1,006.82 kg	4,027,276.60
Mustard	3,154,700	7,174,700	80,80	3,000	1,080.67 kg	3,242,018
Cucumber	3,154,700	3,800,000	76,00	5,000	640 kg	3,203,391.55
Tomato	3,154,700	2,250,000	112,50	2,000	1,671.36 kg	3.342.728.48
Broccoli	3,154,700	2,240,000	149,33	7,000	460.50 kg	3,223,467.30
Native chicken	4,200,000	1,479,000	3,236	150,000	28.62 head	4,292,606

Table 2. Minimal Land Requirement to Achieve the BEP Production

Commodity	Number production per ha	BEP Unit	Minimal land requirements to achieve the BEP production per commodity (ha)
A	**B (*)**	**C**	**D=C/B**
Peanuts	1,25 ton	413,35 kg	0.3307
Corn	1,71 ton	1006,82 kg	0.5888
Mustard	3,39 ton	1080,67 kg	0.3188
Cucumber	3,01 ton	640 kg	0.2126
Tomato	5,25 ton	1671,36 kg	0.3184
Broccoli	2,82 ton	460,5 kg	0,1633
Native chicken	10,000 head	28,62 kg	0,0029

*Source : BPS Papua Barat (2013)

REFERENCES

Alnasser, N. O.S Shaban, and Z.A. Zubi. 2014. The Effect of Using Break-Even-Point in Planning, Controlling, and Decision Making in the Industrial Jordanian Companies. International Journal of Academic Research in Business and Social Sciences May 2014, Vol. 4, No. ISSN: 2222-6990

Bandini, Y. 2003. Sapi Bali. Penebar Swadaya. Jakarta.

BPS Provinsi Papua Barat. 2013. Papua Barat dalam Angka. Badan Pusat Statistik Provinsi Papua Barat.

Garrison , Noreen and Peter C. 2006. *Akuntansi Manajerial*. Edisi Ke-11. Salemba Empat. Jakarta

Resnawati H. dan I. A.K. Bintang. 2005. Produktivitas Ayam Lokal yang Dipelihara Secara Intensif. Proceeding Lokakarya Nasional Inovasi Teknologi Pengembangan Ayam Lokal.

Supriyantono A., T.W. Widayati., O. Yoku and I. Sumpe. 2012. Pengembangan Vilage Breeding Center Sapi Bali dalam Mendukung Program Kecukupan Daging Sapi di Papua Barat. di Kebar. Laporan Penelitian MP3EI. Universitas Negeri Papua.

O-09-5

Cutting Costs and Retail Cuts Incomes in Holstein Bull Carcasses

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INTRODUCTION

Depending on the population and increased income, demand for red meat is expected to increase in the world in 2015-2021. However, during this period it is also estimated that production costs will increase due to higher prices of feed and energy, food safety, environmental pollution, and animal welfare regulations. According to the OECD report; large and small ruminant meat prices will rise 4 % and 11 % respectively, depending on the production costs until 2021. Increases of cost and price will most affected cattle meat production. Because, the share of cattle is 78.3 % in total meat production in the world (MFAL, 2015).

In the total cattle population (0.93 %) and cattle meat production (1.36 %), Turkey ranks 22nd and 16th, respectively in the world. The share of cattle is 87.5 % in total meat production in the country (MFAL, 2015). In recent years, there are production and supply problems in cattle meat sector in Turkey and it is resorted to imports for short-term solutions. The amounts of available production and supply can not meet the demand and cattle meat prices are steadily increasing. According to the producer prices; in Turkey, cattle meat is more expensive than certain countries (France, Germany, Russia and Brazil) between 33-59 % (FAOSTAT, 2015a). Feed costs were calculated between 50-67 % excluding animal material in the production period (Gözener and Sayılı, 2015). Therefore, changes in meat prices are often compared with the feeding costs.

Changes in cattle meat prices also depend on the type of marketing. The marketing methods based on live weight, carcass weight and carcass grading affect meat prices (Boggs et al., 2006). Particularly, carcass grading method determines the quality difference in meat and it provides to sale retail cuts from different price (Polkinghorne and Thompson, 2010). Despite of the improvement in the quality-price relationship in cattle meat consumer market in Turkey; there is no a similar improvement in producer market because of not to use a standard carcass grading method (Kale, 2008). This situation affects the producer's incomes negatively.

On the other hand, selling of the cattle meat as retail cuts that provides increased income in the consumer market (Kale, 2008; Sarözkan et al., 2013). Carcass weight has an significant role in increasing of retail cuts income. It was reported that there is a positive correlation between carcass weight and retail cuts weights (Chen et al., 2007). It is known that average carcass weight in Turkey is lower (253.4 kg) than the developed countries (USA: 350.7 kg; United Kingdom: 322.7 kg; Germany: 317.3 kg; France: 300.7 kg) (FAOSTAT, 2015b). Therefore, it can be said that weights and percentages of the retail cuts are also inadequate in carcass in Turkey.

In addition to cutting costs in cattle carcasses, price margin between carcass and retail cuts also affects retail cuts incomes. Price margin is of 4.23 times between carcass and most valuable retail cuts (tenderloin) in the United States of America (USA) (USDA, 2015). This value varies between 1.98-2.26 times in Turkey (GDMMB, 2015a; GDMMB, 2015b).

In this study, cutting costs and retail cuts incomes were calculated in Holstein bull carcasses that breeding widely held in Turkey.

MATERIALS AND METHODS

Research materials are 47 Holstein bull carcasses on average 21 months of age. The process of slaughter and carcass cutting was performed in a private slaughterhouse in Ankara Province. Slaughter and cutting regulations of General Directory of Meat and Milk Board (GDMMB) were applied in this process (GDMMB, 2000; GDMMB, 2012). After 24 h chilling, cold carcass weights (CCW) were determined and the carcasses were divided into retail cuts (tenderloin, sirloin, rib roast, rump, knuckle, round eye and topside-outside flat, chuck, brisket, shoulder, flank and shank). All retail cuts of each carcass separately were weighed (Çiçek et al., 2015). Also, the percentages of these retail cuts were determined according to CCW.

Statistical analysis

The carcasses were grouped according to CCW. One-way analysis of variance (ANOVA) was used in the evaluation

of the difference between the groups and Tukey's test was applied for the significance control of the difference between the groups.

Calculating of cutting costs and retail cuts incomes

Cutting costs were calculated in 5 items:

Labor costs: Labor expenditures were determined for elapsed time of cutting, production and packaging of minced meat - cubed meat, the retail cuts vacuuming and cleaning. The minimum wage was used according to employer costs (1411.76 Turkish Liras-TL) in the calculation (MLSS, 2015).Energy-water costs: Costs were determined for the purpose spent electricity of cooling (for carcass cooling, cutting, production and packaging of minced meat - cubed meat and the retail cuts vacuuming halls), use of equipment (for retail cuts vacuuming and production and packaging of minced meat - cubed meat), lighting and spent water for the purpose of cleaning.Material costs: The used material costs were determined for cutting (gloves, caps, masks and hand sanitizer), cleaning (disinfectants), packaging of minced meat - cubed meat (plastic containers, food gas, foil and label) and vacuuming (vacuum shrink bags and label).Amortization: It was calculated according to annual declining balance method on the value of buying of each piece of equipment (Yalkın, 1998).Cutting shrinkage: The value of difference was determined between before cutting with carcass weight and total of after cutting retail cuts, crumb and bone weight.

Total incomes were determined as the whole carcass, the retail cuts, cubed meat- minced meat and bones. Calculation was based on the average price of GDMMB and 3 large hypermarkets in the retail sector in Turkey. Shoulder, chuck, and shank were used for cubed meat. Brisket, flank, and crumb were used for minced meat. Other retail cuts were sold in vacuumed pecked. In the study, gross income was determined by subtracting whole carcass income from total retail cuts incomes. Net income is calculated by subtracting total cutting costs from the gross income.

RESULTS AND DISCUSSION

Cold carcass weight, cutting shrinkage and percentages of retail cuts according to weight groups were presented in Table 1. The difference between groups of CCW were found to be significant ($P<0.05$) and difference of cutting shrinkage were found to be insignificant ($P>0.05$). Difference between the groups of percentages of retail cuts was found to be insignificant ($P>0.05$). When CCW increases, percentages of bone decreases and difference between groups was found to be significant ($P<0.05$).

Carcass cutting cost was calculated as 0.969 TL/kg. Cutting costs were listed as material (44.32 %), cutting shrinkage (21.10 %), labor (17.18 %), energy-water (13.92 %) and amortization (3.48 %) (Table 2). The largest share in total income belongs to minced meat - cubed meat (63.03 %).

When the retail cuts incomes are calculated according to the average price of GDMMB and 3 large hypermarkets, gross income was found as 2.73 % and net income was found as 1.00 %. Only when it is calculated according to the price of private sector, gross income was found as 6.81 %, net income was found as 5.08 %.

The increasing of CCW has made a very low impact on the weights of tenderloin, sirloin, rib roast, rump, round eye, topside-outside flat and knuckle. However, brisket, chuck, shoulder, flank and shank has affected more than CCW's increment. As a result, there was no important change for the percentages of total retail cuts. A study reported that retail cuts (brisket, shoulder, chuck), have the highest share, affected more than weight gain. In the same study, it was determined that the retail cuts (tenderloin, topside-outside flat), have the lowest share, affected less than weight gain (Sarıözkan et al., 2013).

It is not possible to say that the percentages of retail cuts are enough both in the income (81 %) and carcasses (63 %). For instance, it was reported that retail cuts constitute 75 % of the carcass weight and 90 % of the carcass value in the USA (Boggs et al., 2006). In our research, it was determined that percentages of crumb (17.51 %) and bone (19.23 %) may have affected the percentages of retail cuts. In a previous study, crumb percentage was calculated as 8.16 % (Sarıözkan et al., 2013). The trimming of retail cuts may have changed this percentage. Thus, applied cutting procedure is important. It was observed that bone percentage decreased depending on the increasing of CCW. However, bone percentages was found to be higher than findings of some studies (14.48-17.73 %) (Kale, 2008; Barton et al., 2006). Different relationships are seen between meat, fat and bone in cattle carcasses in the growth and development period. In maximum growth period, relative increase was observed in bone than amount of meat and fat (Boggs et al., 2006).

In the study, the highest percentage was calculated for cost of material (44.32 %) in carcass costs. In this item, especially, packaging of minced meat - cubed meat (23.64 %) and cleaning (14:49 %) costs seen to be high. The

reason of this, benefiting from the automation in production and packaging of minced meat - cubed meat and using appropriate packaging material (plastic boxes, food gas, foil and label). However, using of separate rooms for cutting, vacuuming and production and packaging of minced meat - cubed meat that was caused to increase cleaning costs.

Despite of cutting shrinkage is determined lower than some studies (0.366 %), it has taken second place in costs. Cutting shrinkage has been reported as 0.70 % in these studies (Kale, 2008; Sarıözkan et al., 2013). Productivity of cutting labor is the most important factor affecting cutting shrinkage. It was determined that a butcher cut daily between 552-598 kg of carcasses in our research. It is stated that a butcher is required to cut daily 600 kg carcass according to GDMMB cutting regulations (GDMMB, 2000). It can be said that labor is productive according to this.

Used carcass amounts were taken into account for each item (labor, energy-water, materials, amortization) in the calculation of unit costs (0.969 TL/kg). Therefore, calculating of the unit cost does not reflect reality by dividing of the total cost to total carcass weight. Kale (2008) was calculated the unit cost as 0.563 TL/kg (not including amortization) using a similar method. When taking into account changes of labor, energy and material prices, it can be said that estimated unit cost is normal.

A large portion (63 %) of the income obtained from minced meat - cubed meat after cutting. It is possible that retail cuts as brisket and shank can be sold directly involved minced meat - cubed meat. Some retailer can increase their income a little more by applying alternative sales methods. However, demand of minced meat - cubed meat are greater because of consumption habits, high meat prices and income level in Turkey (Erdoğan and Çiçek, 2015). This situation often leads to supply of cattle meat as minced meat - cubed meat.

It is very difficult to provide increasing income if minced meat - cubed meat and retail cuts are sold with prices determined by GDMMB calculated cost structures. Because there are differences at a certain rate (20-25 % for minced meat - cubed meat, 21-32 % for the retail cuts) between prices of GDMMB and the private sector. In this study, income calculation was performed considering the average price of GDMMB and 3 large hypermarkets in the retail sector of Turkey. A higher income growth was determined with only in the calculations made by the private sector prices. However, in this study, by-products were not included in the calculation which derived from the carcasses. In a study, the average income of by-products was reported to be between 7.30-8.57 % in purchase price of large ruminant carcasses (Kale et al., 2011).

As a result; it can be said that percentages of retail cuts are sufficient for Holstein bulls under the conditions of Turkey. Also, it was determined that retail cuts sales have provided increased income. However, the shortfalls are available according to abroad percentages of retail cuts both in carcass and income. Carcass meat prices increase due to the high cost of breeding in Turkey. This situation, the price margin reduces between carcass meat and retail cuts. In our research, price margin was determined as 2.19 times between carcass meat and most valuable retail cuts (tenderloin). As mentioned before, the same value is as 4.23 times in the USA.

Buying and selling policy implemented by the GDMMB is important for the regulation of market. However, when considering the cutting costs, it is difficult to provide increasing of income with retail prices of GDMMB. Breeding costs should be reduced as possible and carcass grading method should be applied for solution of problems in red meat sector in Turkey.

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KEYWORD : Holstein bull carcasses, cutting costs, gross income, net income

Table 1. Cold carcass weight, cutting shrinkage and percentages of retail cuts according to weight groups

	Weight groups						Total (n: 47)	
	I. ≤ 275 kg (n: 7)		II. 276-300 kg (n: 23)		III. ≥ 301 kg (n: 17)			
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Cold carcass weight-CCW (kg)	255.286[a]	5.714	288.870[b]	1.318	322.765[c]	4.695	296.128	3.918
Cutting shrinkage (%)	0.31[a]	0.091	0.39[a]	0.040	0.35[a]	0.063	0.36	0.033
Retail cuts (%)								
Tenderloin	1.32[a]	0.047	1.25[a]	0.020	1.25[a]	0.026	1.26	0.015
Sirloin	2.20[a]	0.127	2.16[a]	0.044	2.14[a]	0.040	2.16	0.031
Rib roast	3.26[a]	0.053	3.11[a]	0.049	3.06[a]	0.054	3.12	0.033
Rump	4.28[a]	0.164	3.86[b]	0.076	3.61[bc]	0.098	3.83	0.064
Round eye	3.93[a]	0.075	3.92[a]	0.046	3.80[a]	0.085	3.88	0.040
Topside-outside flat	5.31[a]	0.200	5.18[a]	0.073	5.19[a]	0.066	5.21	0.051
Knuckle	5.87[a]	0.133	5.58[ab]	0.056	5.46[b]	0.074	5.58	0.046
Brisket	9.60[a]	0.317	9.85[a]	0.149	10.04[a]	0.219	9.88	0.117
Chuck	8.21[a]	0.382	7.84[ab]	0.123	9.22[ac]	0.374	8.39	0.181
Shoulder	10.36[a]	0.210	10.89[a]	0.155	10.64[a]	0.192	10.72	0.109
Flank	4.03[a]	0.153	4.56[ab]	0.110	4.95[b]	0.159	4.62	0.092
Shank	4.35[a]	0.121	4.19[a]	0.055	4.29[a]	0.097	4.25	0.048
Total retail cuts	62.74[a]	0.701	62.39[a]	0.345	63.65[a]	0.416	62.90	0.258
Crumb	15.79[a]	0.530	17.60[b]	0.326	18.10[b]	0.368	17.51	0.244
Bone	21.16[a]	0.439	19.62[b]	0.170	17.90[c]	0.389	19.23	0.239

[a, b, c] means with the same superscripts on the same row are different (P<0.05).

REFERENCES

Barton, L., D. Rehak, V. Teslik, D. Bures and R. Zahradkova. 2006. Effect of breed on growth performance and carcass composition of Aberdeen Angus, Charolais, Hereford and Simmental bulls. Czech J Anim Sci 51(2): 47-53.

Boggs, D.L., R.A. Merkel, M.E. Doumit and K. Bruns. 2006. Livestock and carcasses; an integrated approach to evaluation, grading and selection. 6th ed., pp. 111-141. Kendall/Hunt Publishing Company, Iowa, USA.

Chen, Y., C. Li, L. Liu, G. Zhou, X. Xu and F. Gao. 2007. Prediction of yield of retail cuts for native and crossbred Chinese Yellow cattle. Animal Science Journal, 78, 440-444.

Çiçek, H., M. Tandoğan and R. Kara. 2015. Prediction of weights and percentages of retail cuts in Holstein Bull carcasses. Kafkas Univ Vet Fak Derg, (in press), DOI: 10.9775/kvfd.2015.14553.

Erdoğan, N. and H. Çiçek. 2015. A research on determination of animal foods consumption status and consumer preferences: the case in university staff. Kocatepe Vet J, 8(1), 25-31.

FAOSTAT. 2015a. Food and Agriculture Organization of The United Nations Statistics Division. Available from: http://www. http://faostat3.fao.org/download/P/PP/E. Accessed Nov. 05, 2015.

FAOSTAT. 2015b. Food and Agriculture Organization of The United Nations Statistics Division. Available from: http://www. http://faostat3.fao.org/download/Q/QL/E. Accessed Nov 05, 2015.

GDMMB. 2015a. General Directory of Meat and Milk Board. Weekly market bulten (No: 2015/04). Available from: http://www.esk.gov.tr/tr/12365/Haftalik-Piyasa-Bulteni. Accessed Oct 15, 2015.

GDMMB. 2015b. General Directory of Meat and Milk Board. Available from: http://www.esk.gov.tr/tr/12197/Satis-Fiyatlari Accessed Oct 15, 2015.

GDMMB. 2012. General Directory of Meat and Milk Board, Slaughter Regulation (No: 37), 20, Ankara, Turkey.

GDMMB. 2000. General Directory of Meat and Milk Board. Cutting and management regulation, No: 204.

Gözener, B. and M. Sayılı. 2015. Production cost and factors affecting live weight gain in cattle fattening in the Turhal District of Tokat Province. Journal of Agricultural Sciences, 21, 288-299.

Kale M.C., Y. Aral, E. Aydın, Y. Cevger, E. Sakarya and S.C. Güloğlu. 2011. Determination of by-product economic values for slaughtered cattle and sheep. Kafkas Univ Vet Fak Derg, 17(4), 551-556.

Kale, M.C. 2008. The effects of rapid sale of cattle meat in General Directory of Meat and Milk Board slaughterhouses either as whole body or meat pieces on enterprise revenue. Ph.D. thesis, Ankara Univ., Ankara, Turkey.

MFAL. 2015. Republic of Turkey Ministry of Food, Agriculture and Livestock, General Directorate of Livestock. Red meat strategy, p. 75. Ankara.

MLSS. 2015. Republic of Turkey Ministry of Labour and Social Security. Available from: http://www.csgb.gov.tr/csgbPortal/ShowProperty/WLP%20Repository/cgm/asgariucret/2015_birinci_alti_ay. Accessed July 08, 2015.

Polkinghorne, R.J. and J.M. Thompson. 2010. Meat standards and grading A world view. Meat Science, 86, 227-235.

Sarıözkan, S., A. Akçay and D. Bayram. 2013. Carcass traits and economic assessment on carcass cutting of Zavot cattle. Ankara Üniv Vet Fak Derg, 60, 257-262.

USDA. 2015. United States Department of Agriculture Economic Research Service. Available from: http://www.ers.usda.gov/data-products/meat-price-spreads.aspx Accessed Oct 15, 2015.

Yalkın, Y.K. 1998. General accounting principles-practices and uniform accounting system practices. 11th ed., pp. 238-270. Turhan Bookstore, Ankara.

O-09-7

A Comparison of Cattle Fattening System With and Without Credit in Two Districts in East Java, Indonesia

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INTRODUCTION

Cattle fattening plays an important role in increasing the income of farmers in Indonesia. Cattle fattening generates revenue in shorter periods and with higher turnover compared to cow-calf-operations (CCO). Moreover, the expected profit from this cattle fattening is higher than from other types of cattle production. For example, Sugiarto et al. (2014) indicated that farmers who fatten cattle earn about IDR 500 thousand per month while farmers breeding cattle can generate IDR 290 thousand per month.

Cattle fattening can be done in commercial feedlots, but the majority is done by small to medium scale farmers. Research conducted by Priyanti et al. (2012) reported that small scale fattening households can generate relatively high profit compared to medium sized farmers and there is potential to compete with the commercial feedlots. Recently, small to medium sized fattening operations have developed in some areas in Indonesia, such as East Java, where the sources of feed are abundant.

Household cattle fattening is however subject to several limitations that affect profitability. Efficient fattening requires expensive inputs especially feeder cattle and the more intensive use of inputs especially feed, veterinary products and/or other infrastructure such as pens compared to traditional cattle production. The changes in price of inputs (feed, feeder cattle etc.) as well as the output (beef price) change the farmer's profit (Sugiarto et al., 2014) and only farmers who have access to adequate capital can participate in this business. Most farmers in East and Central Java do not reach their capacity to fatten feeder cattle because of limited money to buy feeder cattle and feed concentrates (Adinata et al., 2012; Priyanti et al., 2012). Perdana (2003) and Mukson et al. (2014) also reported that the small number of cattle owned by farmers in Indonesia is caused by lack of capital, skill in that business, and the farmers' aims which usually is to keep cattle for savings and not for profit.

To deal with capital limitations, The Government of Indonesia has established several types of credit scheme to subsidies farmers to develop their fattening activity. One of those credit programs is KKPE (Food Security & Energy Credit) which distributes funds to all provinces in Indonesia, one of which is East Java, the biggest producer of cattle production (53% of total cattle population) in Indonesia. The allocation of KKPE funds for livestock in East Java province until 2015 was about 16 percent of total KKPE while the highest percentage of KKPE funding is for sugarcane (Direktorat Pembiayaan Pertanian, 2014). It is likely that the supply of credit for cattle farming is limited or the willingness of farmers to take out loans is lower.

The survey was conducted during July to September 2015 to assess how the government credit scheme performs in regard to cattle fattening operations in two districts in East Java. The paper describes and compares the characteristics of cattle fatteners who took up credit for cattle fattening with those who did not take any loans in Lamongan and Tuban. It focuses especially on their production practices and feeding management.

METHODOLOGY

Two districts in East Java (Lamongan and Tuban) were selected because beef production is important and local government is actively encouraging and targeting the expansion of cattle fattening in those areas. This means that results for the case study area may not representative of other areas where cow-calf production or other non-cattle activities are encouraged. There were 102 total respondents in this survey of which 54 were cattle fatteners who had experience in obtaining credit for their cattle operations and 48 fatteners who did not borrow money for cattle purchases. The cattle fatteners with loans were chosen randomly, based on information from banks or Dinas Peternakan dan Kesehatan Hewan Kabupaten while fatteners without credit were also chosen randomly around those areas. A structured questionnaire was used to interview the farmers in regard to their cattle fattening practices. Mean values of those parameters were compared between fatteners with and without access to cattle credit. Descriptive analysis is used in this paper.

RESULTS AND DISCUSSION

Characteristics of cattle fatteners

The characteristics of those cattle fatteners who got access to credit are significantly different (P=0.001) to those who did not borrow money in terms of education level. On the average, the education of farmers obtaining credit was higher than those who did not borrow which is indicated by more years of schooling (average nine years) compared to seven years for those fatteners who were doing without credit. It suggests that farmers with high education experience tend to find it easier to get a loan for cattle. Increasing the level of education probably increases the opportunity to get credit because those people can prepare plans and budget required to access bank credit better than others (Nuryartono et al. 2005).

According to the survey, husband's age is not different which was at 47 and 48 years respectively for those who got credit and those who do not. In addition, the number of household members also is not significantly different between the two groups of farmers, which were about four persons per household, similar as household size of fatteners in Malang, Pasuruan and Probolinggo (Priyanti et al., 2012).

Generally, cattle production in Indonesia cannot be separated from the rest of the farming system. Jasila et al. (2012) reported that farmers grow crops and keep cattle at the same time which also happens on cattle fattening farms in Tuban and Lamongan. Most fatteners, both those with credit (45 percent) and those without credit (75 percent) interviewed in this survey, have their primary occupation as farmers and put fattening cattle as their secondary occupation. Interestingly, 44 percent of farmers taking loans had their primary occupation in the non-farm sector, which usually generates a higher level of income. This implies that that group of farmers may access credit because they have a better income situation which can be used to pay back the loan. In contrast, only eight percent of farmers without credit worked in the non-farm sector which may affect their decision to access a loan.

Farming characteristics

The land owned by cattle fatteners consists of rice fields and dry land. On average, fatteners who have used credit have larger areas of land compared to fatteners without using credit which were 1.5 ha and 0.6 ha respectively. On the other hand, the average area of dryland, which less fertile than rice fields, was not significantly different (P=0.870) between borrowing and non-borrowing fatteners (around 0.5 ha).

The crops planted by fatteners with credit are similar to those without credit with three paddy crops per year or two paddies followed by fallowed land (no crops), or peanut. As paddy rice is the mainly production in the research location, both types of cattle fatteners have sufficient rice straw for their (small) cattle herds.

Cattle characteristics and their management

Cattle fatteners with credit have relatively less experience in cattle fattening compared to fatteners without credit who had 11 and 18 years' experience respectively in that activity, although not all respondents in this survey are specializing in fattening cattle. They start their involvement in the beef industry in cow-calf operations and then move into cattle fattening for around seven months period of fattening depending on the age of feeder cattle bought, and the farmer's financial situation.

Most farmers in this study were categorized as smallholder cattle fatteners who have one to five head of cattle. The average number of cattle owned by these fatteners can be seen in Table 1. There were 35 percent of farmers with credit but only five percent of fatteners without credit who have large herds. Most of those cattle are crossbreed cattle including Limousin Cross, Brahman Cross, Simmental Cross and other mixed-breed cattle. Farmers prefer crossbred cattle to local cattle because their average daily rate of gain can be more than 0.5 kg/day (Perdana, 2003).

Fatteners with credit are much more likely to also be involved in cow-calf production (48 percent) compared to non-credit households (29 percent). While the vast majority of cows are held in small herds (1-5 head, with an average of around 1.9), households without credit do not own more cows than this, while farmers with credit hold a significant proportion of cows in higher scale categories (6-20 heads and >20 heads). It is likely that farmers with large herds can generate income that increases their chances of accessing bank loans.

Farmers with credit commonly buy feeder cattle in the market (70 percent), produce their own calves (16 percent) and buy from other farmers, or traders (14 percent). Farmers without credit get feeder cattle from local market (66 percent), produce own calves (7 percent) and buy from other farmers, or traders (27 percent). Even farmers without credit also involved in cow-calf operation, they are not always produce male calves to fatten; they also bought feeder from market.

There is a significant different types of pens used to hold the fattening cattle between farmers with and without credit. Most farmers with credit (about 87 percent) build the trough for feed from concrete as suggesting by the

bank which extends them the credit, although there are 13 percent of farmers with credit still using traditional pens (where feed is placed on the floor). This might be because of the different type of credit received by some farmers with no requirement for standard pens. In contrast, half of the farmers without credit still use traditional pens while the other half already use modern pens (with a concrete feed trough). This might include those farmers involved in group production (76 percent and 52 percent respectively for farmers with and without credit).

Feeding management

There were no significant differences in the amount of feed for cattle given by those two groups of fatteners which are mostly rice straw (7-8 kg per head per day), rice bran (4-4.5 kg per head per day), molasses, and salt although there are other types of feed (maize, peanut straw etc.). Most farmers with credit (57%) and farmers without credit (62%) get rice straw from their own production and also from other farmers, collected either by themselves or in group. The same way to collect rice straw is also reported in Pasuruan and Malang by Hanifah et al. (2010). However, a much larger proportion of farmers with credit (52 percent) also feed elephant grass to cattle compared to farmers without credit that feed more natural grass. This suggests that farmers with credit try to improve the cattle weight gain of their cattle, especially during the dry season through elephant grass.

Less than 40 percent of both groups fattening cattle feed their cattle with concentrate which given to cattle around 6 kg and 8 kg per day for the borrowing group and non-borrowing group respectively. It is interesting that almost 50 percent of farmers with credit make their own concentrate (or in group) using some by-products such as copra, peanut skin, palm oil cake, coffee bean skin, molasses, rice bran, while 35 percent of them buy concentrate from other big firm or other farmers who also produce feed for cattle. On the other hand, most fatteners without credit get concentrate from Dinas Peternakan dan Kesehatan Hewan Kabupaten who has program to distribute or introduce concentrate.

CONCLUSION

In Tuban and Lamongan, East Java, there are significant differences between households with and without credit, especially in terms of scale of cropping and cattle production. Farmers with credit have a main occupation outside cattle production. This suggests that wealthier farmers are more able to access credit, and to increase the scale of their cattle production. In addition, farmers with credit are more likely to have better pen infrastructure required to house more cattle and because this is a condition of accessing credit. It is also important to note that farmers with credit are more diversified into cow-calf production and fattening, while the non-credit group is more specialized in cattle fattening. This is likely to reflect differences in capital demands and turnover for the two different cattle production activities, and suggests that cattle fattening can be a pro-poor activity.

There are few differences in the social characteristics of farmers with and without credit (age, family size), except for higher education levels for farmers with credit. While not necessary a condition of loans, farmers with credit are much more likely to participate in cattle groups that usually involve training programs designed to improve cattle production systems. In terms of production system, both groups base diets on rice straw, rice bran and molasses, but farmers with credit feed more improved forages (elephant grass) while farmers without credit feed more native grass. The two groups have fatten cattle for similar periods, have similar buying and selling channels, and similar ownership structures.

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KEYWORD：Fatteners, Credit, Comparison, Cattle practices

Tabel 1. The average number of cattle owned by fatteners with and without credit

Parameters	Fatteners with credit			Fatteners without credit		
	No of respondents	%	Average number of cattle	No of respondents	%	Average number of cattle
Number of male cattle owned						
Adult cattle						
1-5 head	33	64.7	2.6	40	95.2	2.3
6-20 head	11	21.6	9.1	2	4.8	6.0
> 20 head	7	13.7	60.4	-	-	-
Young cattle						
1-5 head	5	71.4	1.8	4	100.0	1.5
6-20 head	1	14.3	11.0	-	-	-
> 20 head	1	14.3	27.0	-	-	-
Calves						
1-5 head	10	90.9	2.3	4	100.0	1.8
6-20 head	1	9.1	7.0	-	-	-
> 20 head	-	-	-	-	-	-
Number of female cattle owned						
Adult cattle						
1-5 head	18	69.2	1.9	13	100.0	1.9
6-20 head	5	19.2	8.6	-	-	-
> 20 head	3	11.5	47.7	-	-	-
Young cattle						
1-5 head	-	-	-	3	100.0	1.3
6-20 head	1	100.0	10.0	-	-	-
> 20 head	-	-	-	-	-	-
Calves						
1-5 head	5	71.4	1.6	3	100.0	1.0
6-20 head	2	28.6	7.5	-	-	-
> 20 head	-	-	-	-	-	-

Calves = cattle aged less than 1 year; young = cattle aged 1-2 year; adult = cattle aged more than 2 years

REFERENCES

Adinata, KI, Sari, AI & Rahayu, ET 2012, 'Development strategy of beef cattle business at Mojolaban subdistrict of Sukoharjo Regency', *Tropical Animal Husbandry*, vol. 1, no. 1, pp. 24-32.

Direktorat Pembiayaan Pertanian 2014, *Realisasi KKPE Per Bank Pelaksana*, Direktorat Pembiayaan Pertanian, Direktorat Jenderal Prasarana dan Sarana Pertanian, Jakarta.

Hanifah, VW, A. Priyanti, I.G.A.P. Mahendri & Cramb, RA 2010, 'A comparison of feeding management practices of beef cattle smallholders in lowland and upland sites in East Java', in *The 5th International Seminar on Tropical Animal Production Community Empowerment and Tropical Animal Industry*, Yogyakarta, Indonesia, pp. 189-95.

Jasila, I, Priyanti, A, Mahendri, I-GAP & Cramb, RA 2012, 'A Comparison of Small-Scale Brahman Farmers in Lampung and East Java', in *International Conference on Livestock Production and Veterinary Technology 2012*, Bogor, West Java, Indonesia, pp. 334-41.

Mukson, Roessali, W & Setiyawan, H 2014, 'Analysis Development Regional of Cattle Beef in Support Meat Self-Sufficiency in Central Java', *Jurnal Peternakan Indonesia*, vol. 16, no. 1, pp. 26-32.

Nuryartono, N, Zeller, M & Schwarze, S 2005, 'Credit rationing of farm households and agricultural production: empirical evidence in the rural areas of Central Sulawesi, Indonesia', paper presented to Conference on International Agricultural Research for Development, Stuttgart-Hohenheim, 11-13 October 2005.

Perdana, T 2003, 'Competitiveness and comparative advantage of beef cattle fattening in Bandung Regency', Padjadjaran University.

Priyanti, A, Mahendri, IGAP, Cahyadi, F & Cramb, RA 2012, 'Income Over Feed Cost for Small-to Medium Scale Beef Cattle Fattening Operations in East Java', *J.Indonesian Trop.Anim.Agric*, vol. 37, no. 3, pp. 195-201.

Sugiarto, M, Djatmiko, OE & Mastuti, S 2014, 'Investment Risk Assessment of Two Types of Beef Cattle Enterprise in Banjarnegara District, Central Java, Indonesia', in *Proceedings of the 16th AAAP Animal Science Congress Vol. II "Sustainable Livestock Production in the Perspective of Food Security, Policy, Genetic Resources and Climate Change"*, Gadjah Mada University, Yogyakarta, Indonesia, pp. 992-5.

O-09-8 AN INVESTIGATION OF ENTREPRENEURIAL ORIENTATION TO IMPROVE COMPETITIVENESS OF BEEF CATTLE FARMING IN KEBUMEN DISTRICT, INDONESIA

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Introduction

Kebumen District has been growing fast as a production center of beef cattle in Indonesia since 2011. Beef cattle farming has become an important livelihood in the economic dynamics of farmers' family through the beef cattle breeding. Small scale beef cattle farming has a strategic role in enhancing the region\'s economic growth through increased family income, employment and the emergence of other innovations. Katua (2014) stated that today small and medium enterprise (SMEs) spread worldwide plays a significant role in the economy and as the engine of economic growth and poverty eradication in the world. Economic activity of small scale beef cattle farming in the Kebumen District was managed by profit-oriented and business efficiency. However, the business environment faced by farmers and groups of farmers is very dynamic and competitive which requires farmers to have entrepreneurial skills and high business adaptability. Therefore, entrepreneurial activities related to innovation, proactive, and risk taking are crucial to achieve business competitiveness which is indicated by business efficiency. Some entrepreneurial values (innovation, proactive, and risk taking) are combined into an entrepreneurial orientation. Entrepreneurial orientation is a combination of innovation, proactive and risk management to improve the competitiveness and sustainability of the business. Sriprasert (2013) mentioned entrepreneurial orientation is the main factor for the organization to obtain sustained competitive advantages. Saeed et al. (2014) confirmed that entrepreneurial orientation influences firm growth and performance indicators. Entrepreneurial orientation composes of some dimensions such as innovativeness, risk taking, and proactiveness. This study, therefore, investigates the relationships between entrepreneurial orientation and competitiveness of beef cattle farm in Kebumen District. Specifically, this study was aimed to (1) explain the entrepreneurial orientation of beef cattle farmers in Kebumen District (2) to identify the economic efficiency of beef cattle farming in Kebumen District and (3) analyze the relationship between entrepreneurial orientation and economic efficiency of beef cattle farming in Kebumen District, Indonesia.

Methodology

The study was conducted using a survey of 40 respondents were selected using a multistage sampling. Four sub districts which were known as a breeding center of beef cattle were selected purposively and 20 percent of total farmer groups were chosen in each selected sub district. Respondents were chosen randomly 20 percent of the total members of each selected farmers\' group. The data were taken using a questionnaire based on a Likert scale (scale 1-5 which determined strongly disagree to strongly agree). Descriptive statistics (mean and standard deviation) was used to describe the entrepreneurial orientation and economic efficiency of beef cattle farming. Meanwhile, the Spearman rank correlation test was used to identify a relationship between entrepreneurial orientation and economic efficiency of beef cattle farming.

Results

Description of Respondents

Beef cattle farmers in Kebumen District showed a good individual potential based on age and education. Beef cattle farmers were categorized mostly in the productive age (44 years) and holding an adequate basic education (junior high school). Family of farmers does not have a huge economic burden because of the number of families in accordance with the government\'s family planning program (4 people per family). However thus, the number of beef cattle per farmer is still relatively small (less than 2 animal units).

Table 1. Demographic description of beef cattle farmers

Variables

Mean

Standard Deviation
Age of farmers
44.42
9.94
Education attainment
8.85
2.24
Number of family members
1.27
0.69

Entrepreneurial Orientation

Entrepreneurs have a lot of significant roles to develop an economic atmosphere in rural areas such as an increasing number of employment, exploiting human and natural resources and creating new product with higher value. From an attitudinal perspective, entrepreneurs as individuals with: a need for achievement (Miner, 2000), a risk-taker (Kuratko, 2007), passion, desire to innovate (Bolton & Lane, 2012). Bolton & Thompson (2004) stated that entrepreneurs habitually creates and innovates to build something of recognized value around perceived opportunities. They are a particular type of person whose risk-taking and innovative prowess lends itself to identifying and exploiting profitable opportunities resulting in organizational and economic growth (Kuratko, 2007).

The results shown that entrepreneurial orientation of cattle farmers in Kebumen District was classified as moderate (average score of 52.67). The farmers have perceived that innovation would deliver uniqueness and generate a financial capability for their business. They also prefer to act, try, and result oriented instead of waiting for things to happen. In response to the more competitive industry, the farmers have moderate willingness and readiness to commit having a reasonable possibility of losses. Kuratko et al., (2011) stated that innovation involves risk-taking, and the higher an organization innovates, the more risks it takes.

Table 2. Entrepreneurial orientation of beef cattle farmers

Variables
Mean
Standard Deviation
Innovative
15.50
1.06
Proactive
18.75
1.64
Risk taking
18.43
1.94
Entrepreneurial Orientation (EO)
52.67
4.05

Innovative and proactive value was relatively equal among the beef cattle farmers in the rural area of Kebumen District. However, among these three values, the farmers have a quite deviated value of risk taking. This describes a different perception to view a willingness and readiness of farmers to commit a reasonable possibility of losses.

Economic Efficiency of Beef Cattle Farming

Beef cattle population in the District of Kebuman in 2014 reached 64 292 heads rose 2.76% from the previous year (Bureau of Statistics of Kebumen, 2015). The local government give serious attention in order to improve beef cattle population in Kebumen District. Beef cattle production based on breeding pattern is done systematically based community to improve beef cattle population. Economic success of these efforts will be able to increase the passion and motivation of the community. One indicator of economic success of beef cattle farming is economic

efficiency. Daroini and Nafingi (2014) found, the analysis of R / C Ratio is an instrument to compare total revenue and total cost of beef cattle production.

Beef cattle farming in Kebumen District was run by having scales of business was 1.25 Animal Unit (AU). This needs a total cost of Rp 3,231,801.00 per year with revenues generated was Rp 5,295,000.00. The study revealed the beef cattle farming in Kebumen District has an economic efficiency of 1.62. This illustrated the feasibility that any expenditure of Rp 1.00 will get additional revenue of Rp 1.62. Kitsopanidis (2004) stated that comparing total revenue with total costs may highlight that the revenue would be able to cover the total cost of production.

Role of Entrepreneurial Orientation to the Economic Efficiency

The entrepreneurial orientation of a farmer is depicted by the extent to which the farmers are inclined to take business-related risks, to favor change and innovation in order to obtain a competitive advantage for their farm, and to compete aggressively with other farms. It has been referred to as an entrepreneurial mindset, climate, or strategic orientation. Sanchez & Marin (2005) measures the performance of small and medium enterprises (SME\'s) with reference to three aspects, namely profitability, productivity, and market. The study explained that entrepreneurial orientation of beef cattle farmers has significant role to improve economic efficiency of cattle farming in Kebumen District, Indonesia ($P<0.05$). Improving innovativeness, willingness to take risks and pro activeness of farmers would able to increase economic efficiency of beef cattle farming. This is line to the study of Okpara (2009) that SMEs adopted proactive (high) entrepreneurial orientation achieved higher performance, profitability and growth, compared to those that adopted a conservative (low) orientation. Innovatiness, risk taking, and pro activeness of beef cattle farmers in Kebumen District was sufficient to drive change on economic efficiency of farm. Entrepreneurial orientation would drive capacity of farmers to innovate, ability to cope change of competition and survive from the risk of industrial change. Strengthening this entrepreneurial orientation is expected to improve the entrepreneurial behavior of farmers. Values of entrepreneurial orientation have significant influence to the growth of small and medium enterprise Mwangi and Ngugi (2014) and determined the entrepreneurial marketing behaviors (Kilenthong et al, 2016).

Conclusions

The results from this study, therefore, suggest that entrepreneurial orientation plays an important role in improving competitiveness of beef cattle farming in Kebumen District, Indonesia. The important values in strengthening competitiveness of beef cattle farming are risk taking, pro activeness and innovativeness. Hence, the results drive academicians and policy makers that it is possible to promote competitiveness of beef cattle farming by paying attention to the entrepreneurial orientation. Improving innovativeness, willingness to take risks and pro activeness of farmers would able to increase competitiveness of beef cattle farming.

KEYWORD : entrepreneurial orientation, competitiveness, beef cattle farming

REFERENCES

Bolton, W., & Thompson, J. 2004. Entrepreneurs: Talent, temperament, technique. Oxford: Butterworth Heinemann

Bolton, D. L.,& M. Lane. 2012. Individual Entrepreneurial Orientation: Development of a Measurement Instrument. Education + Training , Vol. 54 Iss 2/3 pp. 219 – 233

Bureau of Statistics of Kebumen, 2015. Kebumen in Number. Kebumen, Indonesia

Daroini, A and A.K. Nafingi. 2014. Return and Cost Analysis of Beef Cattle Farm in Tulungagung, Indonesia. Jurnal Cendekia Vol 12 No 2/ 2014 p 98-103

Katua,T.N. 2014. The Role of SMEs in Employment Creation and Economic Growth in Selected Countries. International Journal of Education and Research Vol. 2 No. 12 December 2014. 461-472.

Kilenthong, P., C.M.Hultman., and G.E.Hills. 2016. Entrepreneurial Orientation As The Determinant Of Entrepreneurial Marketing Behaviors. Journal of Small Business Strategy Vol. 26, No. 2/2016 : 1-20

Kitsopanidis, G.I. 2004. Economics of Extensive Beef Cattle Farming in Greece. New Medit N.1/2004.

Kuratko, D. 2007. Entrepreneurial leadership in the 21st Century. Journal of Leadership and Organizational Studies, 13 (4), 1-11

Kuratko, D. F., Morris, M. H., and Covin, J. G. 2011. Corporate innovation & entrepreneursip. Third Edition. Canada: South-Western Cengage Learning.

Miner, J. 2000. Testing a psychological typology of entrepreneurship using business founders. The Journal of

Applied Behavioral Science, 36 (1), 43-69.
Mwangi, M. M. A & Ngugi, K. (2014). Influence of Entrepreneurial Orientation on Growth of Micro and Small Enterprises In Kerugoya, Kenya. European Journal of Business Management, 1 (11), 417-438.
Okpara, J.O. 2009. Entrepreneurial orientation and export performance: evidence from an emerging economy. Int. Rev. Bus. Res. Papers 5(6):195-211
Saeed, S., S.Y.Yousafzai, and A.Engelen. 2014. On Cultural and Macroeconomic Contingencies of the Entrepreneurial Orientation–Performance Relationship. Entrepreneurship Theory and Practice 38 (2): 255-290
Sanchez, A.A. and Marin, G.S. Orientation Management, Characteristic, and Performance. A Study Spanish SME's, Journal of Small Business Management, 43(3), 2005, 287-306
Sriprasert, P. 2013. The Effect of Entrepreneurial Orientation on the Success of Community Enterprise: A Study of Nakhon Si Thammarat, Thailand. International Proceedings of Economics Development and Research (IPEDR) 2013. V59. 33

O-09-9

Decreases of milk yield after conception of mature dairy cattle in tropical environment

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Objective

Holstein-Friesian cattle have been intensively used for in breed improvement programs in Thailand. However, the uses of pure bred Holstein-Friesian become more health problems especially from tick borne diseases. Therefore the cross bred Holstein-Friesian with <25% of zebu cattle have been used for milk production purpose to increase disease prevention efficiency from tropical diseases.

Milk yields of dairy cattle are depended on various factors for example breed, environment, farm managements and cow health. Several studies mentioned the heat stress, illness (Pholpark, 1999) and genetic (Boonkum, 2015) are the factors that can effected milk yields. In general, dairy cattle remain its milk production efficiency through after pregnancy. For zebu, milk yield decreases after conception, but it is in doubt on milk production decrease in crossbred Holstein-Friesian that are mostly <25% of zebu bred. Many Thai dairy farmers observed that many cows reduced their milk yield after pregnancy.

Therefore, the objective of this study was to determine the change of milk yield after successful conception after calving.

Methodology

Data collection

Data from 433 lactations from dairy cows in small holder dairy farms belonging to Mae Wang Dairy Cooperative, Chiang Mai, Thailand, were collected during September 2011 to August 2012. All farms had been in the veterinary herd health management program from Faculty of Veterinary Medicine, Chiang Mai University, Thailand. With the program, the farms were monthly visited by a veterinarian as a farm consultant in both disease prevention program and increase production efficiency including reproductive management program. In addition, reproductive checks including postpartum check and pregnancy check had been performed in all cows. Crossbred Holstein-Friesian cattle have been mostly used in all farms. Most farms were free stall barns using bucket-type milking machines and milking twice daily. Data on calving date and conception date, confirmed by rectal palpation at 60 day after the last artificial insemination without any signs of estrus after insemination, were collected. After calving, milk yield from cows were monthly weighed.

Statistical analysis

As milk yields are related to many factors, the comparison making in this study were to compare between milk production before and after pregnant in which the milk yield were adjusted for month in lactation. Therefore, data on lactation with both before and after pregnant were included in the final analysis. The milk yield data in the final analysis were limited in < 3 months before and after pregnancy. A dependent variable was test-day milk yield in kg/cow/day unit. Independent variables included month in lactation and interaction between conception and cow groups: YOUNG as cows from lactation 1, MATURE as cows from lactation 2-4, and OLD as cows older than lactation 4. To collect the nonlinear curve of milk production, month in milk (MIM), the integral value calculated by division of days in milk with 30, was included in the final model. Repeated measure analysis, the mixed procedure, was used using autoregression type 1 as correlation structure and time at milk weighing as repeated factor. Least square means were calculated for all levels and used to compare between each pair-wise values. The significant value was defined at $P<0.1$.

Results

From all data of 433 lactations, only 291 lactations with 722 milk yield data were included in the final analysis (Table 1). Overall averages of days in milk and milk yield were 106 days and 16.61 ±0.25 kg/cow/day, respectively. For reproductive performance in this study, averaged days to first service and days open were 99.4 ± 2.4 and 155 ± 3 days, respectively.

Results from the final analysis on the effects of MIM and reproduction on milk yield were shown in Figures 1 and 2,

respectively. From Figure 1, peaks of milk yield were during 31-60 to 61-90days postpartum. Milk production in the first 120 days postpartum had higher than that in late lactation or after 180 days postpartum (P<0.05). After conception, MATURE cows had significantly lower milk yield than before conception, but no significant differences was observed between before and after conception of YOUNG and OLD cows, respectively (Figure 2). MATURE cows decreased approximately 1.5 kg/cow/day after conception.

Table 1. Overall means and standard error of means (SEM) for days in milk, days to first service, days open and milk yield.

Factor	N	Mean	SEM
Days in milk (days)	722	106.43	3.94
Days to first service (days)	291	99.39	2.41
Days open (days)	296	154.96	3.01
Milk yield (kg/cow/day)	722	16.61	0.25

Conclusion

Many effects of reproduction on milk yield have been investigated by many researches (Weller et al., 1985; Arbel et al., 2001), but in tropical area was limited numbers of studies. In this study we use the repeated measure analysis in combination with the defined variables as the time that the cows were pregnant or not would be appropriate for analysis the changes of milk yield after conception. Due to the uses of milk yield data that mostly originated from early lactation or during peak of milk yield (days in milk = 109 days), an average milk yield was therefore high as shown in Table 1 at 16.61 + 0.25 kg/cow/day. In considering for this milk yield, the farms in this study might be in ranges of production in other previous studies for example Thailand at 11.6-13.1 kg/cow/day (Buabun et al., 2016), Pakistan at 5.0 -11.3 kg/cow/day (Mushtaq et al., 2010) and Ethiopia at 11.1 kg/cow/day (Meseret et al., 2015).

In Figure 1, an average milk yield was highest at 18.2 kg/cow/day during DIM 61-90. Many factors are involved levels of milk yield such as genetic, lactation periods, age, milking management, environment and health status of dairy cows. Managements and environment might be the most important factors involved milk quality due to heat stress can caused lower dry matter intake and a lower conversion efficiency of feed into milk (Lambertz, 2014; Boonkum and Duangjinda,2015).

Figure 2 showed the lower milk production after conception of cows in lactation 2 to 4. Physiology of conception is related to milk yield reduction from hormonal changes (Bachman et al., 1988; Akers, 2002) and also nutritive requirements of fetus (Bell et al., 1995). In our study, milk production of ADULT cows significantly decreased after pregnancy, but not for YOUNT and OLD cows. The non-significant differences for the old cows (lactation >4) might be due to either technical problems as small sample sizes or physiological problem as old cows might decrease reproductive performances. Cows in first lactation had increased milk production after calving that supported by previous study showing that pregnancy cows had higher milk yield than open cows (Mushtaq et al., 2010). Cows in first lactation had less milk production than when they were getting older. It might be possible that cows in

lactation 2 to 4 had more milk production starting after calving. Consequently, the cows could not balance their nutrition requirements for fetus growth.

In conclusion, In conclusion, only cows in lactation 2 to 4 decreased their milk yield after successful conception, but not for others younger and older cows.

KEYWORD：Milk yield, Conception, Milk lactation

Figure 1. Least square means of milk yield separated by MIM, the integral value calculated by division of days in milk with 30. Differences of superscript letter indicating significant differences at P<0.05.

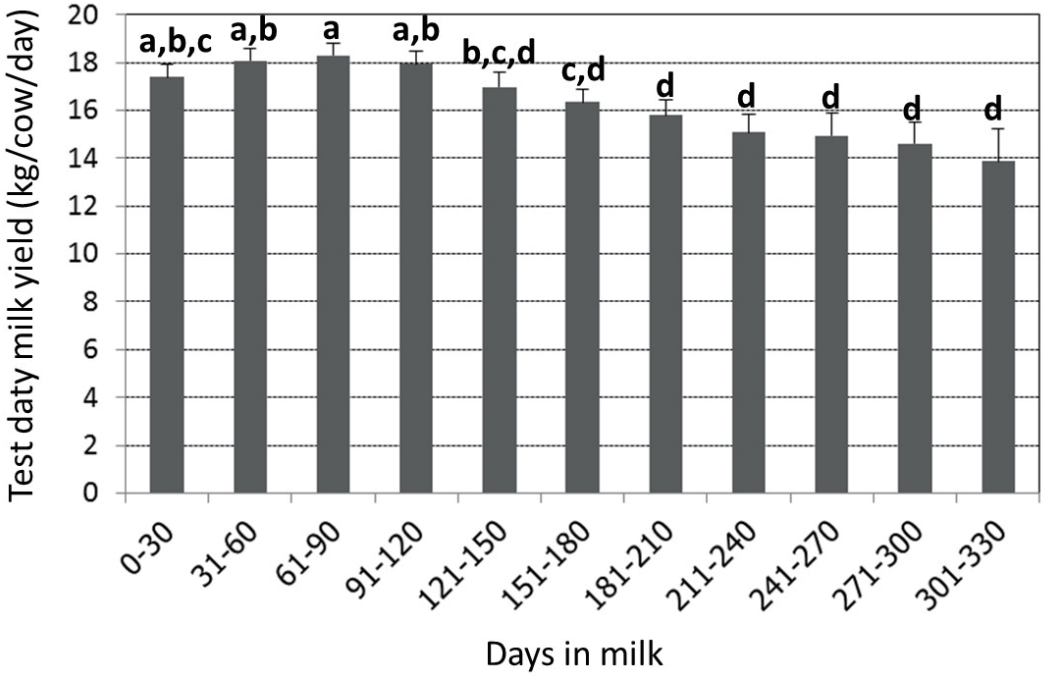
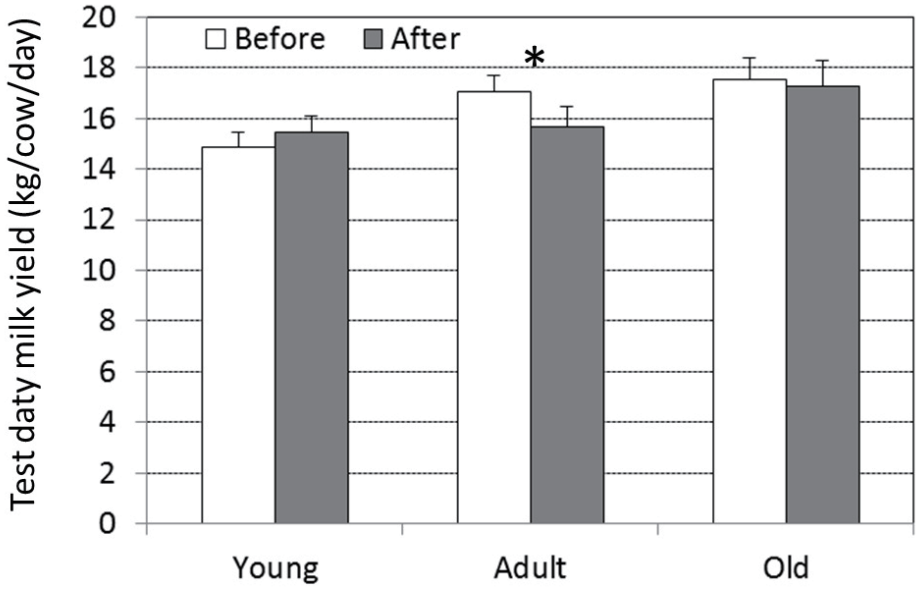


Figure 2. Least square means of milk yield for lactation numbers and pregnant factors corrected for MIM effect. * indicating significant differences at P<0.1



REFERENCES

Akers, R. M. 2002. Lactation and the mammary gland. 1st ed. Iowa State Press, Ames, Iowa.

Arbel, R., Bigun, Y., Ezra, E., Sturman, H., Hojman, D. 2001. The effect of extended calving intervals in high-yielding lactating cows on milk production and profitability. Journal of Dairy Science 84 (3), pp. 600-608

Bachman, K. C., M. J. Hayen, D. Morse, and C. J. Wilcox. 1988. Effect of pregnancy, milk yield, and somatic cell count on bovine milk fat hydrolysis. J. Dairy Sci. 71:925–931.

Bell, A. W., R. Slepetis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954–1961.

Boonkum, W. and Duangjinda, M. 2015. Estimation of genetic parameters for heat stress, including dominance gene effects, on milk yield in Thai Holstein dairy cattle. Animal Science Journal (2015) 86, 245–250.

Buaban S. 2005. Dairy cattle improvement system in Thailand. The experiences of dairy industry development in South East Asia, Hanoi, September 14–15.

Buaban, S., Duangjinda, M., Suzuki, M., Masuda, Y., Sanpote, J., Kuchida, K. 2016. Genetic relationships of fertility traits with test-day milk yield and fat-to-protein ratio in tropical smallholder dairy farms. Animal Science Journal 87 (5), pp. 627-637

Kaewlamun, W., R. Chayaratanasin, P. Virakul, A. A. Ponter, P. Humblot, S. Suadsong, P. Tummaruk and M. Techakumphu. Differences of periods of calving on days open of dairy cows in different regions and months of Thailand. Thai J Vet Med. 2011. 41(3): 315-320.

Lambertz C., Sanker C., Gauly M. Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. J. Dairy Sci. 2014, 97:319–329.

Meseret, S., Tamir, B., Gebreyohannes, G., Lidauer, M., Negussie, E. 2015. Genetic analysis of milk yield in first-lactation Holstein Friesian in Ethiopia: a lactation average vs random regression test-day model analysis. Asian-Australasian Journal of Animal Sciences (AJAS) 2015; 28(9): 1226-1234.

Mushtaq, A., M.S. Qureshi, S. Khan, G. Habib, Z. A. Swati. 2010. Effect of body condition and pregnancy on milk yield and its composition in crossbred dairy cows under tropical. Egypt. J. Exp. Biol. (Zool.), 6(2): 247 – 253

Nebel R. L, McGllliard M. L. Interactions of High Milk Yield and Reproductive Performance In Dairy Cows. J Dairy Sci, 1993, 76:3257-3268.

Qureshi MS, Khan JM, Khan IH, Chaudhry RA, Ashraf K, Khan BD. 2001. Improvement in economic traits of local cattle through crossbreeding with Holstein Friesian semen. Pakistan Vet. J., 22(1): 21-26.

Rukkwamsuk, T. 2009. A field study on negative energy balance in periparturient dairy cows kept in small-holder farms: Effect on milk production and reproduction. African Journal of Agricultural Research Vol. 5(23), pp. 3157-3163,

Sartori, R. M.B., Baruselli P.S., Gimenes, L.U., Ereno, R.L, Barros, C.M. 2010. Physiological differences and implications to reproductive management to Bos Taurus and Bos indicus cattle in a tropical environment. In Reproduction in Domestic Ruminants VII. (Eds Mc Lucy, JL Pate, MF Smith and TI Spencer), Nottingham Univerrsity Press, Nottingham.

SAS Institute, Inc. 1997. SAS/STAT Software: Changes and Enhancements through Release 6.12. Statistical Analysis Systems Institute, Inc., Cary, NC.

Suriyasathaporn, W., M. Nielen, A. Brand, E.N. Noordhuizen-Stassen, S.J. Dieleman, and Y.H. Schukken. 1998. A Cox proportional-hazards model with time-dependent covariates to evaluate the relationship between body-condition score and the risks of first insemination and pregnancy in a high-producing dairy herd. Prev. Vet. Med. 37:159-172.

Suriyasathaporn, W., K. Kanistanon, W. Kowitayakorn, W. Punyapornwittaya, and K. Kreausukon. 2003. Defining the coefficients for the calculation of standard milk production and the average daily milk production for the whole lactation. Thai J. Vet. Med. 33: 63-71.

Suriyasathaporn, W., S. Boonyayatra, K. Kreausukon, T. Pinyopummintr and C. Heuer. 2006. Modification of microclimate to improve milk production in tropical rainforest of Thailand. Asian-Australasian J. Anim. Sci. 19: 811-815.

Suriyasathaporn, W., Vinitketkumnuen, U., Chewonarin, T., Chupia, V., Pinyopummintr, T. 2009. The indicative influence of oxidative stress on low milk yields in dairy cattle. Thai J Vet. Med. 39: 237-243.

Weller JI, Bar-Anan R, Osterkorn K. 1985. Effects of days open on annualized milk yields in current and following lactations. J Dairy Sci. 1985 May;68(5):1241-9.

O-09-10 PIGS DEVELOPMENT ANALYSIS AND TECNOLOGIES INTRODUCTION IN THE VILLAGE OF TEMPOK

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Pigs is one of the commodity reability by several people as a source of income in the North of Sulawesi.Pigs livestock enterprice has potential marked and oportunity for future hope of bussines very encouraging.Enterprice pigs has been known and great desirable of people in Tempok because it easy to sell and potential as well as high demand.The problem is waste of pigs poluting to environtment.Based on the problem waste , this study conducted about the benefit of farmers and the health of environment.

The aim of this research is to analyze the benefit of pigs farmer and environtment.Survey method was used with the determinated by purposive sampling of respondents as members of the group who keep pigs in the village Tempok ,then proceed with the empowerment of group members fot reducing the effect pigs waste to the environtment.Descriptive analysis of data used in this research.The results showed that the pigs are sold by farmer not in flesh pig but intact weight life.The price for catlle over !00 kg is Rp.23.000 life weight and the farmers get benefit Rp.500.000 per head.Otherwise the price under 100 kg is Rp.22.000 per head.The member of group have been trained utilizing waste of pigs.The conclution was that pigs farming can be beneficial assessed on B/C ratio. Biogas unit production as an alternative solution for reducing pigs waste to the health of environment.

Introduction

Pigs are one of livestock commodities reliable by several people of North Sulawesi as a source of income. Pig farming has a potential market which is very encouraging and promising business opportunity. Pigs and or processed products of considerable potential as national export commodities, to various countries such as Singapore and Hong Kong and advantages is that volume of imports can be said to be zero (KementerianPertanian, 2012).

Farm pigs have been known and in demand by public, Tempok village due to its properties, as well as easy to sell because of high demand. Pigs, in village of Tempok, maintained by means stabled. This has been done by farmers to earn higher incomes. Wea and Koten (2013) states that productivity of pigs, lower when cultivated extensively. Intensive pig farming profitable for farmers (Suryadi et al, 2014).

Problem of pigs waste allowed to pollute environment. Cages for pigs is located near kitchen farmers. According to KementerianPertanian (2011), intensive pig farming is conducted in rural settlements can cause environmental problems. Environmental pollution issues often cause unrest in society (Linggotu et al. 2016). Based on this problem, has done research on extent of benefits of pigs for farmers and environment.

Methods of Research

This study was conducted using a survey method. Respondent has done purposive sampling group members who have pigs in Tempok village, then proceed with empowerment of group members to utilize waste of pigs. Empowerment has been done because of their knowledge of pig waste utilization is still low. Empowerment is done with extension methods and application of technology through use of pig waste. Data was analyzed using descriptive analysis.

Results and Discussion

Results from study showed that pigs are sold by farmers in Tempok village not for its meat but in form of live weight. Breeders who sell pigs weighing over 100 kg, price received Rp 23,000 per kg live weight, so that profit receivedRp 500,000 per pig. In contrast, farmers who sell pigs weighing under 100 kg live weight, price received is Rp 22,000 per kg live weight. Members of group have been trained to utilize pigs waste.Pig farming profitable for farmers because of RC value obtained was of 1.68. This is why farmers still maintain their business. Pig farming is a source of income for them. Pigs according Geong and Johanis (2010) are types of pigs that are important to small farmers in province.

Results from study showed that development of pig farming, in village of Tempok lead to pollution of soil, water

and air (primarily causes smell). Waste of pigs allowed to flow in backyard or in channels connected to a public place. Organic waste does not decompose properly can cause environmental problems such as odors, toxic gases, pests and diseases and others. Pig farming, in village of Tempok developed yet environmentally friendly and sustainable. Pig cage was built beside kitchen.

Negative impact of pig farming, in village of Tempok, namely gas that smells. Odor comes from nitrogen, and sulfide in pig manure, which will be formed during decomposition of ammonia gas, nitrite, and hydrogen sulfide gas. Air polluted by sulfide and ammonia gas can cause communities and livestock health problems in pig farming around. Ammonia can inhibit growth of livestock and on humans can cause irritation of eyes and respiratory tract. Waste of pigs, if it goes into the aquatic environment will pollute water bodies. This is due to the bacterium E. coli contained in livestock manure, thus showing the water is polluted. Water contaminated by fecal material according to Susanto (2002), which is the material with feces pigs.

Based on research has been done to pig farmer empowerment of environmentally friendly and sustainable, in village of Tempok. Reason for empowerment due to lack of knowledge of group, on impact of pig farming in settlement of environment. This is why farmers still maintain its business and is beside residence. Knowledge of group members, about pig farming is environmentally friendly and sustainable is still very minimal. Members of group do not have knowledge of management of cages of pigs and pig health. Lack of knowledge and skills of group members on use of livestock waste to organic fertilizer and biogas. Organic fertilizers can be used to improve soil fertility and production costs for organic fertilizer can be suppressed. Biogas, in addition to pressing environmental pollution can also be used to cook so cost of fuel (kerosene) are scarce, and expensive can be suppressed.

Empowerment has been done using two methods, extension and introduction of technology in form of organic fertilizers (Figure 1) and a biogas reactor (Figure 2). According Murbandono (2002), organic fertilizer is end result or intermediate result of changes or decomposition section and remains of plants and animals, such as meal, guano, bone meal, animal waste and so forth. Organic fertilizer is fertilizer made from organic materials are degraded organically. Sources of organic raw materials can be obtained from various sources, such as pig manure. Prihandarini (2004) suggests that it is usually to make this organic fertilizer, was added a solution of microorganisms that help accelerate degradation process.

Main raw material used is a waste of pigs, such as pig manure mixed with rest of food and mixed with urine. The raw material is supplied approximately each 10 bags (weighing 30 kg/sack). Additional materials (substituents) is urea, SP-36, ash, sawdust, calcite. Starter used EM4 (effective microorganism).

Extension is done with hope can lead to changes desired by farmers (Dumaria, 2006). Biogas is useful as an alternative energy source. This management is very profitable for farmers and surrounding communities (Mariawan, 2012; Zukri, 2012). Waste of pigs to produce biogas as many as 151 842 ml with a cooking time of 34 minutes (Takarenguang et al. 2016). Technology to manufacture biogas from pig waste likely to be solution of choice, to limitations of fuel oil (Utomo and Wahyuningsih, 2010).

Biogas is a type of energy in sustainable development are important for energy and environmental planning (Srisertpol et al. 2010). Biogas is a renewable energy source that can address need for energy as well as to provide for needs of soil nutrients in a system of sustainable agriculture. Biogas technology can be applied to scale household, commercial or villages (Eze, 2009). Barnhart (2012) suggested that householdscale biogas technology is used as fuel for cooking replace firewood and to improve human health and environment. Equality biogas produced from livestock manure with other energy sources can be seen in Table 1.

Utilization of biogas as an energy source in small industries based agro-processing can provide multiple effects and can be driving dynamics of rural development. In addition, it can also be used to increase value added by way of green labeling on processed products are in process by use of green energy.

Structuring of cultivation of environmentally friendly and sustainable is big breeding activities carried out by pig farmers. Implementation for better cultivation by implementing pigs waste management technologies and in accordance with the principles of Good Farming Practice (GFP). According to KementerianPertanian (2011), preservation of environment is an effort to protect life of environmental capabilities to pressure changes and or negative impact caused by an activity in order to remain capable of supporting human life and other living beings. According to KementerianPertanian (2012), indicators of successful development of environmentally friendly pig farming is (i) a pattern of livestock pig farming for better; (ii) reduce air pollution caused by smell of sewage / pigs

waste; (iii) support preservation of agricultural farm; (Iv) increase supply of organic fertilizer from animal waste so that dependence on inorganic fertilizer (chemical) will be reduced; (V) improve knowledge of members of group, a group of business management and application of waste treatment technologies that are environmentally friendly.

Conclusion and Suggestion

Based on results of study it can be concluded, farm pigs provide sufficient revenue for farmers seen from RC value. Biogas production is needed to accommodate livestock waste that pollute environment.
Based on research results suggested need for socialization by government to other pig farmers.

KEYWORD : Pigs, Evaluation, Tecnology

Figure 1. Organic Fertilizers

Figure 2.Biogas Reactor

Table1.Equality 1 m^3 of biogas with Other Energy Sources

Souces of other Energy	Number
1. LPG (Kg)	0,46
2. Kerosene (l)	0,62
3. Diesel (l)	0,52
4. Fuel (l)	0,80
5. City Gas (m^3)	1,50
6. Firewood (Kg)	3,50
7. Electric (Kwh)	6,00

Sources :Musanif, *et al.,* 2006

REFERENCES

Barnhart, S. 2012. Teaching Sustainability across Scale and Culture: Biogas in Context.Journal of Sustainability Education Vol. 3, March 2012.

Dumaria, T.R. 2006.Breeders participation in Livestock Extension (Case Pig Farmer groups in Siborong-borong Village, North Tapanuli, North Sumatra Province). Essay. Socio-Economic Studies Program, Faculty of Animal Husbandry, Bogor.

Eze, J.I. and E.O. Uzodinma. 2009. Generation of Methane Gas from Poultry Brooding House. The Pacific Journal of Science and Technology.Vol 10.Number 2. Nov 2009 (Fall). p: 942-948.

Geong, M and L. Johanis. 2010. Commercial Pigs Livestock Farming Pigs by Small Farmers in NTT Opportunities of Better market integration. Research Report.Australian Government Australian Centre for International Agricultural Research.Australian Indonesia Partnership.

KementerianPertanian. 2011. Sustainable Pigs Farming Structuring Guidelines. Directorate General of Livestock and Animal Health Directorate of Livestock Farming, Jakarta.

KementerianPertanian. 2012. Sustainable Pigs Farming Structuring Guidelines. Directorate General of Livestock and Animal Health Directorate of Livestock Farming, Jakarta.

Linggotu, L.O., U. Paputungan and B. Polii. 2016. Livestock Waste Management in Prevention of Environmental Pollution in Kotamobagu City. Journal Zootek. Vol. 36, No.1, Jan., 2016.p:226-237.

Mariawan, I.M. 2012.IbM Biogas. Journal of Community Service.WidyaLaksana. Edition January 2012.p37-44.

Murbandono, H. S. L. 2002. Make Compost. PenebarSwadayaPublisher. Jakarta.

Musanif, J., W.A. Adisasmitaand D.M. Nababan. 2006. Bio Program for Rural Energy (BEP) Household Scale Biogas. Directorate General of Processing Agricultural Products Processing and Marketing of Agricultural Products.Department of Agriculture, Jakarta.

Prihandarini, R. 2004. Waste Management, Waste Recycling Being Organic Fertilizer. PerPod Publisher, Jakarta.

Srisertpol, J., P. Srinakorn., A. Kheawnak and K. Chamniprasart. 2010. Mathematical Modeling and Parameters Estimation of an Anaerobic Digestion of Shrimp of Culture Pond Sediment in a Biogas Process. International Journal of Energy and Environment. Issue 4, Vol. 4, 2010. p: 213-220.

Suryadi, I.M.A., L.E. Tripolupiand K.R. Suwena. 2014. Feasibility Analysis Sancaya Pig Farming in terms of NPV method in BanjarPonggang, Payangan, Gianyar. Department of Economics, Faculty of Economics and Business, University of Education Ganesha, Singaraja,Indonesia.

Susanto, R. 2002. Organic Agriculturetowards Alternative Agriculture, and Sustainable.Yogyakarta.

Takarenguang, E.J., J.E.M. Soputan., V.R.W. Rawungand J.A.D. Kalele. 2016. Breeds Pig Waste Utilization For Producing Biogas. Journal Zootek. Vol. 36, No.1, Jan., 2016.p:113-122.

Utomo, S and V. Wahyuningsih. 2010. Mixture Doses of Cattle and Pigs Waste to Bio Gas Production. Journal Agrisains.Vol 1. 1 March 2010.p:7-14.

Wea, R and B.B. Koten. 2013. Composition of Body of Grower Males Local Pig who consume Fermentation and Acid Seeds Use Aras in rations. Journal of Animal Science. Vol.13.No.1, June 2013.p:8-12.

Zukri, A. 2012.IbMPig Farming Group, in village of Banyuning.Journal of Community Service.WidyaLaksana. Edition January 2012.p80-89.

O-10-1

REPRODUCTIVE PERFORMANCES OF HOLSTEIN FRIESIAN HEIFERS AND COWS INSEMINATED WITH SEXED SEMEN

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INTRODUCTION

The semen which the fractions of X-bearing and Y-bearing sperm have been modified from the natural mix through sorting and selection is called as sexed semen. The sexed semen is widely available and is used to produce a calf of a specific sex. Controlling the sex of offspring prior to conception permits the livestock industry to produce the optimal proportion of males and females to take advantage of sex-limited and sex-influenced traits, thus providing economically flexible management practices for the producer (Rath and Johnson, 2008). Research has consistently demonstrated that the technology used to sort semen produces about 90% of calves with the desired gender (De Jarnette *et al*., 2008). Dairy producers are used sexed semen to obtain more heifer calves to optimize the production. In addition, to increment of the number of replacement dairy heifers, which avoid bio-security issues of purchasing heifers for replacements stocks from outside the herd. Further, there are long-term predictions related to sexed semen on accelerated herd expansions, increased milk production and lower production costs.

In dairy production systems the predictable production of milk and young stock is dependent on calving pattern and for this reason; reproductive performance is a key determinant of profitability (Farin*et al*., 1994). As reproductive performances, conception rate, services per conception, voluntary waiting period and days open affect for the profitability of a dairy farm.

Use of sexed semen in artificial insemination, is a novel technology that practice in Sri Lanka. A preliminary study on the success rate of artificial insemination of sexed semen highlights that sexed semen can be used successfully in large dairy farms to obtain heifers and further, it will help to supply of excess female calves for the dairy industry in Sri Lanka (Disnaka *et al*., 2012). Moreover, the high cost of sexed semen doses and sexing of bovine semen are some limitations of application of sexed semen in dairy industry in Sri Lanka. A preliminary study was carried out in Sri Lanka to demonstrate the discontinuous sucrose density gradients can be considered as low cost tool for sperm sexing of bovine semen (Kanesharatnam *et al*., 2012). Therefore, there are potentials to use sex sorted semen for commercial dairy farms.

The reproductive performances of Holstein Friesian dairy cattle inseminated with sexed semen in commercial dairy farms in Sri Lanka needs to be evaluated. Therefore, the objective of this study was to determine the effect of sexed semen insemination on reproductive performances of Holstein Friesian dairy cattle in Up-Country; Sri Lanka.

MATERIALS AND METHODS

This study was carried out at an intensively managed commercial dairy cattle farm located at Up-Country, Central Province; Sri Lanka. The climate in this area is characterized by the mean annual temperature of 15.5 ^{0}C and mean annual rainfall of 2050 mm. The dairy herd was comprised of Holstein-Friesian cattle that calve throughout the year and re-bred using artificial insemination. Cows were housed in tie stall barns and were fed rations based primarily on rye, clover and concentrates.

Data were collected from 220 Holstein Friesian heifers and 52 cows inseminated with frozen-thawed sperm containing conventional semen and sexed semen. Only inseminations from January 2010 to March 2013 were included. The heifers (17 to 21 months of age and mean body weight 328 +28.59 Kg) and cows (34.16+3.17 months of age) were allocated for two treatment groups as in complete randomized design (CRD). The standing heats of all experimental animals were observed daily.

The semen containers with frozen live bull semen in liquid nitrogen were imported from United States of America and were stored in liquid nitrogen (-196 ^{0}C) until insemination. The concentration of sperms in a sexed semen dose was 2 million where it was 15 million in an unsexed semen dose. Artificial inseminations were performed accordingly with sexed or conventional semen after 12 - 18 hours from standing heat by a same Artificial

Insemination (AI) technician for all animals.
Pregnancy status of heifers and cows were diagnosed by rectal palpation of the uterus, after 60 days from the insemination. The conception rates and number of services per conception were computed using collected data. The number of days from calving to conception (i.e. open days) and voluntary waiting period were recorded for each cows. The Analysis of Variances (ANOVA) were performed to identify the significant differences ($p<0.05$) of parameters and data were subjected to descriptive statistical analysis by the Minitab 14 package.

RESULTS

3.1 Conception rate

The conception rate of first, second and third services were not significantly different ($p<0.05$) in sexed semen and conventional semen inseminated heifers. First-service conception rate for sexed semen insemination was only 48.75% as high as that for conventional semen insemination (44.18%); corresponding percentages were 36.11% for sexed semen insemination and 44.44% for conventional semen insemination in second service. The conception rates in third service for sexed and conventional semen were 42.85% and 53.33% respectively. The mean conception rate of first, second and third services were 42.57% and 47.32% for sexed and conventional semen respectively. Conception rates of field trials, involving virgin heifers have typically ranged from 35 to 40% with sexed semen, as compared with 55 to 60% for unsexed semen (Weigel, 2004).
According to the results of this study, first service conception rate was greater in sexed semen insemination (Figure 1), where it was decreased with the number of the service. The lower conception rate and higher cost of sexed semen has led to its use primarily for nulliparous heifers and first services for which the expected number of offspring per unit of sexed semen used is the highest (DeJarnette *et al*., 2008).

The first service conception rate of cows inseminated with sexed semen was 35.29% where it was 40% for conventional semen. The first service conception rate of sexed semen inseminated heifers (48.75%) was significantly higher than the cows (35.29%). The decreased conception rates experienced in cows with sexed semen, make virgin heifers better suited for insemination with sexed semen than lactating dairy cows (Olynk and Wolf, 2007). Several reports recommended the use of sexed semen in heifers in good standing heat (Olynk and Wolf, 2007; Weigel, 2004). In a survey of Wisconsin dairy producers, majority were using sexed semen with virgin heifers during the first and second services (Sterry *et al*., 2009).
A conception rate of 50.84% was resulted in an ongoing preliminary study of artificial insemination with sexed semen in the large scale Up-Country dairy farms in Sri Lanka. Furthermore, 15 pregnancies were confirmed by performing 34 pregnancy diagnoses (15/34, 44.11 %) in farms in the coconut triangle of Sri Lanka. The pregnancy rate of heifers, inseminated using sexed semen, was 56.60%, whereas in cows the rate was 32.60% (Disnaka *et al.,* 2012). The overall success rate of sexed semen artificial inseminations in cows and heifers was 49.3% (Disnaka *et al.,* 2012). The conception rate of fist service of heifers was resulted 48.75% in this study which is similar to the overall conception rate of study carried out by Disnaka *et al*., (2012).
Conception rates of United States dairy herds at first service averaged 47% for Holstein heifers and 53% for Jersey heifers for sexed semen, which were ~80% of that achieved with conventional semen (DeJarnette *et al.,* 2008). Mean conception rate for heifers was 56% for conventional and 39% for sexed semen; conception rates for cows were 30% and 25% in artificial inseminated Holstein heifers and cows in United State (Norman *et al*., 2010). The number of sexed sperm per straw is usually a low dose of approximately two million sperm, which is considerably less than the approximately twenty million sperm contained in a straw of conventional semen for AI in cattle (Olynk and Wolf, 2007). It may be leads to lower overall conception rate of sexed semen inseminated cows and heifers than the conventional semen insemination.

3.2 Services per conception

Based on mean overall conception rates, 1.79 sexed semen services were needed for a heifer pregnancy but only 1.97 services with conventional semen. The higher number of services per conception required for conventional semen insemination compared to the sexed semen for Holstein Friesian heifers. The services per conception of cows were 4.12 and 2.17 for sexed and conventional semen inseminations respectively. The overall services per conception for heifers and cows were 2.96 and 2.07 for sexed and conventional semen inseminations respectively. According to mean overall conception rates of sexed semen for artificial insemination of Holstein heifers and cows in United States were resulted with 2.6 sexed semen services per conception but only 1.8 services with

conventional semen (Norman *et al*., 2010). Further, based on cow conception rates, 4.0 sexed semen services were needed for a cow pregnancy but only 3.3 with conventional semen (Norman *et al*., 2010).
The goal for services per conception needs to remain lesser than 2.0, greater than 2.0 services per conception may not necessarily indicate a drastic problem if the services are occurring soon enough after the voluntary waiting period to keep days open to a reasonable length (DeJarnette 2008).

3.3 Voluntary waiting period and days open

The voluntary waiting period (VWP) is a key management decision wherein the herd manager designates a target number of days postpartum after which cows will be inseminated. The interval from calving to first insemination provides time for uterine shrinkage. Also, some herds may have a variable VWP that may be longer by choice for high producers or for first-parity cows (Miller *et al*., 2007). In present study, the mean voluntary waiting period of cows was 108.54 +49.94. The VWP is an important fact for lactating cows as the conception rate is expected to increase as days postpartum increase (Tenhagen *et al*., 2003), which may be partly related to milk yield.

According to the table 1, the mean age of cows were 34.16+3.17 months. The days open were 169.6 + 98.4 and 144.1+78.2 for sexed and conventional semen insemination respectively. Further, the days open was higher in sexed semen inseminated cows than conventional semen inseminations. As the number of services per conception was higher in sexed semen inseminated cows, the respective open days has been increased over the conventional semen inseminated cows. The increased number of services may be a result of lower sperm concentration of sexed semen than the conventional semen which may leads to lower conception rate. The longer days open reduce the profitability of a dairy farm by increased breeding cost, increased risk of culling and replacement costs, and reduced milk production.

CONCLUSIONS

The application of sexed semen artificial insemination was effective for heifers in first service than the lactating dairy cows, although there is not a significant difference ($p<0.05$). Services per conception was not significantly different in heifers of two treatments. However, it was higher in sexed semen inseminated cows than conventional semen inseminations. Further, the days open was greater in sexed semen insemination over conventional semen insemination of cows. Therefore, the application of sexed semen artificial insemination was more effective for heifers at first service than cows and further better reproductive performances observed in lactating dairy cows inseminated with conventional semen over sexed semen in Up-Country; Sri Lanka.

KEYWORD : Conception rate, Services per conception, Days open, Holstein Friesian, Sexed semen

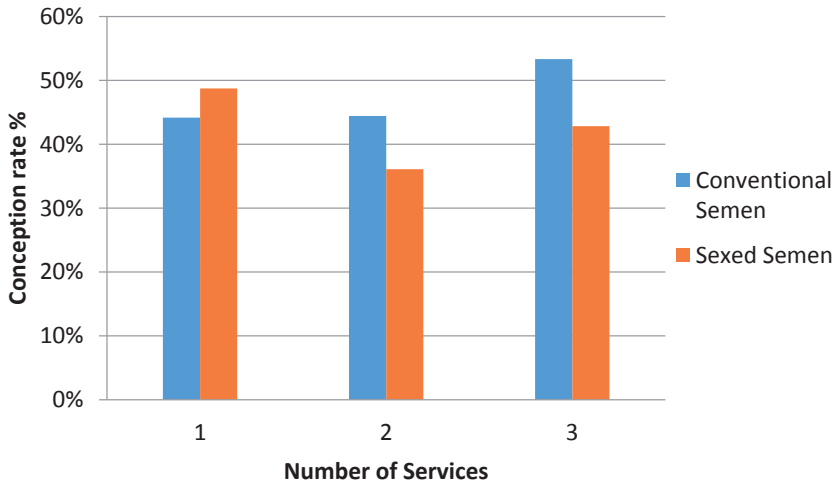

Figure 1. Conception rates of sexed and conventional semen inseminated heifers with respect to number of services

Table 1. The age, voluntary waiting period and days open of the experimental cows

Variable	Treatment	Mean	Standard Deviation	Median
Age at insemination (Months)	Sexed semen	34.07	2.95	35
	Conventional Semen	34.14	3.32	34
	Overall	34.16	3.17	34.5
Voluntary waiting period (Days)	Overall	108.54	49.94	100
Days open	Sexed semen	169.6	98.4	122
	Conventional Semen	144.1	78.2	116
	Overall	151.7	84.6	120

REFERENCES

DeJarnette, J.M., R.L. Nebel, C.E. Marshall, J.F. Moreno, C.R. McCleary and R. Lenz. 2008. Effect of sex-sorted sperm dosage on conception rates in Holstein heifers and lactating cows. J.Dairy.Sci. 91(5):1778–85. http://www.ncbi.nlm.nih.gov/pubmed/18420608.

Disnaka, K. G. J. S., Kaduwela, S. C., Samarawickrama, A. W., Uddkika Senanayaka, Dilan Kumara Silva and Basil Alexander 2012. Success Rate of Artificial Insemination Using Sexed Semenin Large Dairy Farms in Sri Lanka, *Annual Scientific Sessions of the Sri Lanka Veterinary Association*. 29 www.slva.org/scientific_sessions_2012.php?dw=Abstract_Book...pdf

Farin, P., B. Slenninq, M. Correa and J. Britt, 1994. Effects of calving season and milk yield on pregnancy risk and income in North Carolina Holstein cows. J. Dairy Sci., 77: 1848-1855.

Kanesharatnam Nilani, Thampoe Eswaramohan and Kandiah Balasubramaniam 2012. A Preliminary Study for Sperm Sexing by Using Sucrose Density Gradients in Jersey Bull at Artificial Insemination Centre at Thirunelvely (Northern Province of Sri Lanka). International Conference on Bioscience, Biochemistry and Bioinformatics. IACSIT Press, Singapoore. 3:1

Miller, R.H., Norman, H.D. Kuhn, M.T. Clay, J.S. and Hutchison, J.L. 2007. Voluntary waiting period and adoption of synchronized breeding in dairy herd improvement herds. Journal of Dairy Science, 90, 1594—1606.

Norman, H.D., Hutchison, J.L. and Miller, R.H. 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J.Dairy.Sci. 93(8):3880–90.

Olynk, N.J. and C. Wolf, 2007. Expected net present value of pure and mixed sexed semen artificial insemination strategies in dairy heifers. J.Dairy.Sci. 90(5):2569–76.

Rath, D. and L. Johnson. 2008. Application and commercialization of flow cytometrically sex-sorted semen. Reproduction in domestic animals. 43(2):338–46.

Sterry, R., D. Brusveen, V. E. Cabrera, K. Weigel and P. Fricke. 2009. Why they use sexed semen. Hoards Magazine. 205.

Tenhagen, B.-A., C. Vogel, M. Drillich, G. Thiele, and W. Heuwieser. 2003. Influence of stage of lactation and milk production on conception rates after timed artificial insemination following Ovsynch. *Theriogenology*, 60, 1527—1537.

Weigel, K.A., 2004. Exploring the Role of Sexed Semen in Dairy Production Systems. J.Dairy.Sci. 87: 120–130.

O-10-3 Gas Sensors For Electronic Nose And Their Application To Determine Estrus Phase In Cattle.

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OBJECTIVE

Efficient and profitable reproductive performance of cattle requires routine but conscientious heat detection and proper timing of artificial insemination. Failure to detect estrus (heat) is a major factor contributing to low fertility. Unfortunately, not all of cattle can show the clinical sign of estrous, so that the alternative way such as estrus detector using electronic nose should be created. The aims of this research was to determine whether the stage of estrus can be detected using Electronic Nose (EN).

METHODOLOGI

Animals. The sample used in the study was urine cattle of Ongole Crossbred (PO) derived from adult female who had BCS between 3-4, maintained in Kuwang, Sleman district, Yogyakarta. Urine samples of cattle would be collected shortly before injection of Dinoprost as an estrous synchronization material and repeated when cattle was on estrus.

Gas Sensor. The eight element gas sensors arrays were tested such as methane, propane, butane, alcohol, water contaminant, hydrogen sulfide, saturated vapor of organic solvent and ammonia.

Illustration of E-nose Data Logger System. Baseline data is collected for 6 minutes. Ambient air is flowed through sensor chamber. For the first step, sample is placed in sample chamber. Heater is set at around 50 degree Celsius then E-nose will collect the data for 18 minutes. 4^{th} time mode (1 minute for sensing and 1 minute for purging) is used. The first nine peaks of all sensors response are investigated using Principal Component Analysis (PCA) method.

RESULTS

Sensor Arrays. The sensors have been optimized in the sense that sensitivity to the pheromones has been enhanced and sensitivities to other gasses that are typically present in a cow shed (e.g C1 T95813: methane, propane, butane; C2: T95822: saturated vapor of organic solvent; C3: T952600; sensitive to hydrogen sulfide; C4: T95826: ammonia; C5: T952611: methane; C6: T95620; alcohol; C7:T952612: metana propana butana: C8: T952602: water contaminant) have been reduced. The different sensors in the array have been absorbed but have different sensitivities (Figure 1).

Mohammed et al. (2009) reported, beside methane, propane and butane, estrus can be detected using metal-oxide sensor. Lane and Wathes (1998) also reported that acetaldehyde was produced by oxydation of methane or hydration of ethylene is one of good indicator subtance for estrus in cattle. The nose sensors respond to changes in volatile substances with changes in resistance. Molecules causing such a response would be likely to be detectable as an odor signal. Cyclical changes in odor through the cycle, therefore, may be important in determining the behavioral response of a bull to cows at estrus. Sjahfirdi et al. (2011a); Sjahfirdi et al. (2011b) stated blood and urine sample of rat can be used as estrus indicator using Fourier Transform Infrared (FTIR).

Data Analysis. All measurements by the Enose were analyzed using the Principle Component Analysis (PCA) technique. PCA is defined mathematically as the orthogonal linear transformation of data from the 8 sensor patterns to a two- dimensional coordinate system. In this case, every point represent single experiment. There are 27 points in this PCA plot. FC stands for First Component, SC stands for Second Component, and TC stands for Third Component. Then, the data could be analysed using 2 dimension (2D) and 3 dimension (3D) score plot.

From 2D score plot it can be seen separation between estrus and non estrus sample even though there are a certain area of overlapping. The 2D is accounted for 96.1%. However, when Third Component (TC) is included, clear separation among cluster could be seen (Figure 2).

However, when Third Component (TC) is included, clear separation among cluster between estrus and non-estrus could be seen more clearly . The first 3 PC is accounted for 98.9% of the variance in data set (Figure 3.)

CONCLUSIONS

Based on result and discussion, it will be concluded that the E-Nose was able to detect changes in perineal odors associated with estrus and was able to detect estrus day successfully in all studied cattle.

REFFERENCES

Lane AJP, and Wathes, DC. 1998. An Electronic Nose to Detect Changes in Perineal Odors Associated with Estrus in the Cow. J Dairy Sci 81:2145-2150

Luthfiralda Sjahfirdi, Ade Septian, Hera Maheshwari, **Pudji Astuti,** Frans D-Suyatna, Mohammad Nasikin. 2011a. Determination of Estrous Period in Female Rats (Rattus norvegicus) by Fourier Transform Infrared (FTIR) Spectroscopy Through Identification of Reproductive Homrone in Blood Sampel. WASJ 14(4): 539-545

Mohamed, EI, Hadir M. Maher, Asmaa M. Shaban, Fekry M, Hussein. 2009. Electronic Nose Technology for the Accurate Detection of Estrus by Monitoring Changes in Perineal Odors in Dairy Cows. *J. of Biophy and Biomed. Sci.,*2(1): 128-133

Sjahfirdi L, Aziz SN, Maheshwari H, **Astuti P,** Suyatna FD, Nasikin M. 2011b. Estrus Period Determination of Female Rats (*Rattus norvegicus*) by Fourier Transform Infrared (FTIR) through Identification of Reproductive Hormones Metabolites in Urine. *Internt. J. of Basic & Appl Sci IJBAS-IJENS* (11) (03): 158-163

KEYWORD : Electronic Nose, Estrus, Detector, Cattle

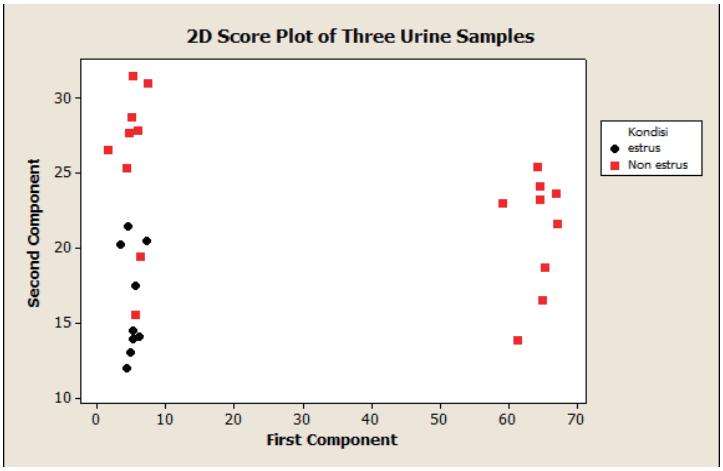
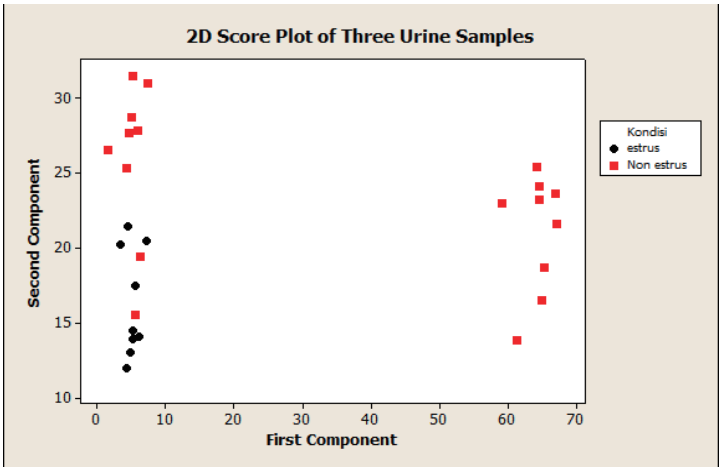


Figure 2. The first 2 PC is accounted for 96.1% of the variance in data set



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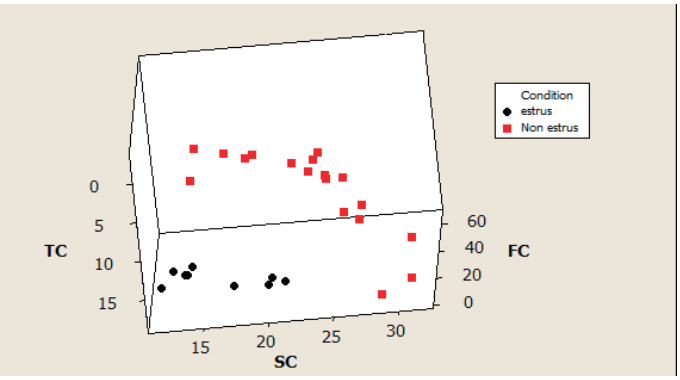

Figure 3. The first 3 PC is accounted for 98.9% of the variance in data set

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Mohamed, EI, Hadir M. Maher, Asmaa M. Shaban, Fekry M, Hussein. 2009. Electronic Nose Technology for the Accurate Detection of Estrus by Monitoring Changes in Perineal Odors in Dairy Cows. *J. of Biophy and Biomed. Sci.*,2(1): 128-133

Sjahfirdi L, Aziz SN, Maheshwari H, Astuti P, Suyatna FD, Nasikin M. 2011b. Estrus Period Determination of Female Rats (Rattus norvegicus) by Fourier Transform Infrared (FTIR) through Identification of Reproductive Hormones Metabolites in Urine. *Internt. J. of Basic & Appl Sci IJBAS-IJENS* (11) (03): 158-163

O-10-4

EFFECTS OF FEED SUPPLEMENT ON THE ARTIFICIAL INSEMINATION EFFICIENCY OF BEEF COWS UNDER SMALL FARMS CONDITION IN INDONESIA

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ABSTRACT

Low artificial insemination (AI) efficiency in beef cattle has been reported in some tropical regions, such in Indonesia. Factors related to this problem are not yet well known. Therefore, the purpose of this study was to identify factors that might affect the AI efficiency and to evaluate the effects of feed supplement on the AI efficiency in beef cows at a tropical area of Indonesia. Two steps of study were conducted. Study 1 was to identify factors that might affect the efficiency of AI. This study was conducted in 623 inseminated cows. The informations related to the farms, inseminated cow, bulls and AI technicians were collected. Interval between calving and first AI, conception rate (CR) and service per conception (S/C) were recorded. Data were tabulated and calculated using Artificial Insemination Database Application (AIDA). Interval between calving and first AI, between calving and conception, CR, and S/C were 142+105 days, 190.9+129.6 days, 39 % and 2.5, respectively. Factors that significantly affected this AI efficiency were BCS during AI, types of estrus, feeding and housing systems, and bulls. Study 2 evaluated the effect of feed supplement on the body condition score (BCS) and CR in the inseminated beef cows. Two groups of 50 postpartum cows were allotted to control and feed supplemented (1 kg rice bran/head/day) groups. BCS and CR were recorded. In supplemented group, although BCS was not improved, but CR was significantly increased the CR (62 vs 42 %). It can be concluded that lower AI efficiency in cows kept under small farm condition could be increased by the supplementation of high quality feed.

Introduction

Artificial insemination (AI) is widely used for both dairy and beef cattle in the developed countries. In the developing countries, however, although numerous projects have been introduced to establish AI services, it seems that only few developing countries have succeeded in establishing an efficient of AI services in comparison to those in the developed countries.

Some developing countries have reported low conception rates (CR) of cows mated by AI techniques. The CR of 30 - 50% in Srilangka (Abeygunawardana, et.al., 2007), 18% in Malaysia (Malik et al., 2012), 38.5% in Bangladesh (Haque, et.al., 2015), 46.5 % in Nigeria with number of services per conception (S/C) was 2.1 (Mai et al, 2014), 30-55% in Suriname (Bastiansen, 1997) , 25-35% in Ethiopia (Woldu et al., 2011), and 23% in Indonesia (Toleng et al, 2001). Based on the category of AI performances presented by Moran (2005), the CR less than 43% was categorized as low conception rate and need to be improved.

Detecting factors limiting CR in artifially inseminated cows is one of the main factors that need to be performed in order to improve AI efficiency. These factors are poorly documented in the developing countries, especially for beef cattle. In Bangladesh, Haque et al. (2015) reported that feeding, body condition score (BCS) and time of AI significantly affected pregnancy rate of dairy cows. In Srilangka (Abeygunawardena et al, 2001), both non technical (farmer's ignorance and low motivation, low motivation and mobility of field staff) and technical (poor heat detection, low quality of semen and inadequate skill of AI technicians) aspects contributed to the low successfull rate of AI program. In Nigeria (Mai et al, 2014), breed of cows, year of AI, adequate nutrition and weaning significantly correlated with the CR of the cows. Supplementation of food for 4 months increased BCS and conception rate of the cows in Ethiopia (Woldu et al., 2011).

The purposes of this study were to identify factors that might affect the AI efficiency and to evaluate the effects of feed supplement on the AI efficiency of beef cows at a tropical area of Indonesia.

Materials and Methods

Two steps of study were conducted. Study 1 evaluated the reproductive performance of artificially inseminated beef cows and factors that might affect the efficiency of AI services. There were 623 cows from various and kept by 544 small farms included in this study. The estrus cows (both under natural and synchronized estrus) were inseminated with frozen semen from various breeds of bull. During the survey, informations related to farms,

AI technicians, inseminated cows and bulls were recorded in accordance with the data entry sheets for AIDA (Artificial Insemination Database Application) introduced by Garcia & Perera (2000). Pregnancy diagnoses were performed 60-70 days after AI by rectal palpation. The efficiency of AI (interval from calving to the first AI, CR and S/C) were calculated. Significant differences at each factor that affected the efficiency of AI were tested by chi-square analysis. Study 2 evaluated the effect of feed supplement on the CR of the inseminated cows. A total of 50 lactating cows were allocated in two groups of treatment. The first group was supplemented with 1 kg rice bran from the first week after calving until the first AI while the second group remained as a control group. Estrous detection, recording of body condition score, procedure for AI, and pregnancy diagnosis were similar to those performed at the first study. The mean difference of BCS among the two groups was analyzed by student's t-test, while the mean difference of CR was analyzed by chi-square analysis.

Results and Discussion

Study 1.

Reproductive performance

The intervals between calving and first service, and between calving and conception were 143 and 190.9 days, respectively, CR at the first and overall services were 40.0 and 39.8% , respectively and S/C was 2.5 (Table 1).

Factors affecting the AI efficiency

Factors affected CR of the inseminated cows are listed in Table 2 . Cows with BCS 4 (scale 1-5) showed significantly higher CR in compared to those with BCS 2,3 and 5 both in the first and overall services. CR for synchronized estrus was significantly higher in compared to that for naturally estrus cows. Food supplementation for the grazing cows either with concentrate or with grasses could signifinatly increase CR in compared to those grazing only. Cows kept in corral during the night showed a significantly higher CR in comparison to other housing systems. Furthermore, semen of local breed (Bali) bull had a significantly higher CR in compared to other semen of some exotis breed bulls.

Feed supplementation could increase significantly ($P<0.05$) conception rate (62 vs. 42%), although there was no significant change of BCS (2.32 vs. 2.48).

In this area, the reproductive performances of beef cattle bred by AI techniques were lower than their genetic potentials. There were several factors related to the lower reproductive performances of these animals such as: BCS, housing system, feeding management, bulls and types os estrus. The supplementation of high quality feed could increase the CR.

Lower CR in this area was similar to those reported in some developing countries such as in Srilangka (Abeygunawardana et al., 2007), in Malaysia (Malik et al., 2012), in Bangladesh (Haque et al., 2015), in Nigeria (Mai et al., 2014), in Suriname (Bastiansen, 1997) and in Ethiopia (Woldu et al., 2011). Higher S/C was similar that reported in Nigeria (Mai et al., 2014). There was an improvement of CR for about 10% in the inseminated cows in this study compared to that in a similar survey conducted in 1995-2000 (Toleng et al., 2001). The improvement of AI efficiency in this area might be due to the impact of various interventions that have been taken both by technical (feeding, semen quality, housing, etc) and non-technical (training for AI technicians and farmers etc.) aspects. In various Asean countries, Boettcher and Perera (2007) reviewed that technician and farmers training, and improved feeding, recording system and semen quality could increase CR by 5 to 27%.

The significant effect of body condition score (BCS) on the CR in this study was similar to that reported in Bangladesh by Haque et al., (2015). Animals kept under the paddock especially in the night and offered them with feed supplement showed higher CR. Feed supplementation significantly increased pregnancy rate in dairy cows (Haque et al.. 2015) and beef cattle (Toleng et al., 2001).

The significant effect of estrus synchronization on the CR was similar to that reported in beef cattle by Martinez et al. (2000). Although these results were in contrast with our previous report (Latief et al., 2001), this discrevancy might be due to the different season at the time of estrous synchronization. In our previous report, the estrus synchronization was conducted during the dry season, where the scores of body condition of the cows were generally low. Rensis and Scaramuzzi (2003) have reviewed that heat stress could adversely affect of hormonal secretion of hypothalamo-hypophiseal - gonadal axis and fetal development after insemination.

Feed supplementati*on could increase conception rate of the cows. This result was similar to our previous report (Toleng et al., 2001), and those were reported in dairy cows by Haque et al. (2015) and* Woldu et al., 2011. Cows

gaining weight during early lactation have a higher conception rate and need fewer services per conception compared to those losing weight (Amaral-phillips and Heersche Jr, 1997). The significance increased of CR without changing BCS might be due to the effects of micro minerals (cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc) as reported by Amaral-Phillips and Heersche, Jr (1997) and Anomin (2009).
It can be concluded that body condition score, feeding system, housing system and estrus synchronization were the factors affecting the efficiency of artificial insemination in cows. Feed supplementation could increase the conception rate of the cows.

KEYWORD : Beef Cattle, Artificial Insemination, Conception Rate, Feed Supplement, Small Farm

Table 1. Reproductive performance of cows

Parameters	
Interval (days) from calving to:	
- First service	143±103.8
- Conception	190.9±129.6
Conception rate (%)	
- At first service	40.0
- Overall service	39.8
Service per conception (S/C)	2.5

Table 2. Factors affecting conception rate during the first and the overall services

Parameters	First service			Overall service			
	No. of cows inseminated	No. of pregnant cows	CR (%)	No. of cows inseminated	No. of pregnant cows	CR (%)	S/C
Cow breed							
Bali	429	184	42.9	542	227	41.9	2.4
Brahman	39	13	33.3	75	27	36.0	2.8
Limousin	44	17	38.6	69	25	36.2	2.8
Simmental	58	18	31.0	77	28	36.4	2.8
Body condition score							
2	36	10	27.8[a]	52	11	21.2[a]	-
3	296	88	29.7[a]	400	136	34.0[ab]	-
4	182	119	65.4[b]	232	142	61.2[c]	-
5	12	7	58.3[a]	13	7	53.8[ac]	-
Synchronized							
Yes	166	91	54.8[a]	203	106	52.2[a]	1.9
No	457	158	34.6[b]	634	227	35.8[b]	2.8
Feeding system							
Grazing + concent	193	90	46.6[a]	268	133	49.6[a]	2.0
Grazing + roughage	146	80	54.8[a]	179	92	51.4[a]	1.9
Grazing only	279	77	27.6[b]	385	106	27.5[b]	3.6
Housing							
Corral/paddock	195	76	39.0[a]	248	106	42.7[a]	2.3
Loose barn	209	52	24.9[b]	298	73	24.5[b]	4.1
Night paddock	182	103	56.6[c]	237	129	54.4[a]	1.8
Tie stall	37	18	48.6[ac]	52	24	46.2[a]	2.2
Bull							
Angus	94	20	21.3[a]	102	24	23.5[a]	4.3
Bali	285	124	43.5[bd]	353	151	42.8[b]	2.3
Limousin	412	115	27.9[c]	680	172	25.3[a]	4.0
Simental	127	39	30.7[cd]	198	69	34.8[ac]	2.9
Type of employer							
Goverm AI center	596	220	36.9	744	285	38.3	-
Private AI center	26	13	50.0	51	25	49.0	-
Self employ	31	16	51.6	40	22	55.0	-

Different superscript within a column in each parameter indicate significant differences $P<0.05$

Study 2

Table 3. Body condition score (BCS) and conception rate of the cows in control and feed supplemented groups.

Group	BCS	CR (%)
Control	2.32	42[a]
Supplemented	2.48	62[b]

Different superscript within a column indicate significant differences P<0.05

REFERENCES

Abeygunawardena, H., Alexander, P.A.B.D. and Abeygunawardena, I.S., (2001*).* Artificial Insemination of cattle in Sri Lanka: Status, performance and problems. In. Radioimmunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and sub-tropical conditions. IAEA-TECDOC-1220. P. 51-66.

Abeygunawardena, H., Abeygunawardena, I.S., and Pushpakumara, P.G.A. (2007*).* Improving the performance of artificial insemination services through application of radioimmunoassay and computerized data management programmes. In. Improving the Reproductive Management of Dairy Cattle Subjected to Artificial Insemination. IAEA-TECDOC-1533. P. 113-124.

Amaral-phillips, D.M. and Heersche, G. Jr, (1997). Role of nutrition on reproductive performance. Cooperative extension service, Univ. of Kentucky, college of Agriculture. ASC-138.

Anomin (2009). Effects of nutrition on beef cow reproduction. Agri-Facts, Practice information for Alberta's Agriculture Industry. Agdex 420/51-1.

Bastiaensen, P. (1997). Preliminary results on artificial insemination of cattle in Suriname. Case Study: Commewijne district.

Boettcher, P.J. and Perera,B.M.A.O. (2007*).* Improving reproductive management of smallholder dairy cattle and the effectiveness of artificial insemination: a summary. In. Improving the Reproductive Management of Dairy Cattle Subjected to Artificial Insemination. IAEA-TECDOC-1533. P. 1-4.

De Rensis,F and Scaramuzzi,R.J (2003). .*Heat stress*and*seasonal effects*on*reproduction*in the *dairy cow*--a*review*. *Theriogenology*.*60*(6): *1139*-1151

Garcia, M. and Perera, O. (2000). Artificial insemination database application (AIDA). User manual. Animal Production and health section, IAEA, Vienna.

Haque, M.N., Gofur, M.R., Asaduzzaman, K.M. and Bhuiyan, M.M.U. (2015). Factors Limiting the Pregnancy Rates in Artificially Inseminated Cows in Bangladesh. *International Journal of Dairy Science, 10: 278-287*

Mai, H.M., Voh, Jr., A.A. and Deshi, P.S. (2014). Some fertility indices in an artificial inseminated Bunaji and Bokoloji herds in Kaduna State, Nigeria. Global Veterinaria 12 (2): 171-175.

Malik, A., Haron, A.W., Yusoff, R., Kasim, A. and Yusoff, A.M., (2012), Pregnancy rate following artificial or natural service in postpartum estrus synchronized beef cattle. Turk. J. Vet. Anim. Sci. 36(4): 451-455.

Martinez, M.F., Kastelic, J.P., Adams, G.P., Janzen, E., McCartney, D.H., and Mapktoft, R.J. 2000, Estrus synchronization and pregnancy rate in beef cattle given CIDR-B, prostaglandin and estradiol, or GnRH. Can Vet J. 41. P. 786-790.

Moran. J. (2005). Tropical dairy farming: feeding management for small holder dairy farmers in the humid tropics. Landlinks Press.

Toleng, L., Sonjaya, H., Yusuf, M. and Hamid, A. (2001). The use of progesterone RIA to increase efficiency and quality of artificial insemination services of beef cattle in South Sulawesi, Indonesia. In. Radioimmunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and sub-tropical conditions. IAEA-TECDOC-1220. P. 37-44.

Woldu, T., Giorgis, Y.T and Haile, A. (2011). Factors affecting conception rate in artificially inseminated cattle under farmers condition in Ethiopia. Journal of Cell and Animal Biology Vol. 5 (16), pp. 334-338.

O-10-6

Potential of Evaporative System Improving Pregnancy Rate in Recipient Heifers after Embryo Transfers in Thailand

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ABSTRACT

In Thailand, hot and humid climate attenuates the reproductive performance, particularly decreasing pregnancy rate and increasing fetal loss of potential of embryo transfer (ET) in dairy cattle. The use of an evaporative cooling system to control temperature and humidity environment in stall barn can solve these problems after ET in recipient cattle. The objective of this study was to evaluate the efficacy of evaporative cooling system to enhance the pregnancy rate after ET in Holstein Friesian heifer recipients in Thailand. Ten donors of Japanese Black cows were estrus synchronized and artificial inseminated (AI) using commercial sex-sorted Japanese Black sperm. Day 7 after AI, each donor cow was embryos flushed and selected only the excellent and good grades of embryos for fresh embryo transferring. The forty Holstein Friesian heifer recipients were divided into two groups (twenty heifers per group). These twenty heifers were separated to be three replicates in each group. Group 1 was housed in an evaporative stall barn and Group 2 was held in free-stall barn. All recipient heifers were estrus induced and had to occur the matured corpus luteum on the same day of ET procedure. Each recipient would be transferred as one embryo. Pregnancy diagnosis was detected by ultrasonography in the 2nd and 4th month after ET. The result showed the temperature and relative humidity (RH) in the evaporative stall barn, were controlled at 26.0-29.0℃ and 70.0-80.0% RH, respectively. The pregnancy rate of recipient those were kept in the evaporative stall barn (80.0%; 16/20) was significantly ($p<0.05$) higher than those recipients in a free-stall barn (50.0%; 10/20). Additionally, every pregnant recipient in the two groups had no fetal lost and delivered healthy calves. In conclusion, the evaporative cooling system can improve the pregnancy rate after ET in Holstein Friesian heifer recipients using Japanese Black embryos in Thailand.

INTRODUCTION

In the recent years, the top quality of beef meat is very in demand in Thailand. Japanese Black cattle is one of the best choices for meat production due to highly intramuscular fat (marbling), tender texture, juiciness and tasty beef flavor (Gotoh et al., 2014) but the head numbers of Japanese Black cattle is still low. The use of the combined efficient techniques such as superovulation, estrus synchronization, artificial insemination (AI) and embryo transfer (ET) can enhance the production outcome effectively (Farin and Farin, 1995). However, the important crisis for cattle producing in this region is hot ambient temperatures and unstable of relative humidity (RH) environment which are dramatically negative effect on the embryonic loss (Ealy et al., 1993) and pregnancy rate from heat stress (Ahmadi and Ghaisari, 2007, Hossein-Zadeh et al., 2013, Khan et al., 2013). Cows begin to present the stress behaviors e.g., increase in respiratory rate and blood vessels dilation when Temperature Humidity Index (THI; the index for evaluation heat stress in cows) is higher than 72. The reproductive system performance could be significantly decreased when the value of THI is over than 89 (Armstrong, 1994). Therefore, the utility of evaporative cooling system conducted with the animal stall can practically reduce temperature and control RH. They can improve the success of embryo production (Ferreira et al., 2011) and increase the pregnancy rate. The aim of this study was to evaluate the efficacy of evaporative cooling system to enhance the pregnancy rate after ET in Holstein Friesian heifer recipients in Thailand.

MATERIALS AND METHODS

This experiment was conducted at P.N. Farm, Kanchanaburi Province, Thailand. The average temperature all year round is 23.38 ± 2.36 to 34.18 ± 2.34 ℃ (the maximum temperature in summer season is approximately 43.9 ℃) and average relative humidity (%RH) is 69.42 ± 8.06 % (latitude 14.01° N and longitude 99.32° E).

Experimental animals

Ten healthy and fertile pure blood Japanese Black cows [age: 3-7 years old; body condition score (BCS) 3.0-3.5; scale 1-5] were used as donors. Forty crossbred Thai-Holstein heifers (age: 2 years old; BCS 3.0-3.5) were used

as recipient-heifers. Donors and recipients were fed concentrate with 14.0% protein, corn silage, and roughage *ad libitum*. The donors were housed in an evaporative stall barn while recipient-heifers were assigned randomly to one of two housing experimental groups; 1) the evaporative stall barn and, 2) free-stall barn (20 recipients per experimental group). Temperature and RH in an evaporative stall barn were controlled at 26.0-29.0℃ and 70.0-80.0% RH, respectively.

Donor and Recipient managements

The protocols of superovulation for donors and estrus synchronization for recipients from Baruselli et al. 2011 were chosen for this study but with minor modification (modified: an amount of FSH was injected into donors without decreasing volume). Ten donors of Japanese Black cows were induced superovulation (Fig. 1) by the transvaginal insertion of CIDR (1.9 g progesterone CIDR®; Eazi-Breed®, Pfizer Animal Health, Hamilton, NZ) for 12 days and together injected with 500 μg $PGF_{2\alpha}$ (Estrumate®, Vet Pharma Friesoythe GmbH, Friesoythe, Germany) at the time of CIDR insertion. On Day 7, an amount of 0.008 μg of GnRH was once injected (Receptal®, MSD Animal Health, Wellington, NZ). Follicle Stimulating Hormone (Folltropin®-V, Bioniche Canada Inc., Belleville, Ontario, Canada) was injected 8 times with equal volume, total FSH dosage was 400 mg per donor (Day 8: PM, from Day 9 to Day 11: twice daily and Day 12: AM). The CIDR was removed on Day 12 and 500 μg $PGF_{2\alpha}$ was injected. The second injection of GnRH was given on Day 13 (AM) following twice fixed time artificial inseminated (FTAI) on Day 13 and Day 14 with 12 h interval using commercial sex-sorted Japanese Black sperm (Sexing Technologies, Texas, United States; imported by Pornchai Intertrade Limited Partnership, Thailand). To collect the embryo, embryo flushing was processed as nonsurgical method on Day 20 from inseminated donors. The embryo grading criteria according to Bó and Mapletoft 2013 was used to evaluate the quality of embryos in this study. Only the excellent and good grades embryos (grade 1; scale 1-5) were used for fresh embryo transfer. On the other hand, the estrus synchronization of recipient heifers was started on Day 1 which was straight to Day 4 of CIDR treatment in superovulation protocol (Fig. 1). The recipient heifers were received CIDR (1.9 g progesterone CIDR®; Eazi-Breed®, Pfizer Animal Health, Hamilton, NZ) for 5 days and 0.008 μg of GnRH (Receptal®, MSD Animal Health, Wellington, NZ) was once injected on the first day of CIDR insertion. An amount of 500 μg $PGF_{2\alpha}$ (Estrumate®, Vet Pharma Friesoythe GmbH, Friesoythe, Germany) was injected at the day of CIDR withdrawal. The second injection of 0.008 μg of GnRH was injected (Receptal®, MSD Animal Health, Wellington, NZ) on Day 13. On the same day of embryo flushing, the synchronized recipients were rectal palpated to examine the functional corpus luteum on ovaries. An embryo was transferred using an ET gun and embryo was liberated on the uterine horn with corpus luteum appearance. Pregnancy diagnosis was detected by ultrasonography in the 2^{nd} and 4^{th} month after ET.

Statistical analysis

The pregnancy rate subjected to be statistically analyzed by T-test with paired two samples for means. P-value ≤ was regarded as statistically significant.

RESULTS AND DISCUSSION

Embryo collection was operated on Day 21 from donors using nonsurgical method, approximately 5.2 ± 1.03 excellent and/or good embryos were collected from each donor. The result of the current study was shown in Table 1. The pregnancy rate of the experimental group 1 (80.0%) was significantly higher than the experimental group 2 (50.0%, p=0.0302). According to this finding, it could be proved that the cattle stall barn conducted with an evaporation cooling system can increase the pregnancy rate. Temperature and relative humidity in an evaporative stall barn were controlled at 26.0-29.0℃ and 70.0-80.0% RH, respectively. These numbers could be compared in the THI table of heat stress estimate for dairy cows. The THI of an evaporative stall barn in this study presented in between 76-81 which were in the range of mild to moderate stress. In contrast, temperature and relative humidity of free-stall barn were 36.5℃ and 74.36 %RH, respectively that revealed the THI of 93 which indicated that cattle were in severe stress (Wiersma, 1990 cited by Armstrong, 1994). In donor cows, it has been reported that hot temperature declines the development of embryo to blastocyst stage effect to low number of embryo recovery during embryo flushing (Krininger et al., 2003). The pregnant cows, heat stress influence to pregnancy rate due to the induction of body temperature resulted to the degenerated embryos and embryo loss after embryo transferring (Hansen, 2007, Ono et al., 2016). Heat stress during gestation period might reduce the blood progesterone level that leads to early embryonic death and fail to implantation of the embryo (Mann et al., 1999). On the contrary, the study of Katanani et al. 2002 showed that heat stress conditions could improve the

pregnancy rate following transfer of only fresh embryo. At the end of gestation period of this study, all pregnant cows gave birth to healthy calves. In conclusion, the evaporative cooling system can improve the pregnancy rate after embryo transfer in Holstein Friesian heifer recipients using Japanese Black embryos and reduce the THI index. The use of a cooling system conduct with the animal housing has great benefit for cattle production.

KEYWORD : Embry transfer, Evaporative system, Holstein Friesian, Pregnancy rate

Figure 1. Superovulation and estrus synchronization protocol (minor modified from Baruselli et al. 2011) for donors and recipients.

Treatment Day		0	4	7	8	9	10	11	12	13	14	20
Donors	AM	CIDR insertion +$PGF_{2\alpha}$		GnRH		FSH	FSH	FSH	CIDR removal +FSH +$PGF_{2\alpha}$	GnRH	FTAI (I)	Embryo flushing
	PM				FSH	FSH	FSH	FSH +$PGF_{2\alpha}$		FTAI (II)		
Recipients	AM		CIDR insertion +GnRH					CIDR removal + $PGF_{2\alpha}$		GnRH		FTET

FTAI = Fixed time artificial insemination, FTET = Fixed time embryo transfer

Table 1. The number of the pregnant recipients after embryo transferred for 60 and 90 days and number of the calves were born. The results are presented as percentage (%). Three replicates/group and 6-7 recipients/replicate had been used for analysis.

Animal barn	No. of recipient (n)	No. (%) of pregnant recipient	No. (%) of Calf
Evaporative stall barn	20	16 (80.0)[a]	16 (100.0)
Free-stall barn	20	10 (50.0)[b]	10 (100.0)
P-value	N/A	0.0302	N/A

[a, b] superscription in the same column as significant difference ($p<0.05$), N/A is not applicable

REFERENCES

Al-Katanani, Y. M., M. Drost, R. L. Monson, J. J. Rutledge, C. E. Krininger III, J. Block, W. W. Thatcher and P. J. Hanse. 2002. Pregnancy rates following timed embryo transfer with fresh or vitrified *in vitro* produced embryos in lactating dairy cows under heat stress conditions. Theriogenology 58 (1): 171-182

Ahmadi, M. R. and H. R. Ghaisari. 2007. Heat stress and different timed-AI methods influence on pregnancy rates of dairy cows. Vet. Arhiv. 77 (4): 327-335

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77: 2044-2050

Baruselli, P. S., R. M. Ferreira, J. N. S. Sales, L. U. Gimenes, M. F. Sá Filho, C. M. Maritns, C. A. Rodrigues and G. A. Bó. 2011 Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology 76: 1583-1593

Bó, G. A. and R. J. Mapletoft. 2013. Evaluation and classification of bovine embryos. Anim. Reprod. 10 (3): 344-348

Ealy, A. D., M. Drost and P. J. Hansen. 1993. Development changes in embryonic resistance to adverse effects of maternal heat stress in cows. J. Dairy Sci. 76: 2899-2905

Farin, P. E. and C. E. Farin. 1995. Transfer of bovine produced in vivo or in vitro survival and fetal development. Biol. Reprod. 52: 676-682

Ferreira, R. M., H. Ayres, M. R. Chiaratti, M. L. Ferraz, A. B. Araújo, C. A. Rodrigues, Y. F. Watanabe, A. A. Vireque, D. C. Joaquim, L. C. Smith, F. V. Meirelles and P. S. Baruselli. 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. J. Dairy Sci. 94: 2383-2392

Gotoh, T., H. Takahashi, T. Nishimura, K. Kuchida and H. Mannen. 2014. Meat produced by Japanese Black cattle and Wagyu. Anim. Front. 4 (4): 46-54

Hansen, P. J. 2007. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 68 S: S242-S249

Hossein-Zadeh, N. G., A. Mohit and N. Azad. 2013. Effect of temperature-humidity index on productive and reproductive performances of Iranian Holstein cows. Iran. J. Vet. Res. 14 (2): 106-112

Khan, F. A., S. Prasad and H. P. Gupta. 2013. Effect of heat stress on pregnancy rates of crossbred dairy cattle in Terai region of Uttarakhand, India. Asian Pacif. J. Reprod. 2 (4): 277-279

Krininger III, C. E., J. Block, Y. M. Al-Katanani, R. M. Rivera, C. C. Chase Jr. And P. J. Hansen. 2003. Differences between Brahman and Holstein cows in response to estrus synchronization, superovulation and resistance of embryos to heat shock. Anim. Reprod. Sci. 78: 13-24

Mann G. E., G. E. Lamming, R. S. Robinsons and D. C. Wathes. 1999. The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. J. Reprod. Fertil. 54: 317–228

Ono, T., T. Isobe, Y. Morita, L. T. K. Do, F. Tanihara, M. Taniguchi, M. Takagi and T. Otoi. 2016. Effects of parity and season on pregnancy rates after the transfer of embryos to repeat-breeder Japanese Black beef cattle. Arch. Anim. Breed. 59: 45-49

O-10-7

Factor affecting sperm uptake into the sperm storage tubules in Japanese quail (Coturnix japonica)

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INTRODUCTION

Fertilization is an indispensable step for formation of a zygote in sexual reproduction, leading to species survival. In birds, several unique mechanisms such as polyspermic fertilization and sperm storage in the oviduct are employed in the process of the fertilization. The ejaculated sperm are stored in the oviductal sperm storage tubules (SST) in a quiescent state until use for fertilization. Because of the presence of SSTs, once ejaculated sperm have entered the female reproductive tract, they can survive up to 2-15 weeks in domestic birds, including chickens, turkeys, quail and ducks for various periods depending on the species (Bakst *et al*., 1994; Bakst, 2011) in contrast to the relatively short life span in mammalian spermatozoa (i.e., several days). Although extensive investigations concerning the function of the SST in birds have been performed since its discovery in the 1960s by means of ultrastructural analysis (Bobr *et al*., 1964; Schuppin *et al*., 1984; Van Krey *et al*., 1967), the specific mechanisms involved in the sperm uptake into the SST, the sperm maintenance within it, and the controlled sperm release from it are long standing enigma. We previously demonstrated that SST exist under hypoxic condition to produce and secret mass amounts of lactic acid *in vivo*, and it established cytoplasmic acidification of the resident sperm leading to sperm motility inactivation (Matsuzaki *et al*., 2015). The resident sperm are squeezed out from the SST by contraction like morphological changes of the SST under the control of the elevated level of circulating progesterone (Ito *et al*., 2011). Although we observed prostagladin $F_{2\alpha}$ in the seminal plasma of Japanese quail enhances sperm uptake into the SSTs by opening of the entrance of the SSTs (Sasanami *et al*., 2015), the mechanism of how the spermatozoa are navigated to the SSTs after insemination is not fully understood yet.

In this study, we investigated a factor affecting the sperm uptake into the SST in Japanese quail.

MATERIALS AND METHODS

Male and female Japanese quail, *Coturnix japonica*, 15-30 weeks of age (Quail cosmos-farm, Toyohashi, Japan), were maintained individually under a photoperiod of 14L: 10D (with the light on at 0500) and were provided with water and a commercial diet (Motoki-corp, Saitama, Japan) *ad libitum*. The hens were decapitated, and the UVJ was immediately dissected and placed in physiological saline. The adhering connective tissues were removed, and each UVJ was excised longitudinally. They were then divided into several pieces, and were used for the following experiments.

The ejaculated sperm were obtained from 2 males and each ejaculate was divided into two separate tubes containing 1 x 10^7 cells/ml. They were then stained with 1 mg/ml Hoechst33342 (H33342) or pHrodo-AM (pHr) and were combined for the incubation in Hanks\' balanced salt solution (HBSS) with the isolated UVJ tissues. After the incubation, the tissues were washed in PBS and were cut into small pieces, and were mounted in glycerol. The specimens were observed under fluorescent microscope and the sperm fluorescence derived from H33342 (blue) or pHr (red) in the SSTs was counted to calculate the sperm filling rate. In some experiments, monensin, an inhibitor of intracellular transport of protein at the level of Golgi apparatus (Kääriäinen et al., 1980), or brefeldin A (BFA), a specific inhibitor of membrane transport (Fujiwara et al., 1988) was included in the incubation mixture.

RESULTS AND DISCUSSION

When the ejaculated sperm were incubated in HBSS with the isolated UVJ tissues *in vitro*, sperm entry into the SSTs was observed within 1 hr. The sperm filling rate was about 30%, demonstrating that *in vitro* assay system that evaluate sperm uptake into the SSTs was established. When the formalin- or methanol-fixed UVJ was incubated with ejaculated sperm, a significant decrease in the filing rate compared with that of the use of fresh UVJ was observed. This indicates that the structure of the SST itself does not simply contribute to the sperm entry into the SSTs. Next, we tested if chemoattractant released from the SSTs may contribute to the sperm uptake into the SSTs. Because secretory granules were found in the SST by ultrastructural analysis, we observed the effects of

monensin and BFA on sperm filling rate. As the results, no inhibitory effect was found, suggesting that secretory activity by the SSTs may not be important for sperm uptake into the SSTs. Further, we compared sperm filling rate of the SST tissues of which were incubated with the spermatozoa obtained from different male. The sperm filling rate was significantly correlated with sperm motility score, indicating that the intrinsic sperm motility appears to be important for sperm entry. Moreover, when the filling rate was compared between each female, a considerable variation was found in spite of the use of same ejaculates. These results indicate the possibility that compatibility between male and female in terms of the sperm uptake into the SST exists in birds.

CONCLUSION

In sexual reproduction, sperm are subject to selection by the female and sperm competition, and these factors drive the development of various reproductive tactics through evolution. During the last decade, several key molecules such as $PGF_{2\alpha}$, lactic acid and progesterone that contribute to sperm uptake into, maintenance within and release from the SST, respectively were discovered in Japanese quail (Ito *et al*., 2011; Sasanami *et al*., 2015; Matsuzaki *et al*., 2015). However, we are unable to answer many important questions on the mechanism of sperm storage. The elucidation of this process may directly lead to our understanding on the molecular mechanism of cryptic female choice in which unknown female factors control fertility of the male animals whose sperm were proven to have fertilizability with certain partners but not with another female (Parker, 1970). An elucidation of the mechanism is difficult to uncover due to the lack of a suitable experimental model. In this study, we showed that avian sperm storage is undoubtedly a suitable model for this aim. Further studies will be required for the elucidation of this mechanism in birds.

KEYWORD：Sperm storage tubules, Utero-vaginal junction, Sperm, Japanese quail

REFFERENCES

Bakst MR. (2011) Physiology and endocrinology symposium: role of the oviduct in maintaining sustained fertility in hens. J. Anim. Sci. 89(5):1323-1329.

Bakst MR, Wishart G, and Brillard JP. (1994) Oviducal sperm selection, transport, and storage in poultry. Poult. Sci. Rev. 5:117-143.

Bobr LW, Lorenz FW and Ogasawara FX. (1964) Distribution of spermatozoa in the oviduct and fertility in domestic birds. I. Residence sites of spermatozoa in fowl oviducts. J. Reprod. Fertil. 8: 39-47.

Fujiwara T, Oda K, Yokota S, Takatsuki A and Ikehara Y. (1988) Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. J. Biol. Chem. 263(34): 18545-18552.

Ito T, Yoshizaki N, Tokumoto T, Ono H, Yoshimura T, Tsukada A, Kansaku N, and Sasanami T. (2011) Progesterone is a sperm-releasing factor from the sperm-storage tubules in birds. Endocrinology. 152(10): 3952-3962.

Kääriäinen L, Hashimoto K, Saraste J, Virtanen I and Penttinen K. (1980) Monensin and FCCP inhibit the intracellular transport of alphavirus membrane glycoproteins. J Cell Biol. 87(3 Pt 1): 783-791.

Matsuzaki M, Mizushima S, Hiyama G, Hirohashi N, Shiba K, Inaba K, Suzuki T, Dohra H, Ohnishi T, Sato Y, Kohsaka T, Ichikawa Y, Atsumi Y, Yoshimura T and Sasanami T. (2015) Lactic acid is a sperm motility inactivation factor in the sperm storage tubules. Sci. Rep. 5:17643.

Sasanami T, Izumi S, Sakurai N, Hirata T, Mizushima S, Matsuzaki M, Hiyama G, Yorinaga E, Yoshimura T, Ukena K and Tsutsui K. (2015) A unique mechanism of successful fertilization in a domestic bird. Sci. Rep. 5:7700.

Schuppin GT, Van Krey HP, Denbow DM, Bakst MR and Meyer GB. (1984) Ultrastructural analyses of uterovaginal sperm storage glands in fertile and infertile turkey breeder hens. Poult. Sci. 63(9): 1872-1882.

Van Krey HP, Balander RJ and Compton MP. (1981) Storage and evacuation of spermatozoa from the uterovaginal sperm host glands of the domestic fowl. Poult. Sci. 60: 871-877.

Parker GA. (1970) Sperm competition and its evolutionary consequences. Biol. Rev. 45: 525-567.

O-11-1

Effect of Kaempferia galanga L. on in vitro nutrients digestibility, ruminal fermentation and methane production

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Objective

Antibiotics such as monensin have been used to manipulate rumen fermentation and successfully increase feed efficiency and animal production. The effect of monensin on rumen fermentation was briefly explained by Russell and Strobel (1989). The inclusion of antibiotics in animal rations as has been limited due to the occurrence of multi-drug resistant bacteria which may be a risk to human health (Gustafson and Bowen, 1997). In recent years, plant bioactive compounds as natural feed additives have been studied as antibiotic alternatives for improving ruminal fermentation and nutrient utilization. The antimicrobial effects of some phytochemical compounds such as essential oils have been previously demonstrated (Janssen et al., 1987)

Several plants containing phytochemical reduced methane production and increased VFA production (Bodas et al., 2008). Plant essential oil from varies of sources has been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation. Blended plant essential oil, cinnamaldehyde, eugenol, and capsicum in feedlot cattle diet have the similar effect with monensin (Geraci et al., 2012) improve growth performance and health, by optimizing rumen fermentation and increase immune system status (Compiani et al., 2013). Among the essential oil, thyme and cinnamon oil and their main active components (thymol and cinnamaldehyde, respectively) have potential antimicrobial activity against ruminal microorganisms (Calsamiglia et al., 2007; Benchaar and Greathead, 2011) and have potency as monensin alternate (Khorrami et al., 2015). The effects of essential oil in characteristics of rumen fermentation depend on dose. Varies response were showed among different natural extracts and pure essential oil as well as the concentration of essential oil which included in the fermentation (Macheboeuf et al., 2008).

Kaempferia galanga L. is a plant which widely used as herb in cooking in Indonesia, where it is called *kencur*, and especially in Javanese cuisine and Balinese cuisine. It has different essential oil component from other herbs previously mentioned. The major chemical constituents of volatile oil obtained by water distillation of dried rhizome of *Kaempferia galanga* L. were identified as ethyl-*p*-methoxycinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively (Tewtrakul et al., 2005). Whereas Kumar (2014) reported the major constituents of the *Kaempferia galanga* L oil were ethyl cinnamate (29.48%), ethyl-p-methoxycinnamate (18.42%), γ - cadinene (9.81%), 1, 8-cineole (6.54%), δ -carene (6.19%), borneol (5.21%), ethyl-m-methoxycinnamate (2.15%), camphene (1.58%), linoleoyl chloride (1.35%) and α -pinene (1.32%). Differences plant propagation resulting the different concentration of essential oil component. The main component of *Kaempferia galanga* L. ethyl p-methoxy cinnamate in quantity were 82.01% and 71.77% respectively for essential oil component of *Kaempferia galanga* L. rhizomes with conventional and in vitro propagation (Sahoo et al., 2014). Essential oil from *Kaempferia galanga* L. had antimicrobial activity toward Gram positive and Gram negative bacteria (Tewtrakul et al., 2005). Moreover, to our knowledge, no research has done on *Kaempferia galangal* L. related to rumen fermentation. Therefore this research was conducted to studied the effect of *Kaempferia galangal* L. in ruminal feed fermentation and methane production.

Methodology

Feed, treatments and in vitro fermentation

The effect of Kaempferia *galanga* L. on nutrients digestibility, ruminal fermentation, and methane production were studied in this research using batch culture of in vitro gas production technique. Feed sample for in vitro fermentation consist of *Pennisetum purpureum*, which cut before flowering stage, rice bran and wheat pollard, obtained from feed shop, with ratio 60:20:29 based on dry matter. *Kaempferia galanga* L. meal was prepared by drying fresh rhizomes in dryer incubator at 55℃ and grounded to pass through a 1 mm pore size sieves. Additions of *Kaempferia galanga* L. were based on the final concentration of essential oil in fermentation media i.e. 0, 25, 50, 75, and 100 mg/L.

Inoculum for the in vitro gas production was obtained from two ruminal canulated Ongole grade cattle fed a diet

consisting of *Pennisetum purpureum* and beef cattle concentrate 60:40 DM basis TDN 88.57% and CP 9.34%. Rumen fluid was collected before morning feeding, and squeezed through polyester cloth into a vacuum flask thermos, and immediately sent to the laboratory.

Serum bottles, 125 ml, were used for in vitro incubations. Bottles were set into three triplicate bottles, one set for dry matter digestibility (DMD) and organic matter digestibility (OMD) determination, gas and methane production, one set for crude protein digestibility (CPD), and one set for rumen fermentation parameter. Sufficient anaerobic media was prepared the day before the incubation according to Theodorou *et al*. (1994). Sixty three milliliters of media was added into serum bottles which previously filled with 700 mg of substrate and *Kaempferia galanga* L. powder according to the treatments and continue flushed by ooxygen-free carbon dioxide. Bottles were sealed immediately with butyl rubber stopper plus aluminum crimp cap and pre-warmed overnight at 39℃ . In the next morning, rumen fluid were collected, and 7 ml was added into each bottle using 10 ml plastic syringe. Bottles then incubated for 24 h at 39℃ . Bottle head space gas pressure were zeroing before incubation by inserting 0.6 mm needle attached to a pressure transducer.

At the end of incubation gas were collected using calibrated syringe and 5 ml of gas were transferred into 5 ml plain vacuum tube (Becton Dickinson Vacutainer System) for methane analysis. DMD, OMD and CPD were determined by filtered the bottle content, and residual feed were collected for residual nutrients analysis, including DM, OM and CP. Procedure for nutrient analysis according to AOAC (2005). Sample for protozoa calculation were prepared by pipetting 1 ml of bottle content and be added to 0.8ml of formaldehyde saline (1ml of 37%formaldehyde + 9 ml 0.9% NaCl). One microliter sample then transferred to haemocytometer for direct calculation under microscope according to method explained by Diaz et al. (1993). For ammonia measurement 1 ml of bottle content were preserve with 1 ml NaCl 20% and be frozen until later analysis of ammonia base on phenol hypochlorite reaction as explained by Chaney and Marbach (1962). Media, as much as 1 ml for VFA analysis were added into tube containing 1 ml of 20% metha-phosphoric acid and stored in freezer for further analysis using gas chromatography. Prior to sampling for ammonia, VFA, microbial protein and protozoa, pH media were measured. Rumen microbial protein was determined by Lowry method (Alexander and Griffiths, 1993). Microbial cell were separated from residual feed by centrifugation 1.5 ml of bottle content at 500g. Cell were precipitated from supernatant by spin down at 15.000 g. Pellets were re-suspension in physiology solution and re centrifuge. Re-suspension was repeated for twice. The last suspension was subjected for protein determination.

Calculation and statistical analysis

Parameters studied were nutrients digestibility including dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) (expressed in %), carboxymethyle cellulose (CMCase) (expressed in U), total gas production (ml), total VFA, acetate, propionate and butyrate concentration as mmol/100mL, rumen microbial protein, ammonia concentration as mg/100L, methane production as ml/g DM digested, and protozoa number. Data were subjected to one-way analysis of variance with level of *Kaempferia galanga* L. as the treatment factor. Comparisons between means were analysis using *t*-test of Duncan Multiple Range test.

Result

Dry mater, organic matter and crude protein digestibility were not affected by addition of *Kaempferia galanga* L. at all tratments level (Table 1). CMCase, represent fibrolitic enzymes also was not affected by the treatments even there was a tendency in treatments groups have lower activity except treatment group of *Kaempferia galanga* L. addition equal to 75 mg/L of essential oil. Fermentations with addition of *Kaempferia galanga* L. have not differences on volume of gas production per dry matter digested compared to control ($P<0.01$) (Table 1.) Pattern of DMD fluctuation similar with the pattern of gas production per DM degraded (Figure 1.).

Effect of essential oils on nutrient digestibility were varies. Talebzadeh et al. (2012) reported addition of *Zataria multiflora* essential oils at level 150 to 600 mg/L have no effect on IVDMD but linearly decreased IVOMD. Main component of *Zataria multiflora* essential oil were carvacrol, p-cymene and thymol. Other result from screening plant experiment in 'rumen up' project showed varies value of IVDMD by addition of different plant on in vitro rumen fermentation, some plant showed have no different effect on IVDMD compared to control, some plants increase and some plants decreased (Bodas et al., 2008). Plant secondary metabolites activities were influenced by their chemical nature and concentration. Activities of essential oils also depend on their component and dose. Thymol have no effect on NDF and ADF digestibility at level 5, and 50 mg/L but significantly reduced at level 500 mg/L, while eugenol have no effect on NDF and ADF at dose 5, 50 and 500mg/L (Castillejos et al., 2006). *Zataria*

multiflora essential oil reduced gas production at level 300 mg/L and up, reduced VFA and ammonia at level 600 mg/L, and reduction of rumen biomass start at level 150 mg/L (Talebzadeh et al., 2012). Unlike of *Zataria multiflora*, *Kaempferia galanga* L. did not affect DMD digestibility and other nutrients as well as gas production. Gas production commonly used to predict DMD, in this research DMD pattern parallel to gas production pattern. Main component of *Kaempferia galanga* L. essential oil were ethyl-*p*-methoxycinnamate, and methylcinnamate, carvone, eucalyptol and pentadecane, respectively (Tewtrakul et al., 2005; Kumar, 2014) which differ from previous study. The differences of essential oil component are responsible for their differences effect on enzyme activity, gas production, pH, VFA, ammonia, microbial protein and protozoa number.

CMCase activity was not differing among treatments. Unaltered of CMCase activity by essential oil treatments suggested that essential oils may not affect fibrolitic microbes but may depressed activity of amilolytic and proteolitic bacteria by suppressed the colonization and digestion of readily degrades substrates (Wallace et al., 2002).

pH, total and individual VFA as well as ratio of acetate to propionate, microbial protein and ammonia concentration as shown in Table 2., they were not affected by increasing level of *Kaempferia galanga* L. in the diet. Overall, methane produced in fermentation with *Kaempferia galanga* L. were lower compared to control. Significant decreasing of methane occurred at level of *Kaempferia galanga* L. addition equal to final essential oil concentration 50 and 100mg/L media ($P<0.01$). Protozoa number in treated fermentation have higher protozoa number ($P<0.05$). Significant higher number appeared at level 50 mg/L and up.

Essential oil did not affect proteolysis, the first step of protein degradation in rumen, but decreased rate of ammonia production, deamination step (Wallace et al., 2002), in contrast, *Kaempferia galanga* L. did not alter ammonia might due to it concentration and structure of main essential oil component. Major constituents of *Kaempferia galanga* L. essential oil, is esters and terpenoid compounds (Kumar, 2014), ethyl-*p*-methoxycinnamate, and methylcinnamate, derivatives of cinamic acid (Baser and Buchbauer, 2010). Cinnamaldehyde other derivative of cinamic acid which given in in vitro fermentation at doses 1 to 5 mmol/L equal to 132.16 to 660.88 mg/L reduced ammonia significantly (Macheboeuf et al., 2008), in agreement with Busquet et al. (2006), high level of cinamaldehyde, 300mg/L and 3000mg/L reduced ammonia concentration while at low level 3 and 30 mg/L did not effect on ammonia. Structure changes of essential oil component will alter its activity. Biohydrogenation transform of Ethyl *p*-methoxycinnamate a major constituent of the *Kaempferia galanga* L. rhizome essential oil, to ethyl *p*-hydroxycinnamate increased the potential inhibition, bactericidal, and fungicidal toward bacteria and fungi (Omar, 2014).

Total VFA, acetate, propionate, butyrate, ratio acetate to butyrate, and rumen microbial protein did not affected by *Kaempferia galanga* L. Cinamaldehyde reduced VFA and its component at level 3000 mg/L and up (Busquet et al., 2006) 300 mg/L and up except acetate did not affected up to level 660 mg/L (Macheboeuf et al., 2008). Dose up to 100 mg/L of *Kaempferia galanga* L. essential oil might not enough to change the VFA production and profile, but methane production and protozoa number had been modify. Methane productions per digested dry matter decrease around 14.98% to 32.29% due to the addition of *Kaempferia galanga* L. Several essential oil component reduced methane at different level, carvacrol at 225mg/L, cinnamaldehyde at 265 mg/L, and thymol at 300mg/L (Macheboeuf et al., 2008).

Addition of *Kaempferia galanga* L. increase the number of protozoa level 50 and 75mg/L of essential oil. At level 75 mg/L protozoa number was the highest, then a little bit decreased at level 1000 mg/L. Essential oil at low level might become stimulator for some species of protozoa, and at high level the effect on protozoa population is not drastic (Patra and Xaxena, 2009).

Conclusions

Kaempferia galanga L. in the diet equal to essential oil level 25 to 100mg/L does not effect on DMD, OMD, CPD, gas production, pH, total VFA, acetate, propionate, butyrate, rumen microbial protein, and ammonia concentration, whereas methane production were lower in fermentation with addition of *Kaempferia galanga* L. in the diet, and protozoa number increase at dose 50 up to 100mg/L.

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KEYWORD : Kaempferia galanga L., Essential oil, Ruminal fermentation, Methane production, nutrients digestibility

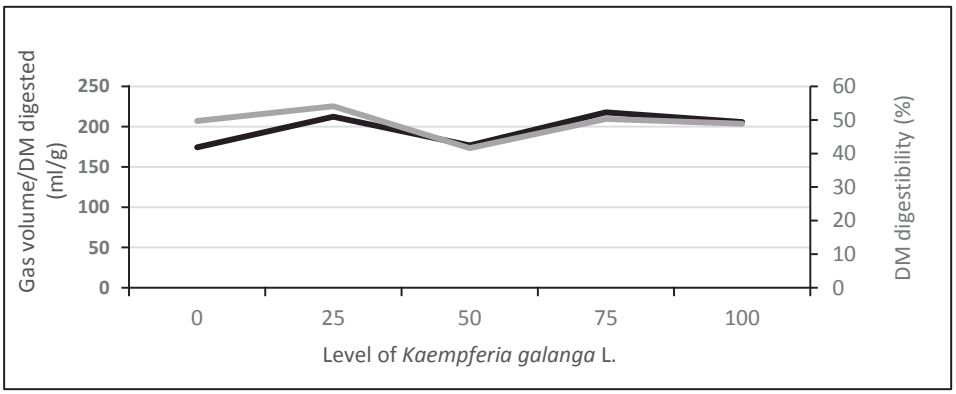

Figure 1. Patterns of dry matter digestibility and gas production of in vitro rumen fermentation added *Kaempferia galanga* L. (gas Production (▬▬), dry matter digestibility (▬))

Table 1. Effect of *Kaempferia galanga* L. on nutrients digestibility and carboxymethyle cellulase activity

Parameters	Level of essential oil (mg/L)				
	0	25	50	75	100
Dry matter digestibility (%)	49.72± 6.637	54.08± 2.043	41.60± 5.844	50.30± 5.882	48.85± 1.292
Organic matter digestibility (%)	49.12± 5.543	45.34± 1.817	56.69± 4.809	50.46± 5.165	51.36± 1.052
Crude protein digestibility (%)	56.02± 3.949	39.05± 3.038	34.75± 4.907	53.58± 3.567	46.31± 5.540
Gas production/DM digested (ml/g)	174.36± 23.487	212.39± 1.220	176.67± 16.148	217.78± 18.493	205.75± 0.888
CMC ase	2.30± 0.130	1.96± 0.014	1.68± 0.270	2.18± 0.660	1.30± 0.559

Table 2. Effect of *Kaempferia galanga* L. on parameters of rumen fermentation

Parameters	Level of essential oil				
	0	25	50	75	100
pH	6.77± 0.049	6.73± 0.042	6.76± 0.007	6.76± 0.021	6.80± 0.057
Total VFA (mmol/100mL)	15.11± 2.477	15.97± 2.741	14.35± 0.029	20.00± 0.627	25.40± 4.452
Acetate	10.77± 1.536	11.52± 1.859	10.44± 0.072	14.68± 0.299	19.92± 12.466
Butyrate	1.34± 0.095	1.34± 0.003	1.35± 0.061	1.65± 0.193	1.60± 0.549
Propionate	3.00± 0.846	3.10± 0.880	2.56± 0.104	3.66± 0.733	3.88± 1.437
Acetate/propionate	3.66± 0.522	3.78± 0.473	4.08± 0.193	4.10± 0.902	4.87± 1.409
Rumen microbe (mg/L)	319.36± 9.400	258.48± 28.576	278.42± 30.456	238.27± 61.663	238.27± 10.528
Ammonia (mg/10mL)	25.44± 0.076	25.15± 0.278	26.79± 1.842	28.76± 2.902	27.04± 1.943
Methane production/DM digested (ml/g)*	8.61± 0.897^{b}	7.32± 0.303^{b}	5.83± 0.572^{a}	7.15± 0.205^{b}	5.23± 0.023^{a}
Protozoa (cel x 10^{4})*	8.52± 0.080^{a}	8.45± 1.850^{a}	10.86± 0.080^{b}	11.87± 0.119^{b}	10.56± 0.497^{b}

REFFERENCES

Alexander, R. R., Griffiths, J. M., 1993. Basic Biochemical Methods. 2nd ed. Wiley-Liss, Inc. New York. Pp, 28-29.

AOAC. 2005. Offocoal method of analysis of the association official analytical chemistry. 18th ed. Maryland, USA.

Baser, K.H.C., and G. Buchbauer. 2010. Handbook of essential oils: science, technology, and applications.CRC Press. Taylor & Francis Group. Broken Sound Parkway. New York.

Benchaar, C., Greathead, S., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166–167, 338–355.

Bodas, R., López, S., Fernández, M., García-González, R., Rodr ´ ıguez, A.B., Wallace, R.J., González. J.S., 2008. *In vitro* screening of the potential of numerous plant species as antimethanogenic feed additives for ruminants. Anim. Feed Sci. Technol. 145, 245–258

Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C., 2006. Plants extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89, 761-771.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J.Dairy Sci. 90, 2580–2595.

Chaney, A.L., Marbach, E. P., 1962. Modified reagents for determination of urea and ammonia. Clinical Chem. 8, 130-132

Castillejos, L., Calsamiglia, S., Ferret, A., 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. J. American Dairy Sci. 89, 2649-2658.

Diaz, A., Avendano, M., Escobar, A., 1993. Evaluation of sapindus saponaria as defaunating agent and its effect on different ruminal digestion parameters. Livestock research for Rural Development. 5, 33-39.

Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316.

Gustafson, R.H., Bowen, R.E., 1997. Antibiotic use in animal agriculture. J. Appl. Microbiol. 83, 531–541

Janssen, A.M., Scheffer, J.J., Baerheim, S.A., 1987. Antimicrobials activities of essential oils. Pharm. Weekblad. 9, 193–197.

Kumar, A. 2014. Chemical composition of essential oil isolated from the rhizomes of *Kaempferia galanga L.* Int J Pharm Bio Sci 5, 225- 231

Macheboeuf, D., Morgavi, D.P., Papon, Y., Mousset, J.-L., Arturo-Schaan, M., 2008. Dose–response effects of essential oils on in vitro fermentation activity of the rumen microbial population. Anim. Feed Sci. Technol 145, 335–350

Omar, M.N., Hasali, N. H. M., Alfarra, H. Y., Yarmo, M. A., Zuberdi, A. M., 2014. Antimicrobial Activity and Microbial Transformation of Ethyl p-Methoxycinnamate Extracted from *Kaempferia galanga Orient. J. Chem*., Vol. 30, 1037-1043

Patra, A.K., Saxena, J., 2009. Dietary phytochemicals as rumen modifier: a review of the effects on microbial populations. Antonie van Leeuwenhoek. 96, 363-375.

Talebzadeh, R., Alipour, D., Saharkhiz, M.J., Azarfar, A., Malecky. M., 2012. Effect of essential oils of *Zataria multiflora* on in vitro rumen fermentation, protozoal population, growth and enzyme activity of anaerobic fungus isolated from Mehraban sheep Anim. Feed Sci. Techol. 172,115– 124

Theodorou, M. K., Williams, B. A., Danoa, M. S., McAllan, A. B., Fance, J., 1994. A simple gas production method using pressure transducer to determine the fermentation kinetics of ruminant feed. Anim. Feed Sci. Technol. 48, 185-197.

Tewtrakul, S., Yuenyongsawad, S., Kummee, S., Atsawajaruwan, L., 2005. Chemical components and biological activities of volatile oil of *Kaempferia galanga* Linn. Songklanakarin J. Sci. Technol. 27(Suppl. 2): 503-507

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. Asian-Aust J Anim Sci 15, 1458– 1468

O-11-4

Effects of nitrate, extracted chitosan or shrimp shell meal on degradability and In Vitro gas production

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Introduction

Methane production through enteric fermentation is of concern worldwide for its contribution to the accumulation of greenhouse gases in the atmosphere, as well as its waste of fed energy for the animals (Johnson and Johnson, 1995). Developing feeding strategies of ruminants with the methane suppressing impact are desirable and inhibition of methanogensis has long been considered as a strategy to improve animal productivity.

Enteric methane production in ruminants can be reduced in 3 ways: by removing methanogens from the rumen, by reducing H2 production, or by providing an alternative H2 sink (Joblin, 1999). Nitrate is an electron sink, reported to reduce methane production in sheep (Takahashi and Young, 1991; Sar et al., 2004, 2005). Leng (2008) has emphasized that nitrate as a feed component replacing urea has a dual role as an electronic sink for hydrogen produced by fermentation, and the ammonia produced is the preferred source of fermentable nitrogen in diets having a low content of crude protein. It is thus logical to test whether nitrate can be used to lower enteric methane production and at the same time maintain or promote microbial growth in the rumen.

Chitosan (*N*-acetyl-D-glucosamine polymer**;**CHI), is a natural biopolymer derived through the deacetylation of chitin, a major component of the shells of crustaceans. As a nontoxic, biodegradable carbohydrate polymer, CHI has received much attention for diverse potential applications in medicine and food preservation because of its antimicrobial properties, against bacteria, molds, and yeasts (Jeon et al., 2002). Moreover, CHI may provide an alternative to antimicrobial growth promoters in the diets of ruminants, as suggested byGoiri et al. (2009). Benefits observed in vitro seem to be caused by changes in ruminal fermentation, in particular by increased propionate proportion and decreased methane production.Therefore, the objective of this study was undertaken to investigate the effect of levels of nitrate, extract chitosan or shrimp shell meal on difference roughage and concentrate ratio diets.

Materials and methods

Briefly, shrimp shell from black tiger shrimp (*Penaeus monodon*) were provided from the local market. The shrimp shell meal was prepared by grinded the sun-dried shrimp waste. The chitosan was obtained from treated fresh shrimp shell by demineralization, deproteination, and deacetylation steps according to the process provided by Toan (2009).

This study was conducted using an *in vitro* gas fermentation technique at various incubation time intervals. Two, 1-year-old, rumen fistulated dairy steers with an initial BW of 250 ± 15 kg were used as rumen fluid donor. Steers were fed with R:C at 60:40 (14% CP and 78.6% TDN, dry matter basis). The experiment design was a 2x7 factorial arrangement in CRD. Factor A was 2 levels of R:C ratio (60:40, 40:60) and factor B was 7 kinds and levels of additives (NON: non supplementation; Low-Ni: supplementation with 7 mg of KNO3; High-Ni: supplementation with 14 mg of KNO3; Low-Chi: supplementation with 4.8 mg of extract chitosan; Low-SSM: supplementation with 4.8 mg of shrimp shell meal and High-SSM: supplementation with 24 mg of shrimp shell meal). Substrates and additives were milled to a 1-mm screen, and weighed (total substrate mixture 200 mg of DM) into 50 ml bottles for various time incubations. The method used for in vitro fermentation based on the technique described by Menke et al. (1979). The gas production was recorded at 0, 2, 4, 6, 8, 12, 24, 36, 48, and 72 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and Mc Donald (1979). The fermented contents were sampled at 12 and 24 h after incubation for analyses of in vitro digestibility according to Tilley and Terry (1963). Ruminal pH was measured and protozoa population was determined by direct count technique described by (Galyean, 1989).

Results and discussion

Effects of Nitrate, extract chitosan and shrimp shell meal on *in vitro* gas production

Cumulative gas production for each of substrate treatments in each time of measurement is presented as gas production curves and kinetics of gas production are given in Table 1. Increasing levels of concentrate affected on cumulative gas production at 96 hours after incubation (P<0.001), gas production from the immediately soluble fraction (a) (P<0.001), gas production from the insoluble fraction (b) (P<0.001), gas production rate (c) (P>0.05) and the potential extent of gas production (a+b) (P<0.001). These findings suggest that the inclusion of high proportion of concentrate results in an increasing rate and extent of fermentation of the inoculums. These results agreed with Lunsin and Wanapat (2010); Anatasook and Wanapat (2012) who reported that cumulative gas production were higher when decreased R:C ratio from 70:30 to 30:70.

Feed additives supplementation were also impacted on cumulative gas production (P<0.05). Cumulative gas production was highest on treatment with Low-Chi and lowest at High-Ni addition. In the rumen, continuous microbial fermentation is contingent on the reoxidation of cofactors generated when organic matter is fermented to volatile fatty acids (VFA) with the synthesis of microbial cells. Nitrate can replace carbon dioxide as an electron acceptor with the generation of another reduced product in this such as nitrate is reduced to nitrite and then to ammonia (Leng, 2008). The reactions by which electrons are transferred to produce methane or ammonia, therefore hydrogen sinks in the rumen were trapped and resulted in lower rumen gas production.

Chitosan has the antimicrobial activity of being against different groups of microoganisms (Goiri et al., 2009; Benhabiles et al., 2012) which produce enzymes to hydrolyze the forage and that results in the reduction of the total gas production and decreased of IVDMD, IVDOM. The results of present study also in agreement when supplement high levels of chitosan and shrimp shell meal on *in vitro* fermentation.

Effects of nitrate, extracted chitosan and shrimp shell meal on in vitro degradability, pH and protozoa population.

The effects of nitrate, extracted chitosan and shrimp shell meal on *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), pH, and protozoa population in diets with difference R:C ratios were shown on table 2. The incubated R:C ratio diets significantly affected IVDMD (P<0.001), IVDMD (P<0.001), pH (P<0.001) and protozoa population (P<0.001). Feed additives were also significantly reduced IVDMD (P<0.01), IVODM (P<0.001), and protozoa population but did not influence on pH parameter.

The*in vitro*degradability of both DM and OM increased with an increasing level of concentrate. This could be due to the high soluble carbohydrate contained in the treatments at the high level of concentrate ratio. Results of the current experiment agreed with the finding of Arriola et al. (2011) who found that when the dairy cows were fed lower F:C ratios diets, the digestibility of dry matter increased and Anatasook and Wanapat (2012) who reported that the*in vitro*degradability of both DM and OM increased with an increasing level of concentrate. A diet with increasing of the R:C ratios could increase the pH (Aguerre et al., 2011). In the present study, the high R:C ratio had higher rumen pH than low R:C ratios diet. Similarity, The R:C ratio affected the protozoal population which was lower in a R:C of 60:40. This observation is supported byCherdthong et al. (2010)who found that protozoal populations linearly increased with an increasing level of concentrate.

Marais et al. (1988) reported that nitrate or its reduced product temporarily present in the digesta would decrease ruminal dry mater digestibility. Dai et al. (2010) also showed that ruminal IVDMD changed in a quadratic fashion with the rise of nitrate-N addition level. Similarity, the present study shown that nitrate supplement resulted on IVDMD and IVOMD reduction.

In general, in the current *in vitro* trials, chitosan reduced IVDMD and IVOMD. This could be in agreement with the chitosans' previously described antimicrobial action (Jeon et al., 2002). The mechanism of the antimicrobial activity of chitosan polymers has not yet been fully elucidated, but the most feasible hypothesis is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces (Fang et al., 1994)

Conclusion

The feed additives evaluated in the current experiment can impact on *in vitro* gas production, nutrient degradability, protozoa population. This study is an important reference for further research on animal performances.

Acknowledgments

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KEYWORD : nitrate, chitosan, shrimp shell meal, rumen degradability

Table 1. Effects of nitrate, extract chitosan and shrimp shell meal on *in vitro* gas production

R:C ratio	Supplementations	Gas kinetic				Cumulative gas (mL) produced at 96h
		a(mL)	b (mL)	c (mL/h)	a+b (mL)	
60:40	Non	0.7	60.5	0.044	61.2	61.6
	Low-Ni	-0.4	57.0	0.048	56.6	60.0
	High-Ni	1.2	59.4	0.032	60.6	58.1
	Low-Chi	1.4	61.9	0.044	63.4	63.7
	High-Chi	1.6	59.8	0.044	61.4	61.8
	Low-SSM	2.2	57.9	0.039	60.1	59.3
	High-SSM	0.5	61.3	0.041	61.9	61.0
40:60	Non	0.8	68.4	0.073	69.2	71.9
	Low-Ni	-1.3	69.1	0.067	67.8	69.7
	High-Ni	-0.1	65.8	0.061	65.7	67.3
	Low-Chi	-0.6	68.5	0.071	67.9	69.9
	High-Chi	-0.3	67.8	0.067	67.5	68.7
	Low-SSM	-0.9	67.8	0.064	66.9	68.5
	High-SSM	1.0	67.1	0.060	68.2	69.0
SEM		0.06	0.25	0.0003	0.24	0.17
Comparison						
Ratio		***	***	***	***	***
Supplementation		*	ns	***	ns	*
Ratio*Supplementation		*	ns	ns	ns	ns

Non = Control; Ni = KNO_3; Chi = Chitosan; SSM = shrimp shell meal; ns = non-significant; * P<0.05; *** P<0.001; SEM = standard error of the mean

Table 2: Effects of nitrate, extracted chitosan and shrimp shell meal on *in vitro* degradability, pH and protozoa population

R:C ratio	Supplementations	IVDMD (%)	IVOMD (%)	pH	Protozoa (10^5 copies/mL)
60:40	Non	62.71	70.13	6.78	2.5
	Low-Ni	62.68	70.84	6.79	2.0
	High-Ni	62.65	68.11	6.69	1.5
	Low-Chi	61.18	67.65	6.70	2.0
	High-Chi	57.24	66.67	6.71	1.5
	Low-SSM	61.64	65.99	6.73	1.7
	High-SSM	58.71	63.53	6.74	1.7
40:60	Non	70.54	83.72	6.69	5.0
	Low-Ni	69.49	81.93	6.68	3.7
	High-Ni	69.6	80.15	6.70	3.0
	Low-Chi	68.47	77.72	6.67	3.7
	High-Chi	65.09	75.40	6.68	2.7
	Low-SSM	68.20	75.86	6.68	3.0
	High-SSM	65.37	74.40	6.65	2.2
SEM		0.12	0.11	0.002	0.03
Comparison					
Ratio		***	***	***	***
Supplementation		**	***	ns	***
Ratio*Supplementation		ns	ns	ns	ns

Non = Control; Ni = KNO_3; Chi = Chitosan; SSM = shrimp shell meal; ns = non-significant; ** P<0.01; *** P<0.001; IVDMD: *in vitro* dry matter degradability; IVOMD: *in vitro* organic matter degradability; SEM = standard error of the mean

REFFERENCES

Anantasook, N and Wanapat M., 2012. Influence or rain tree pod meal supplementation on rice straw based diets using in vitro gas fermentation technique. Asian-Aust.J.Anim.Sci. 25, (3): 325-334.

AOAC, 1990. Official Methods of Analyses, 15th edn. Assoc. Offic. Anal. Chem, Arlington, VA.

Boadi, D., C. Benchaar, J. Chiquette, and D. Masse. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can. J. Anim. Sci. 84: 319-335.

Benchaar, C., and D. Greahead. 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166-167: 338-355.

Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci. 146:213–233.

Galyen, M., 1989. Laboratory Procedures in Animal Nutrition Research. New Mexico State University.

Goiri, I., A. Garcia-Rodriguez, and L. M. Oregui. 2009. Effect of chitosans on in vitro rumen digestion and fermentation of maize silage. Anim. Feed Sci. Technol. 148:276–287.

Jeon, Y.-I., J. Y. V. A. Kamil, and F. Shahidi. 2002. Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. J. Agric. Food Chem. 20:5167–5178.

Joblin, K. N. 1999. Ruminal acetogens and their potential to lower ruminant methane emissions. Aust. J. Agric. Res. 50:1307–1314.

Johnson, K. A., and D. E Johnson. 1995. Methane emissions from cattle. J. Anim. Sci.73: 2483-2492.

Leng R. A. 2008. The potential of feeding nitrate to reduce enteric methane production in ruminants. A Report to The Departmernt of Climate Change Commonwealth Government of Australia. ACT Canberra Australia. http://www.penambulbooks.com.

Lusin, R., and M. Wanapat. 2010. Effect of roughage to concentrate ratio and rice bran oil supplementation on rumen fermentation characteristics using in vitro gas production technique. 14th AAAP conference at Pingtung university, Taiwan.

McAllister, T. A., and C. J. Newbold. 2008. Redirecting rumen fermentation to reduce methanogenesis. Aust. J. Exp. Agric. 48:7– 13.

Menke, K. H., L. Raab, H. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuff from the gas production when they were incubated with rumen liquor in vitro. J. Agric. Sci. 93: 217–222.

Ørskov, E. R., and I. Mcdonald. 1979. The estimation of protein degradability in the rumen from incubation measurement weighted according to rate of passage. J. Agric. Sci. (Camb.) 92: 499-503.

Sar, C., B. Mwenya, B. Pen, K. Takaura, R. Morikawa, A. Tsujimoto, K. Kuwaki, N. Isogai, I. Shinzato, Y. Asakura, Y. Toride, and J. Takahashi. 2005. Effect of ruminal administration of Escherichia coli wild type or a genetically modifi ed strain with enhanced high nitrite reductase activity on methane emission and nitrate toxicity in nitrate-infused sheep. Br. J. Nutr. 94:691–697.

Sar, C., B. Santoso, B. Mwenya, Y. Gamo, T. Kobayashi, R. Morikawa, K. Kimura, H. Mizukoshi, and J. Takahashi. 2004. Manipulation of rumen methanogenesis by the combination of nitrate with β 1-4 galacto-oligosaccharides or nisin in sheep. Anim. Feed Sci. Technol. 115:129–142.

SAS, 1998. User's Guide: Statistic, Version 6, 12th Edition. SAS Inst. Inc., Cary, NC.

Takahashi, J., and B. A. Young. 1991. Prophylactic effect of L-cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. Anim. Feed Sci. Technol. 35:105–113.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the digestion of forage crops. J. Br. Grassland Soc. 18: 104-111.

Toan N. V. (2009). Production of chitin and chitosan from partially autolyzed shrimp shell materials. The Open Biomaterial Journal, 1, 21-24.

Wanapat, M., P. Kongmun, O. Poungchompu, A. Cherdthong, P. Khejornsart, R. Pilajun, and S. Kaenpakdee. 2012. Effects of plants containing secondary compounds and plant oils on rumen fermentation and ecology. Trop. Anim. Health. Prod. 44: 399–405.

O-11-6

METAGENOMIC ANALYSES OF RUMEN FIBROLYTIC BACTERIA SWAMP BUFFALO

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INTRODUCTION

The world buffalo (*Bubalus bubalis*) population is estimated at 168 million. Of that, 95.83% (161 million) are found in Asia while the remaining comes from Africa, Egypt, South America, Australia and Europe. The research on rumen microorganisms in swamp buffalo has been neglected and not much emphasis has been done on this species. Traditionally the buffalo has been a draught animal pulling ploughs in the rice fields, and lately transporting oil palm fruits in difficult terrain in oil palm plantation. The buffalo is commonly slaughtered for its meat. In fact, in some regions in Malaysia, the buffalo meat is more cherished by consumers than beef from cattle. The buffalo meat has been shown to be high in protein, low in fat and is palatable.

The swamp buffalo feed on a greater range of food than cattle. They tend to eat dry weeds along with aquatic plants. Surveys of the 16S rRNA gene diversity showed that microorganisms in the swamp buffalo rumen were diverse than those in cattle rumen (Wanapat, 2010). More, specifically, water buffalo rumen contains more bacteria and fungus, but lower amounts of protozoa. However, a greater proportion of the rumen microorganism was unable to be cultured.

Characterization of the bacteria genes that cannot be cultured remains a significant challenge. Genomics, the study of the entire genome of an organism, uses DNA isolated from pure cultures of the microbe. Such a procedure is not possible with microbes that cannot be cultured, thus making traditional genomic analyses of as many as 40% of microbes in the rumen impossible. However, recent advances in genomics of bacteria in water and soil have confirmed the utility of metagenomics, a technique of studying the genomes of all microbes, regardless of their ability to be cultured, using a whole-genome shotgun sequencing approach. Metagenomics is a cost-effective, culture independent approach to identify microbes and analyze microbial genomes. Metagenomics treats the microbial community as a single dynamic entity. It explores the genome content of the community and leads to analysis of changes in content and expression as a function of site, time, and various states of perturbation, e.g., progression towards and regression from disease following treatment. To our knowledge, the study using metagenomics analysis to reveal total rumen bacterial population in the swamp buffalo is not available publicly. Many studies were focus on cattle (Ross, *et al*., 2012) and wild ruminants (Pope *et al*., 2012; Dai *et al*., 2012). Hence, this study aims at improving the knowledge on the rumen bacterial community of the swamp buffalo associated with low quality forages.

METHODOLOGY

The sampling site of rumen fluid was conducted at Seri Menanti, Negeri Sembilan, Malaysia. Rumen contents (RC) and rumen fluid (RF) were collected from five swamp buffaloes free grazing at the area of paddy field. Prior to sequencing, all RC and RF were underwent DNA extraction using QIAamp Fast DNA Stool Kit (QIAGEN, USA) by following the manufacturer instruction. Each sample of RC and RF DNA were extracted in five replicates and the extracts were subsequently pooled. Each DNA concentration and quality was determined by using Qubit 2.0 flourometer. While the band size (approximately 1.5 kb) were analysed on 0.8% agarose gel electropherosis.

The V3 and V4 hypervariable region was amplified from DNA. PCR reaction were carried out an initial denaturation step at 95℃ for 3 minutes followed by 25 cycles of 95℃ for 30 seconds, 55℃ for 25 seconds and 72 ℃ for 30 seconds and final extension program step at 72 ℃ for 5 minutes. The PCR product were analysed on 1.5% agarose gel and bands of desired size were purified. DNA quality and concentration were checked using spectrophotometer. The amplicons were normalized, pooled and sequenced on the Illumina MiSeq desktop sequencer at the UPM Institute of Bioscience.

Illumina reads were firstly analysed by adapter remover using Paried-End Adapter Trimmer, PEAT v1.2 to improve cluster identification. A threshold of phred quality score Q20 of the base were chose and filter for merged fragments and salvage food quality of unpaired read 1 that is longer than 100bp. Hence, any contigs with ambiguous base and longer than 200 bp were culled. To detect putative chimeric sequences in the filtered data, sequences were subjected to USEARCH uchime_ref chimera check against RDP_GOLD version 9 with default

parameters. Representatives assigned as chimeras propagate the assignment across their clusters and a single list of all putative chimeras is output. All chimeric sequences were removed from consideration before the next step in the pipeline. Open reference OTU picking method in Qiime version 1.8 was used. It is due to its ability to detect reference based collection and novel diversity. The input reads undergone another pre-filtering process by searching reads against the reference set with a low percent identity threshold to discard sequences that were not representatives of the targeted marker gene. Sequences were then clustered using UCLUST version 1.2.22 in parallel by a closed-reference OTU picking workflow, where sequences are queried against the reference database at percent identify 97%. If a read matches a references sequence at greater than or equal to 97% identity, it is assigned to the OTU defined by that reference sequence.

Next, a random subsample of 0.1% of the sequences that failed to match the reference sequence collection are clustered *de novo*, and the cluster centroids for all resulting OTUs are used to define a new reference sequence collection. These OTUs are referred to as new reference OTUs. The sequences that were not included in the random subsample that was clustered *de novo* then go through an additional process of parallel closed-reference OTU picking, where they are clustered against the new reference OTUs based on matching a sequence in the new reference OTUs based on matching a sequence in the new reference sequence collection at greater or equal to 97% identity. This creation of a new reference database allows us to harness the parallelization of closed-reference OUT picking pipeline, greatly decreasing the time it takes to sequence that failed to hit the initial reference database to be clustered into OTUs. In the final clustering step, sequences that were failed to hit a reference sequence during final closed-reference OTU picking step were clustered to *de novo* which referred as clean-up OTUs. Finally, the reference OTUs, new reference OTUs and clean-up OTUs are combined into a single OTU table. This table is a filtered table excluding OTUs with counts less than or equal to a threshold 5. By threshold 5, each OTU is observed at least 5 times before being qualified.

RESULTS AND DISCUSSION

Initially there are 510436 of paired-end reads. Upon FASTQ screening, a total of 434498 reads had passed the criteria of Q20 phred quality score and sequence bigger than 100bp. After FASTA pre-processing, a number of 19902 reads has been flagged as chimeric leaving out 414596 reads for downstream analysis. The analysis has gone through Open Reference OTU picking where the output of reads is 304146, while the number of observations is 3889 and table of density is 1.0.

Rarefraction curve is the basis for calculating diversity metrics which reflected the diversity within the sample based on the abundance of various taxa within a community. The rarefraction curve generated from the OTU suggested that high sampling coverage was achieved in sample. Two observed non phylogenetic approach parameters were used are Chao1 (Figure 1) and observed species (Figure 2). Hence, a steady increase in species richness was found in this sample.

Comparison of significant presence of the phylum proportions was common in microbial ecology research (Ellis *et al*., 2013). Overall, there were 16 identified phyla in the sample. Among all phylum categories, taxa within the Bacteroidetes were the most dominant at 40% and followed by Firmicutes as the second dominant at 38.9%. A distinct feature was present by Fibrobacteres with relative abundance of 5.4%. A further observation of taxa in the swamp buffalo rumen was carried out at family level. Total of 50 identified family and the most dominant family was shown by Prevotellaceae (19%) derived from Bacteroidales order. While second dominant family is shown by Ruminococcaceae which derived from Clostridiales order and Firmicutes phylum. *Prevotella* was the most abundant genera (19%) (figure 3) in a total of 37 identified genus which plays significant role in utilize carbohydrate (Herbert, 1992) and also metabolism of protein and peptides in the rumen (Wallace *et al.*, 2007). This followed by genera *Fibrobacter* (5%) and *Ruminococcus* (4%). According to Hungate (1966), *F. succinogenes* and the *Ruminococcus* spp are mostly limited to cellulose and its hydrolytic products (i.e. cellobiose and glucose) as growth substrates.

CONCLUSION

Based on the data obtained, general outline of this microbiota at higher taxonomic level analyses has shown that the same two phyla which are Bacteroidetes and Firmicutes were the most abundant within the bacterial community. This concludes that genes related to fibrous degrading were expressed by the microbiome.

KEYWORD : Metagenomics,, Swamp buffalo,, Fibrolytic bacteria, Genera

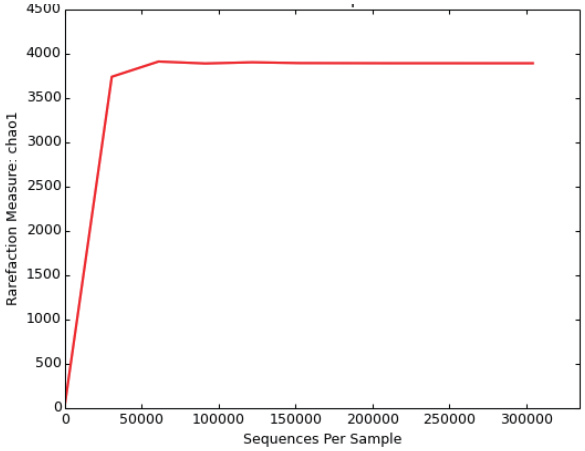

Figure 1: Rarefraction curve of sample at 97% sequences identity.

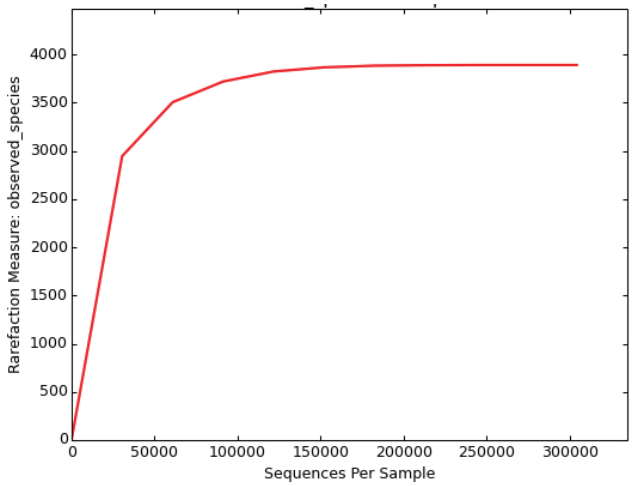

Figure 2: Observed species shows count of unique OTUs found in the sample.

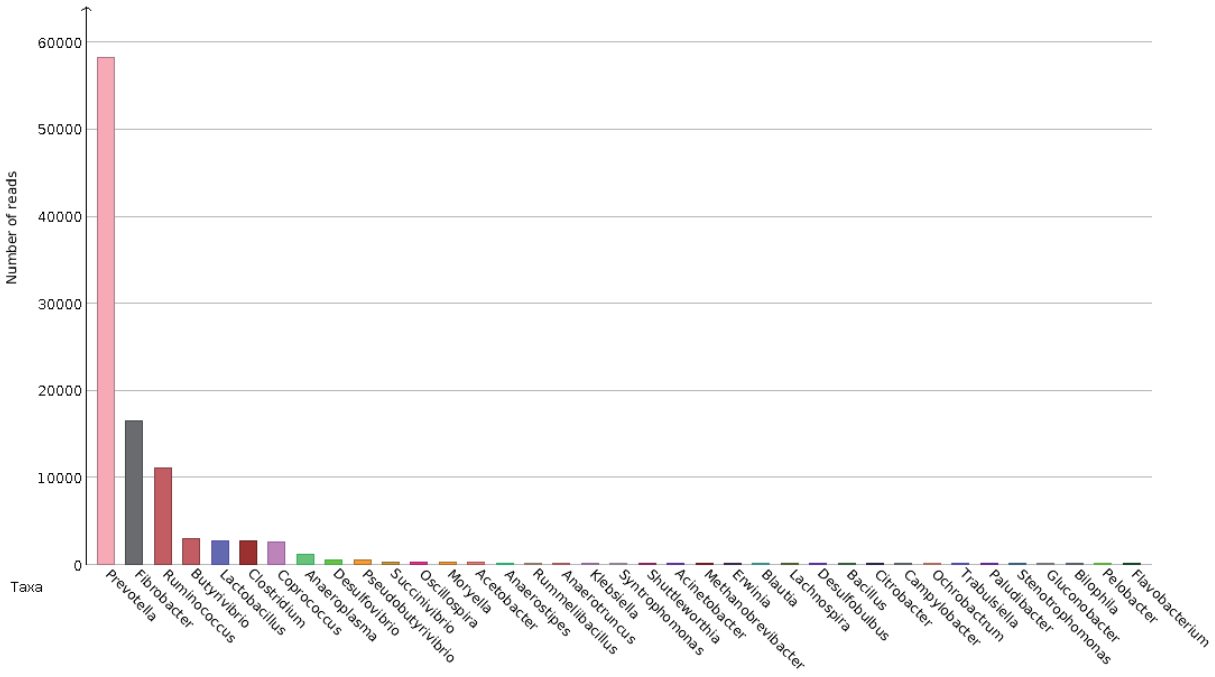

Figure 3: Bacteria distribution at genus level.

REFFERENCES

Dai, X, Zu, Y, Luo, Y, Song, L, Liu, D et al., (2012). Metagenomic insight into the fibrolytic microbiome in Yak rumen. PLos ONE 7(7): e40430. Doi: 10.17371/journal.pone.0040430

Ellis, JL, Dijkstra, J, Kebreab, E, Bannink, A, Odongo, NE, McBride, BW & France, J 2008, 'Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle', *Journal of Agricultural Science,* vol. 146, pp. 213-33.

Herbert J, S,.1992. Vitamin B12-Dependent Propionate Production by the Ruminal Bacterium *Prevotella ruminicola* 23. Applied And Environmental Microbiology, p. 2331-2333.

Hungate, RE 1966, in *The rumen and its microbe*, Academic Press, New York, p. 533.

Pope, PB, McKenzie, AK, Gregor, I, Smith,W, Sundset, MA, et al., (2012). Metagenomics of the Svalbard Reindeer rumen microbiome reveals abundance of polysaccharide utilization loci. PLoS ONE 7(6):e38571. doi: 10.1371/journal.pone.0038571.

Ross, EM, Moate, PJ, Bath, CR, Davidson, SE, Sawbridge, TI, et al., (2010). High throughput whole rumen metagenome profiling using untargeted massively parallel sequencing. BMC Genetics 2012, 13:53.

Wallace R, J, McKain N, Broderick G,A, Rode L,M, Walker N,D , Newbold C,J, Kopecny, J. 2007, Peptidases of the Rumen Bacterium, *Prevotella ruminicola*. Anaerob Volume 3, Issue 1, Elsvier Ltd. p. 35-42.

Wanapat, M (2010). Current researches towards rumen fermentation and microbial ecology of swamp buffaloes. Proceeding of the International Conference on Biochemistry and Medical Chemistry, Feb 23-25, Cambridge, UK, pp:431-435.

O-11-8

Bacterial communities of milk and cow shed samples in relation to changes of the somatic cell count

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Objective

Mastitis in dairy cows as one of the most serious diseases in today's dairy production, has always been the focus of attention and exploration by researchers all over the world. With the development of science and technology, more and more pathogenic bacteria were found with mastitis. In addition, many non-pathogenic bacteria are also common in milk. However, there is no culture independent data to study the sources of bacteria in milk, it is necessary to determine whether there is a certain relationship between the bacteria and somatic cells in milk. Therefore, our experiments are devoted to examine environmental impact on microbiota of dairy cow milk in relation to changes in the somatic cell count.

Methodology

Milk samples were collected from the dairy cow herd of Okayama Livestock Research Institute. All cows were managed by automatic milking systems (Astronout A4, Liley) and were offered total mixed ration silage for a whole year. Likewise, environmental samples, i.e. aerosol, tap water, feed, feces, bedding, and udder skin, were collected to compare microbiota with manually collected milk one. Bacterial community was examined by DGGE and total population was quantified by real time PCR. Principal coordinate analysis was employed to describe similarity and diversity of microbiota.

Results

Automatic milking system collected milk total bacterial population was linearly correlated with SCC, whereas typical contagious mastitis-related pathogens were not found in any milk samples. Many bacteria are common in milk, i.e. *Pseudomonas fluoresces*, *Enterococcus casseliflavus*, *Acinetobacter lwoffii, Pseudomonas putida*, and *Propionibacterium acnes*. Although milk microbiota differed between sampling times, no significant differences were seen between cow's milk collected at the same time. However, no bacterial species detected in the DGGE analysis were suggested to be related to the SCC. Microbiota of milk resembled with those of udder skin, aerosol, and tap water, but appeared unrelated with those of feed, feces, and bedding. In addition, the bacteria on the skin of the nipple does not have obvious similarities with the bedding, feces and feed bacteria flora.

Conclusion

Milk bacteria flora are most similar to the udder skin , the bacterial population in milk may cause changes in the milk somatic cell count BReasonable and effective cleaning nipple skin may have a positive effect on prevention of mastitis.

KEYWORD：SCC, Bacterial communities, environmental

O-12-1 The effects of dietary spirulina (Spirulina platensis) on growth performance, skin color and nutritional digestibility in Thai native chicken

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INTRODUCTION

Thai native chicken meat is preferred by the consumers according to the good taste and low cholesterol (Jutarasitha, 2008). Pradu Hangdum Chiangmai is one of the Thai native chickens which very famous in northern part of Thailand. They are generally raised under free range and in farm system by the small famers. The consumers in northern part of Thailand preferred the yellow skin of Pradu Hangdum Chiangmai. Skin color is the one of important factors for consumer decision.

Spirulina is the one of natural pigmenting source in poultry (Tasaki, 2003; Mariey *et al*., 2012) addition to protein source in poultry (Ross and Dominy, 1990) and fish feed (Lu and Takeuchi, 2004; Ungsethaphand *et al*., 2010). In Thailand, the information about the using of spirulina in Thainative chicken feed was not found. Therefore, the spirulina were use as feed additive to improve product performance. The effects of spirulina on the growth performance, skin color and nutritional digestibility were observed.

MATERIALS AND METHODS

Animals and treatment

The 240 two-week-old chicks (Pradu Hangdum) were randomly assigned to 5 experimental groups (Completely randomized design; CRD), 4 replicates of 12 chicks. In group 1, the chicks were fed control diet without spirulina (0%). In groups 2, 3, 4 and 5 the chicks were fed the diet containing spirulina at 0.25, 0.50, 0.75 and 1.00% respectively. The chicks were fed *ad libitum* with experimental diets and had free access to water for 12 weeks. Feed intake and body weight gain were measured weekly for 12 weeks period. At the end of experimental period, twelve birds from each group were humanely slaughtered for carcass observation and twelve birds from each group were separated for digestibility observation.

Statistics

All data were statistically analyzed using one-way ANOVA, and significant differences among the treatments were determined with Duncan's multiple range test using the SPSS (SPSS Inc., Chicago, USA) software program, with 95 % confidence.

RESULTS

Growth performance and carcass quality: After feeding the experimental diets for 12 weeks, feed intake, body weight gain and feed conversion ratio were not significantly different (P> 0.05) (Table 1). The carcass qualities of all experimental groups were not significantly different (P> 0.05) (Table 2) excepted that the skeleton frame in the supplementation with spirulina at the level of 0.25 and 0.50% groups were higher (P< 0.05) when compare with control.

Skin color: The lightness of the 0.50% spirulina group was lowest (P<0.05) (Table 3). The redness did not show a significant difference (P>0.05) among the groups but the yellowness of the 0.75% spirulina group was higher (P<0.05) than those of the others groups excepted that of 1.00% spirulina group.

Nutritional digestibility: The spirulina was not affected on fat and phosphorus digestibility but decreased in the digestibility of dry matter, crude fiber and ash in the 0.05, 0.75 and 1.00 % spirulina groups (P<0.05). The crude protein digestibility was decreased in 1.00% spirulina group (P<0.05); and calcium digestibility were decreased in 0.50 and 1.00% spirulina groups (P<0.05). (Table 4).

DISCUSSION

The using of spirulina in Pradu Hangdum diet at the levels of 0.25-1.00% could not enhance growth performance, carcass quality and nutrient digestibility these results might due to Pradu Hangdum strain has been improved for raising in a farm system and local condition and under free range system. Pradu Hangdum had the good product performance even they were fed by fermented diets (Verrarux *et al*., 2013) or crops residues (Chawut, 2015) as

the main ingredient of the feed. However, spirulina at the level of 0.75% dramatically increased the yellowness of the skin in which meet the consumer preference. The same phenomena were observed in laying hen fed 0.2-0.8% spirulina, yolk color were increased (Zahroojian et al., 2011). According to that spirulina contain the carotenoid (Devanathan and Ramanathan, 2012) in which absorb from the intestine to blood system (Yonekura and Nagao, 2007) and deposit in fat tissue under the skin (Na *et al.*, 2004). Therefore, it is profitable to use spirulina to improve the skin color of Pradu Hangdum.

KEYWORD : Spirulina, Thai native chicken, Growth performance

Table 1 The effects of spirulina on growth performance from 2-14 weeks of age (n = 48)

Spirulina (%)	Feed intake (g/bird/week)	Weight gain (g/bird/week)	FCR
0	307.87	75.38	4.08
0.25	295.81	67.71	4.47
0.50	293.16	69.88	4.21
0.75	314.6	72.39	4.33
1.00	286.41	68.97	4.22

Table 2 The effects of fermented feeds on carcass quality (% live weight) (n = 12)

Items	Spirulina (%)				
	0 .00	0.25	0.50	0.75	1.00
Live weight (g)	1105.00	1071.67	1119.17	1127.50	1089.17
Carcass yield	80.22	80.10	79.48	76.41	70.28
Wings	9.73	9.73	9.76	9.59	9.40
Drumstrick	10.85	10.54	10.63	10.28	9.41
Thighs	10.54^{ab}	10.73^{a}	11.03^{a}	11.27^{a}	9.48^{b}
Pectoralis Major	8.88	9.24	9.58	8.88	8.02
Pectoralis Minor	3.10	3.13	3.23	3.26	2.85
Skeletal frame	17.05^{c}	19.71^{a}	19.56^{ab}	17.19^{abc}	15.94^{bc}
Shanks and feet	4.85	4.88	4.65	4.97	4.47
Head and neck	8.68	8.74	8.35	8.69	7.93
Liver	2.36	2.41	2.26	2.40	2.40
Gizzard	4.12	3.78	4.15	4.31	4.06
Heart	0.90	0.58	0.58	0.60	0.53
Spleen	0.48	0.53	0.48	0.51	0.53

$^{a-c}$Means followed by different letters are significantly different ($P < 0.05$)

Table 3 The effects of spirulina on skin color

Spirulina (%)	L*(lightness)	a*(redness)	b*(yellowness)
0	64.30^{bc}	3.27	7.30^{b}
0.25	65.39^{ab}	3.65	4.42^{c}
0.50	65.96^{a}	3.51	3.56^{c}
0.75	63.38^{c}	3.26	10.76^{a}
1.00	64.95^{ab}	3.11	9.45^{ab}

$^{a-c}$Means followed by different letters are significantly different ($P < 0.05$)

Table 4 The effects of spirulina on nutritional digestibility (%)

Items	Spirulina (%)				
	0	0.25	0.50	0.75	1.00
Dry matter	82.09^{ab}	82.87^{a}	75.65^{c}	78.18^{bc}	70.99^{d}
Crude protein	68.16^{a}	63.27^{a}	57.98^{a}	60.11^{a}	47.29^{b}
Crude fiber	49.50^{a}	48.97^{a}	12.05^{c}	29.97^{b}	14.14^{c}
Ash	48.35^{a}	51.22^{a}	31.37^{bc}	40.57^{b}	24.52^{c}
Ether extract	85.88	86.78	83.00	84.18	78.45
Calcium	72.42^{a}	71.87^{a}	60.80^{b}	75.16^{a}	57.50^{b}
Phosphorus	93.45	93.08	91.74	92.34	88.47

$^{a-d}$Means followed by different letters are significantly different ($P < 0.05$)

REFERENCES

Chawut, T. 2015. Study of cost structure of farmers raising Chiang Mai Pradu-Hangdum chicken as supplementary livelihood integrated with agriculture based on royal philosophy of economic sufficiency in Ban Nam-Sup community, Phetchaburi Province. Khon Kaen Agri. J. 43: 65-69.

Devanthan, J. and N. Ramanathan. 2012. Pigment production from Spirulina platesis using seawater supplemented with dry poultry manure. J. Algal Biomass Utln. 3: 66-73.

Jaturasitha, S., T. Srikanchai, M. Kreuzer and M. Wicke. 2008. Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (black-bone and Thai native) and imported extensive breeds (Bresse and Rhode Island Red). Poult. Sci. 87: 160-169.

Lu, J. and T. Takeuchi. 2004. Spawning and egg quality of the tilapia Oreochromis nilotcus fed solely on raw Spirulina throughout three generations. Aquaculture. 234: 625-640.

Mariey, Y. A., H. R. Samak and M. A. Ibrahem. 2012. Effect of using spirulina platensis algae as a feed additive for poultry diets: 1- productive and reproductive performances of local laying hens. Egypt. Poult. Sci. 32: 201-215.

Na, J.C., J.Y. Song, B.D. Lee, S.J. Lee, C.Y. Lee and G.H. An. 2004. Effect of polarity on absorption and accumulation of carotenoids by laying hens. Anim. Feed Sci. and Technol. 117: 305-315.

Ross, E. and W. Dominy. 1990. The nutritional value of dehydrated, blue-green algae (Spirulinaplatensis) for poultry. Poult. Sci., 69: 794-800.

Takashi, S. 2003. Effect of administration of Spirulina on egg quality and egg componests. Anim. Husb. 57: 191-195.

Ungsethaphand, T., Y. Peerapornpistal, N. Whangchai and U. Sardsud. 2010. Effect of feeding Spirulina platensis on growth and carcass composition of hybrid red tilapia (Oreochromis x O. niloticus). Maejo Int. J. Sci. Technol. 4: 331-336.

Verarux, N., S. Pipatpongchai and S. Kiratikrankul. 2013. Production performance and cost of Thai Black Tail (Pradu Hangdum) chicken fed different fermented ingredient and raised in natural system by Maehongson farmers. Proceeding of the 4th meat science and technology 19 July 2013, Bangkok, Thailand. 8-15

Yonekura, L. and A. Nagao. 2007. Intestinal absorption of dietary carotenoids. Mol. Nutr. Food Res. 51: 107-115.

Zahroojian, N., H. Moravej and M. Shivazad, 2011. Comparision of marine algae (spirulina platensis) and synthe tic pigment in enhancing egg yolk colour of laying hen. Br. J. Poult. Sci. 52: 584-588.

O-12-2 Effect of Palm Oil and Free Fatty Acid Substitution in Diets on Growth Performance and Carcass Yield of Broilers

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Abstract

This study was conducted to evaluate the effects of palm oil and Free Fatty Acids (FFAs) substitution in diet on growth performance and carcass yield of broilers. A total of 900 male broiler chicks were divided into 5 groups with 6 replicates of 30 birds each. The birds received a control diet using palm oil as dietary oil supplementation and other groups were substituted with 25, 50, 75 and 100 % of FFAs for 35 days. At the end of feeding trial, the results indicated that 50% FFAs substitution not influenced the body weight when compared to the control group, however, substitution FFAs at 25, 75 and 100% in diets significantly decreased the body weight ($P<0.01$). In the other hand, the dietary treatments not influenced the feed intake, FCR and carcass yield of broilers. It is believed that FFAs is useful as dietary oil supplementation up to 50% substitution of palm oil without the negative effect the growth performance and carcass yield of broilers.

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INTRODUCTION

Fats are an excellent source of energy, and their inclusion in the diet is the main method of increasing the energy content of poultry diets (Yacowitz, 1953; Sunde, 1954). Several fat sources are available for poultry, such as animal fats (e.g., lard and tallow) and vegetable oils (e.g., palm oil and soybean oil). Dietary fats and oils, consisting mainly of triacylgecerols, are relatively large molecules and cannot be absorbed intact in the small intestines. The triacylgecerols must be broken down (hydrolyzed) by lipase and colipase, both of which are enzymes produced in the pancreas. The result of the hydrolysis is two free fatty acids and one 2-monoacylglycerol, which can be absorbed by the small intestines.

Free Fatty acid (FFA) is a fatty acid which is part of triacylglycerol with no glycerol. Fatty acids play a number of key roles in metabolism - major metabolic fuel (storage and transport of energy). The digestion and absorption of fat is a complex process requiring amounts of bile salts, lipase and colipase. Overall digestion and absorption was higher in triglyceride-fed chicks than chicks receiving fatty acids and the poorer fat absorption observed on feeding free fatty acids instead of triglycerides is partially explained by less efficient micellarization (Sklan, 1978). Van Kuiken and Behnke (1994) reported that the activity of lipase can be inhibited by FFAs. Lack of any one of these enzyme will impair the digestion and absorption processes. Therefore, the objectives of this study were to evaluate the effect of palm oil and free fatty acid substitution in diets on growth performance and carcass yield of broilers.

MATERIALS AND METHODS

This study was conducted at Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Animals and management

A total of 900 one-day-old male ROSS 308 broiler chicks were used in this trial. Chickens were randomly divided into five treatments and each treatment consisted of six pens (thirty birds per pen). Feed and water were provided *ad libitum*, with water supplied by nipple drinkers. All chicks were inoculated with Newcastle disease vaccine and inactivated infectious bursal disease vaccine on day 10.

Experimental diets

The experimental diets (Table 1 and 2) were satisfying the nutritional requirements described in strain's and based on corn and soybean meal. Five dietary treatments were provided to chickens for 35 days as follows; basal diet (control), and substitution of palm oil by 25, 50, 75, 100 % FFAs, respectively.

The specifications of FFAs used in the experiment was liquid substance, brown in color, 99% minimum of crude fat, 1% maximum of moisture, 70% minimum of free fatty acids, 80 minimum of iodine value, 30°C of melting point and fatty acid composition: 18% palmitic acid, 5% stearic acid, 60% oleic acid, 14% linoleic acid and 3% others.

Parameters

Growth performance: The initial body weight of each chick was recorded at the beginning of feeding trial (day 1). The body weight, body weight gain and feed intake were measured on the feed switching time basis which means evaluating on 10, 24 and 35 days in order to calculation of average daily gain and feed conversion ratio and the mortality of broilers were observed.

Carcass quality: At the end of finisher periods (35 days), 2 birds per replicate (showing closest to the average body weight in the pen) were selected in order to determine the carcass quality as following; live weight, slaughter weight, carcass weight, inner breast weight, outer breast weight, thigh weight, drumstick weight, wing weight and abdominal fat.

Statistical analysis

Analysis of variance was conducted using pen means. All statistical analyses were performed by ANOVA using SAS statistical programs (SAS Institute, 1996). Differences among treatments were determined with Duncan's multiple range test (Duncan, 1955). Probability values less than 0.05 were considered statistically significant. Then the data were analyzed by regression analysis based on the data that was in response to the increasing levels of FFAs in linear and quadratic equation to predict the levels of FFAs.

RESULTS AND DISCUSSIONS

Growth performance

The growth performances of animals are shown in Table 3. All animals remained in good health throughout the trial. The substitution of FFAs in 25, 75 and 100 % results in significantly decreased the body weight ($P<0.01$) of finisher period and overall trial (35 days) when compared with control group and linearly increased the FCR of the overall trial. Substitution of FFAs linearly increased the feed intake and FCR in starter period. However the feed intakes of the overall trial (35 days) were not significantly influenced by the FFAs.

The results of this study indicated that FFAs was affected bird performance negatively, which is in agreement with the report of Pesti *et al*. (2002). The body weight of broilers fed diets containing FFAs in finisher and overall performance were significantly lower than those of birds fed with control diet, suggesting that the energy in the diets with a higher FFAs content was not utilized as efficiently as the energy in diets with a lower FFAs content (Wu *et al*., 2011). Shannon (1979) and Sklan (1979) reported that the absorption of fatty acids was higher in chicks fed triglycerides than in chicks fed FFAs. Their results suggested a need for glycerides for the efficient solubilization and absorption of FFAs. The cause of lower body weight could be that the diets with higher FFAs levels were associated with some chemical, physical, or physiological characteristic that reduced palatability and feed intake (Rossell, 1994).

There were many factors shown to affect the utilization of fats such as the age of the chick (Whitehead and Fisher, 1975; Wiseman and Salvador, 1989), the degree of saturation of fatty acids (Renner and Hill, 1961a, b; Young, 1961; Young and Garrett, 1963; Garrett and Young, 1975; Ketels and Groote, 1989), and the chain length of the fatty acid (Renner and Hill, 1961a), as well as positional effects of fatty acids on the triglyceride molecule (Renner and Hill, 1961b). Therefore, in this study, the cause of lower body weight may due to the high levels of FFAs and the high ration of U: S.

Carcass quality

Substitution of FFAs linearly reduced slaughter weight, carcass weight and outer breast weight (Table 4) and there were no significant difference in the other carcass yields (Inner breast weight, tight weight, wing weight, drumstick weight abdominal fat weight and liver weight). Peebles *et al*. (1999) reported that birds fed with less saturated fatty acid in diet improved slaughter yield. Since the carcass quality was effected by fatty acids profile, therefore, the carcass quality had no significant different in broilers fed with FFAs.

CONCLUSION

In conclusion, substitution FFAs at 25, 75 and 100% in diets had a negative effect to the body weight. However 50% FFAs substitution not influenced the body weight when compared to the control group. The carcass quality was not effected by FFAs.

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KEYWORD：Free fatty acid, Growth performance, Carcass yield, Broiler

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Table 1 Experimental diet composition

Ingredient name	Starter	Grower	Finisher
Corn Thai	55.01	56.39	62.35
Palm oil	3.48	5.06	4.74
Soybean meal (48% CP)	36.07	34.00	28.72
L-Lysine HCL 78%	0.23	0.08	0.08
DL-Methionine	0.33	0.25	0.21
L-Threonine	0.08	0.01	0.01
Monodicalciumphosphate21	2.19	1.92	1.77
Calcium carbonate	1.51	1.25	1.22
Salt	0.30	0.30	0.30
PX[a] Broiler+Antioxidant+ Anticoxidial+Mold inhibitor	0.55	0.53	0.40
Choline Chloride 75%	0.25	0.23	0.22
Total	100.00	100.00	100.00

[a]Premix

Table 2 Calculated nutrient content of experimental diet

Nutrients	Unit	Starter	Grower	Finisher
ME. for Poultry	Cal/Kg.	3,025	3,150	3,200
Protein	%	22.10	21.00	19.00
Fat	%	5.75	7.32	7.17
Fiber	%	3.54	3.45	3.28
Calcium	%	1.05	0.90	0.85
Total Phosphorus	%	0.85	0.78	0.73
Avail. P for Poultry	%	0.50	0.45	0.42
Salt	%	0.32	0.32	0.32
Arginine	%	1.39	1.33	1.18
Isoleucine	%	0.94	0.90	0.79
Lysine	%	1.27	1.10	0.97
Met+Cys	%	0.94	0.84	0.76
Methionine	%	0.63	0.54	0.48
Threonine	%	0.83	0.73	0.65
Tryptophan	%	0.24	0.23	0.20
Valine	%	0.95	0.91	0.82

Table 3. Effect of free fatty acids on growth performance of broilers.

Item	Free fatty acids					P-Value	SEM	Contrast, P<	
	0%	25%	50%	75%	100%			Linear	Quadratic
Starter (d 1 to 10)									
BW (g)	344.41	345.6	342.94	345.37	347.64	0.77	1.15	0.44	0.45
FI (g/b/d)	34.97	35.01	35.36	36.11	36.68	0.15	0.26	0.01	0.48
BWG (g)	297.77	298.96	296.31	298.75	301.02	0.83	1.21	0.49	0.50
ADG	29.78	29.90	29.63	29.88	30.10	0.82	0.12	0.49	0.50
FCR	1.17	1.17	1.19	1.21	1.22	0.27	0.01	0.03	0.80
Grower (d 11 to 24)									
BW (g)	1375.36	1350.59	1351.61	1358.09	1347.17	0.24	4.29	0.11	0.36
FI (g/b/d)	99.11	96.34	97.75	97.75	97.80	0.40	0.43	0.69	0.25
BWG (g)	1030.84	1005.09	1009.08	1012.73	999.53	0.25	4.58	0.09	0.51
ADG	73.63	71.79	72.08	72.34	71.40	0.25	0.33	0.09	0.51
FCR	1.35^{b}	1.34^{b}	1.36^{ab}	1.35^{b}	1.37^{a}	<0.01	0	<0.01	0.26
Finisher (d 25 to 35)									
BW (g)	2432.14^{a}	2382.63^{b}	2405.32^{ba}	2364.68^{b}	2363.6^{b}	0.01	7.44	0.003	0.59
FI (g/b/d)	165.36	163.33	162.94	161.75	163.25	0.69	0.75	0.30	0.34
BWG (g)	1057.27	1032.14	1057.07	1006.62	1016.57	0.51	11.14	0.19	0.96
ADG	96.11	93.83	96.10	91.51	92.42	0.51	1.01	0.19	0.96
FCR	1.72	1.74	1.70	1.77	1.77	0.50	0.01	0.23	0.63
Overall (d 1 to 35)									
BW (g)	2432.14^{a}	2382.63^{b}	2405.32^{ba}	2364.68^{b}	2363.6^{b}	0.01	7.44	0.003	0.59
FI (g/b)	3556.23	3495.46	3514.36	3508.79	3531.69	0.50	11.15	0.66	0.14
BWG (g)	2385.87	2336.19	2362.46	2318.10	2317.13	0.40	13.01	0.10	0.81
FCR	1.49	1.50	1.49	1.52	1.53	0.21	0.01	0.04	0.47

a,b,c,d Means within a row with different letters differ highly significant (P<0.05).

Table 4. Effect of free fatty acids on carcass quality of 35 days old broilers.

Item (g)	Free fatty acids					P-Value	SEM	Contrast, P<	
	0%	25%	50%	75%	100%			Linear	Quadratic
Live weight	2369.17	2309.25	2321.67	2287	2290.75	0.25	12.75	0.05	0.45
Slaughter weight	2205.08	2152.5	2163.92	2115.92	2119.83	0.11	12.04	0.02	0.57
Carcass weight	1935.5	1890.42	1901.83	1855.17	1848.75	0.09	11.41	0.01	0.83
Inner breast weight	82.67	84.08	84.5	83.42	80.83	0.82	1.01	0.57	0.29
Outer breast weight	420.75	418.33	406.67	395	398.42	0.20	4.21	0.03	0.73
Thigh weight	290.83	279.5	291.17	279.67	277	0.28	2.69	0.15	0.79
Drumstick weight	227.25	221.75	228.42	221.08	222.42	0.78	2.19	0.53	0.99
Wing weight	174.17	171.83	174.83	162.92	166.83	0.20	1.87	0.08	0.87
Abdominal fat weight	48.77	46.43	44.81	48.77	45.72	0.76	1.14	0.66	0.68
Liver	46.82	45.01	45.8	43.76	45.71	0.68	0.65	0.47	0.41

REFFERENCES

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1–42.

Garrett, R. L., and R. J. Young. 1975. Effect of micelle formation on the absorption of neutral and fatty acids by the chicken. J. Nutr. 105: 827–838.

Ketels, E., and G. D. Groote. 1989. Effect of ratio of unsaturated to saturated fatty acids of the dietary lipid fraction on utilization and metabolizable energy of added fats in young chicks. Poult. Sci. 68: 1506–1512.

Peebles, E. D., S. M. Doyle, T. Pansky, P. D. Gerard, M. A. Latour, C. R. Boyle, and T. W. Smith. 1999. Effect of breeder age and dietary fat on subsequent broiler performance and slaughter yield. Poult. Sci. 78: 512–515.

Pesti, G. M., R. I. Bakalli, M. Qiao, and K. G. Sterling. 2002. A comparison of eight grades of fat as broiler feed ingredients. Poult. Sci. 81: 382–390.

Renner, R., and F. W. Hill. 1961a. Utilization of fatty acids by the chicken. J. Nutr. 74: 259–264.

Renner, R., and F. W. Hill. 1961b. Factors affecting the absorbability of saturated fatty acids in the chicks. J. Nutr. 74: 254–258.

Rossell, J. B. 1994. Measurement of rancidity. Pages 22–53 in Rancidity in Foods. 3rd ed. J. C. Allen and R. J. Hamilton, ed. Chapman and Hall, London, UK.

SAS Institute. 1996. SAS User's Guide: Statistics. Version 8 ed. SAS Inst. Inc., Cary, NC.

Shannon, D. W. F. 1979. The effect of level of intake and free fatty acid content on the metabolizable energy value and net absorption of tallow by the laying hen. J. Agric. Sci. (Camb.). 75: 217– 221.

Sklan, D. 1978. Digestion and Absorption of Lipids in Chicks Fed Triglycerides or Free Fatty Acids: Synthesis of Monoglycerides in the Intestine. Poult. Sci. 58 (4): 885-889.

Sklan, D. 1979. Digestion and absorption of lipids in chicks fed triglycerides or free fatty acids: Synthesis of monoglycerides in the intestine. Poult. Sci. 58: 885–889.

Sunde, M. L. 1954. Use of animal fats in poultry feeds. I. Am. Oil Chem. Soc. 31: 49–52.

Van Kuiken, B. A. and Behnke, W. D. 1994. The activation of porcine pancreatic lipase by cis-unsaturated fatty acids. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 1214: 148-160.

Whitehead, C. C., and C. Fisher. 1975. The utilization of various fats by turkeys of different ages. Br. Poult. Sci. 16: 481–485.

Wiseman, J., and F. Salvador. 1989. The effect of age and rate of inclusion on the apparent metabolisable energy values and apparent fat availability. Br. Poult. Sci. 30: 653–662.

Wiseman, J., and F. Salvador. 1990. The Influence of Free Fatty Acid Content and Degree of Saturation on the Apparent Metabolizable Energy Value of Fats Fed to Broilers. Poult. Sci. 70: 573-582.

Wu, H., L. M. Gong , L. Guo , L. Y. Zhang, and J. T. Li. 2011. Effects of the free fatty acid content in yellow grease on performance, carcass characteristics, and serum lipids in broilers. Poult. Sci. 90: 1992–1998.

Yacowitz, H. 1953. Supplementation of corn-soybean oil rations with penicillin and various fats. Poult. Sci. 32: 930. (Abstr.)

Young, R. J. 1961. The energy value of fats and fatty acids for chicks. I. Metabolizable energy. Poult. Sci. 40: 1225–1233.

Young, R. J., and R. L. Garrett. 1963. Effect of oleic and linoleic acids on the absorption of saturated fatty acids in the chicks. J. Nutr. 81: 321–329

O-12-6

Comparative the Dietary Protein and Energy Basis in Feed Formulation on Production Performance and Carcass Yield of broilers

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INTRODUCTION

Poultry feeds are often formulated to meet nutrient requirements. Formulation optimization can also be achieved by maximizing the benefits of the whole production system, including both feed and animal production, which needs the investigation of formulations with deficient or excessive nutrient levels (Jackson et al., 1982; Pesti and Fletcher, 1983). In comparison with ME, NE is a more precise measure for energy availability for growth performance. It is the part of the dietary energy which is available to the animal for maintenance and production. Feed formulated on the basis of NE, as opposed to that formulated on AME, resulted in savings of over 80 g feed per kg live weight over a rearing period of 35 d in broiler chickens (Choct, 2005). A previous experiment conducted with 30 varying diets to measure and predict the AMEn (Carr'e *et al*., 2013) and net energy (NE) values of diets (Carr'e *et al*.,2014) in broilers supplied a great amount of data that represented a unique opportunity to investigate these relationships. The current study shows the predictions of growth performance and body composition from the feed composition(Smith *et al*., 1998). In parts of protein requirement of chickens is, in fact, a requirement for amino acids (AA; NRC, 1994; Leeson and Summers, 2001). Therefore, the use of digestible AA for feed formulation has been recognized as being preferable to formulating diets on a total AA basis (Rostagno *et al*., 1995). The reduction excess dietary amino acids via decreasing dietary crude protein (CP) and meet the amino acid needs of the bird more accurately. A method to determine which amino acids are limiting in dietary formulation is the ideal amino acid concept (Emmer and Baker, 1997) It is known that the CP and amino acid (AA) status of a diet influences the carcass composition of broilers (Si *et al*., 2001). Therefore, the present study was planned to comparative the dietary protein and energy basis in feed formulation on production performance and carcass yield of broilers.

MATERIALS AND METHODS

This study was conducted at Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Birds and Management

A total of 450 one-day-old Ross 308 broiler male chicks, weighing an average of 43.06 g, were randomly distributed in four treatments with six replicate pens (25 birds per pen). Chickens were reared and housed in battery brooders in a room with continuous fluorescent lighting. From d 1 to 35, chickens had ad libitum access to conventional corn-SBM diets and water, as recommended by Ross 308 manual.

Experimental diets

The experimental diets (Table 1 and 2) based on corn and soybean meal. Four dietary treatments were provided to chickens for 35 days as follows. There were 3 dietary treatments as follows; 1) Control diet (conventional diet) using Metabolizable Energy (ME) and Crude Protein (CP) basis or ME&CP, 2) Net Energy (NE) and CP basis or NE&CP, 3) NE and protein as essential amino acids basis or **NE&AA.**

Parameters

Production performance: The initial body weight of each chick was recorded at the beginning of feeding trial (day 1). The body weight, body weight gain and feed intake were measured on the feed switching time basis which means evaluating on 35 days in order to calculation of average daily gain, average daily feed intake and feed conversion ratio and the mortality of broilers were observed.

Carcass quality: At the end of finisher periods (35 days), 2 birds per replicate (showing closest to the average body weight in the pen) were selected in order to determination of carcass quality as following; live weight, slaughter

weight, carcass weight, inner breast weight, outer breast weight, thigh weight, drumstick weight, wing weight and abdominal fat.

Statistical analysis

Analysis of variance was conducted using pen means. All statistical analyses were performed by ANOVA using SAS statistical programs (SAS Institute, 1996). Differences among treatments were determined with Duncan's multiple range test (Duncan, 1955). Probability values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Production performance

In this study show that the BW, BWG and ADG of the control group were significantly higher than the other groups whereas the FI was significantly higher than Net Energy (NE) and CP basis but had no different when compared with NE and protein as essential amino acids basis. This is consistent with Griffiths *et al.* (1977), who suggested that a reduction in energy intake is due to physical limitations, because birds cannot eat more to compensate for reduced energy density of the diet during early age. As the birds become larger, the physical constraint on the amount of feed intake is decreased. While there was no different between control group and Net Energy (NE) and CP basis in FCR and significantly lower than NE and protein as essential amino acids basis. Mortality was not significantly different among treatments overall experimental periods (Table 3). The results of this study indicated that energy and protein was affected bird performance negatively, which is in agreement with the report of Hidalgo *et al.* (2004), who found that birds fed the lowest dietary regimen had increased their feed intake during the finisher and overall experimental period. Weight gain and FCR were severely depressed during all the growth periods. The birds provided low-CP and low-ME diets had increased feed consumption, but this increase could not compensate for the reduced growth and did not allow for complete recovery of final BW. The difference in rate and efficiency of growth probably occurred due to poor efficiency of utilization of ME and CP, although critical AA were according to the requirements (Kamran *et al*., 2008). This observation was in agreement with reports by Golian and Maurice (1992) and Leeson et al. (1993), who reported that birds consume feed to primarily meet their energy requirements. Therefore, in this study, the cause of lower body weight, body weight gain, ADG and FCR may due to the reduce levels of energy and protein.

Carcass Yield

Concentrations of energy and protein did not alter yield of the carcass, outer breast weight, inner breast weight, wing weight, thigh weight, and drumstick weight but significantly increased the abdominal fat (Table 3). The increased accumulation of abdominal fat in birds fed low-protein diets was the only consistent observation reported in these studies. Nevertheless, the preponderance of information suggests that the rate and efficiency of growth is reduced, and carcass composition is inferior when the dietary CP level is reduced by more than 3%, even when all known nutrient requirements are met (Aletor *et al.,* 2000; Bregendahl *et al*., 2002; Sterling *et al.,* 2005; Waldroup *et al.,* 2005). Similarly, Leeson *et al*. (1996) reported no differences in carcass and breast fillet weights in broilers fed gradient concentrations of ME. However, Dozier and Moran (2001; 2002) reported feeding broilers diets formulated to contain sub optimum concentrations of CP and ME impaired the amount and yield of carcass parts and the dimensions of breast fillets (Dozier and Moran. 2002).

CONCLUSION

The growth performance of broilers in this study show that the BW, BWG and ADG of the control group were significantly higher than the other groups whereas the FI was significantly higher than Net Energy (NE) and CP basis but had no different when compared with NE and protein as essential amino acids basis. While there was no different between control group and Net Energy (NE) and CP basis in FCR and significantly lower than NE and protein as essential amino acids basis. And this study found that there were no different in the carcass yield of broilers but NE and protein as essential amino acids basis led to the higher abdominal fat than control group and Net Energy (NE) and CP basis.

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throughout this trial.

KEYWORD : Protein, Amino acid, Energy, Broiler

Table 1 Experimental diet composition

Ingredient name	starter			grower			finisher		
	ME&CP	NE&CP	NE&AA	ME&CP	NE&CP	NE&AA	ME&CP	NE&CP	NE&AA
Corn	55.07	55.12	56.64	57.96	58.14	61.55	62.47	62.77	65.67
Palm oil	2.80	2.75	2.50	3.77	3.62	3.057	4.48	4.23	3.75
SBM 48%	37.64	37.63	36.27	34.25	34.21	31.17	29.34	29.28	26.70
L-Lysine	0.19	0.19	0.23	0.13	0.13	0.23	0.14	0.14	0.22
DL-Methionine	0.32	0.32	0.33	0.27	0.27	0.30	0.24	0.24	0.27
L-Threonine	0.09	0.09	0.12	0.05	0.05	0.09	0.04	0.04	0.07
MCP 21%	1.68	1.68	1.69	1.49	1.49	1.51	1.34	1.34	1.35
Calcium carbonate	1.34	1.34	1.34	1.22	1.22	1.23	1.12	1.13	1.14
Salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Choline Chorine 60%	0.28	0.283	0.28	0.27	0.27	0.27	0.25	0.25	0.25
Premix[1]	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Total	100	100	100	100	100	100	100	100	100

[1] Premix 1 kg containing : vitamin A 4 MIU, vitamin D 0.64 MIU, vitamin 24,000 IU, vitamin K 1.4 g, vitamin B1 0.6 g, vitamin B2 0.3 g, vitamin B6 0.75 g, vitamin B12 14 mg, nicotinic acid 20 g, pantothenic acid 10 g, folic acid 0.44 g, biotin 0.04 g, choline 60 g, iron 45 g, copper 40 g, manganese 15 g, zinc 40 g, cobalt 0.2 g, iodine 0.4 g, selenium 0.06 g, carrier added to 1.00 kg.

Table 2 Calculated nutrient content of experimental diet

Nutrients	starter			grower			finisher		
	ME&CP	NE&CP	NE&AA	ME&CP	NE&CP	NE&AA	ME&CP	NE&CP	NE&AA
ME. for Poultry (kcal/Kg)	3,000	-	-	3,100	-	-	3,200	-	-
NE. for Poultry (kcal/Kg)	-	2,289.99	2,289.99	-	2,366.63	2,366.63	-	2,443.27	2,443.27
Protein (%)	23	23	22.533	21.50	21.50	20.46	19.50	19.50	18.61
Fat (%)	5.29	5.25	5.04	6.32	6.18	5.70	7.12	7.88	6.47
Fiber (%)	2.56	2.56	2.54	2.47	2.47	2.40	2.33	2.34	2.28
Calcium (%)	0.96	0.96	0.96	0.87	0.87	0.87	0.79	0.79	0.79
Total Phosphorus (%)	0.73	0.73	0.73	0.68	0.68	0.68	0.62	0.62	0.62
Avail. P for Poultry (%)	0.48	0.48	0.48	0.44	0.44	0.44	0.40	0.40	0.40
Salt (%)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Arg (%)	1.44	1.44	1.41	1.34	1.34	1.25	1.20	1.20	1.20
Ile (%)	0.90	0.90	0.88	0.84	0.84	0.79	0.76	0.76	0.71
Leu (%)	1.81	1.81	1.76	1.72	1.72	1.65	1.60	1.60	1.54
Lys (%)	1.28	1.28	1.28	1.15	1.15	1.15	1.03	1.03	1.03
Met+Cys (%)	0.95	0.95	0.95	0.87	0.87	0.87	0.80	0.80	0.80
Met (%)	0.63	0.63	0.63	0.56	0.56	0.58	0.52	0.52	0.53
Thr (%)	0.86	0.86	0.86	0.77	0.77	0.77	0.70	0.70	0.70
Trp (%)	0.25	0.25	0.24	0.23	0.23	0.22	0.21	0.21	0.20
Val (%)	0.98	0.98	0.96	0.92	0.92	0.87	0.84	0.84	0.79

Table 3 The dietary protein and energy basis in feed formulation on growth performance of broilers. (1-35 days)

Item	ME&CP	NE&CP	NE&AA	P-Value	SEM
Starting body weight (g)	43.06±0.10	43.05±0.09	43.05±0.08	0.99	0.02
Final body weight (g)	2588.45±64.93^{a}	2515.22±35.04^{b}	2526.36±30.15^{b}	0.03	12.84
FI (g/b)	3671.55±109.38^{a}	3544.35±45.21^{b}	3646.54±55.08^{a}	0.02	21.37
BWG (g/b)	2545.39±65.01^{a}	2472.16±35.07^{b}	2483.30±27.51^{b}	0.03	12.85
ADG (g/b/d)	72.73±1.86^{a}	70.63±1.00^{b}	70.95±0.86^{b}	0.03	0.37
FCR	1.44±0.01^{b}	1.43±0.03^{b}	1.47±0.02^{a}	0.03	0.01
Mortality (%)	2.06±2.26	2.03±3.41	1.39±2.15	0.89	0.60

$^{a, b, c}$ Meanwithin a row with different letters differ highly significant (P<0.05).

Table 4 The dietary protein and energy basis in feed formulation on carcass yield of broiler 35 days

Item	ME&CP	NE&CP	NE&AA	P-Value	SEM
Carcass weight	2041.08±71.72	2010.75±27.65	2013.25±34.59	0.50	11.28
Outer breast weight	524.67±35.83	521.75±10.56	494.17±10.76	0.06	5.98
Inner breast weight	92.33±5.28	88.75±3.49	91.25±3.74	0.35	1.01
Wing weight	185.42±10.15	178.17±9.75	174.33±5.39	0.11	2.23
Thigh weight	287.83±5.33	287.75±18.06	295.17±15.51	0.59	3.23
Drumstick weight	220.67±5.87	220.00±11.15	225.42±9.46	0.54	2.10
Abdominal fat weight	41.07±8.70 [b]	37.52±3.57 [b]	51.68±5.76 [a]	0.00	2.03

[a, b] Meanwithin a row with different letters differ highly significant (P<0.05).

REFERENCES

Aletor, V. A., I. I. Hamid, E. Nieb, and E. Pfeffer. 2000. Low protein amino acid-supplemented diets in broiler chickens: Effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. J. Sci. Food Agric. 80:547–554.

Bregendahl, K., J. L. Sell, and D. R. Zimmerman. 2002. Effect of low protein diets on growth performance and body composition of broiler chicks. Poult. Sci. 81:1156–1167.

Carré, B., M. Lessire, and H. Juin. 2013. Prediction of metabolisable energy value of broiler diets and water excretion, from dietary chemical analyses. Animal 7:1246–1258.

Carré, B., M. Lessire, and H. Juin. 2014. Prediction of the net energy value of broiler diets. Animal. 8:1395–1401.

Choct, M. 2005. The Net Energy Value for Poultry Diets of Commonly Used Feed in Australia (Kingston, ACT, Research Rural Industries Research and Development Corporation).

Dozier, W. A., III, and E. T. Moran, Jr. 2001. Response of early- and late-developing broilers to nutritionally adequate and restrictive feeding regimens during the summer. J. Appl. Poult. Res. 10:92–98.

Dozier, W. A., III, and E. T. Moran, Jr. 2002. Dimension and light reflectance of broiler breast fillets: Influence of strain, sex, and feeding regimen. J. Appl. Poult. Res. 11:202–208.

Emmer4 J.L andD.H. Baker, 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poultry Res. 6462-470.

Golian, A., and D. V. Maurice. 1992. Dietary poultry fat and gastrointestinal transit time of feed and fat utilization in broiler chickens. Poult. Sci. 71:1357–1363.

Griffiths, L., S. Leeson, and J. D. Summers. 1977. Influence of energy system and level of various fat sources on performance and carcass composition of broilers. Poult. Sci. 56:1018–1026.

Hidalgo, M. A., W. A. Dozier III, A. J. Davis, and R. W. Gordon. 2004. Live performance and meat yield responses to progressive concentrations of dietary energy maintained at a constant metabolizable energy-to-crude protein ratio. J. Appl. Poult. Res. 13:319–327.

Jackson, S., J. D. Summers, and S. Leeson. 1982. Effect of dietary protein and energy on broiler performance and production costs. Poult. Sci. 61:2232–2240.

Kamran, Z., M. Sarwar, M. Nisa, M. Nadeem, S. Mahmood, M. Babar and S. Ahmed. 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poultry science. 87 (3): 468-474.

Leeson, S., and J. D. Summers. 2001. Scott's nutrition of the chicken. 4th ed. Univ. Books, Guelph, Ontario, Canada.

Leeson, S., J. D. Summers, and L. Caston. 1993. Growth response of immature brown-egg strain pullet to varying nutrient density and lysine. Poult. Sci. 72:1349–1358.

Leeson, S., L. Caston and J. Summers. 1996. Broiler response to energy or energy and protein dilution in the finisher diet. Poultry Science. 75 (4): 522-528.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Pesti, G. M., and D. L Fletcher. 1983. The response of male broiler chickens to diets with various protein and energy contents during the growing phase. Br. Poult. Sci. 24:91–99.

Rostagno, H. S., J. M. R. Pupa, and M. Pack. 1995. Diet formulation for broilers based on total versus digestible amino acids. J. Appl. Poult. Res. 4:293–299.

Si, J., C. A. Fritts, D. J. Burnham, and P. W. Waldroup. 2001. Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. Poult. Sci. 80:1472–1479.

Smith, E. R., and G. M. Pesti. 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. Poult. Sci. 77:276–281.

Sterling, K. G., D. V. Vedenov, G. M. Pesti, and R. I. Bakalli. 2005. Economically optimal dietary crude protein and lysine levels for starting broiler chicks. Poult. Sci. 84:29–36.

Waldroup, P. W., Q. Jiang, and C. A. Fritts. 2005. Effects of glycine and threonine supplementation on performance of broiler chicks fed diets low in crude protein. Int. J. Poult. Sci. 4:250–257.

O-12-7

Effect of Nutrient Density and Phytase Supplementation in Diets on Growth Performance, Carcass Yield and Bone Condition of Broilers

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ABSTRACT

The study was carried out to determine the effect of nutrient density andphytase supplementation in diets on growth performance, carcass yield and bone condition of broilers. A total of 10 days old 144male broilers (Ross 308) were divided into 3 groups and each group consisted of 4 replicates of 12 birds each. Birds fed a control diet or low nutrient density diet (phosphorus, calcium, protein, amino acids and dietary energy) with or without 500 FTU/kg phytase supplementation for 23 days. The results indicated that at the end of the experiment, the dietary treatments not influenced the feed intake, body weight and feed conversion ratio of broilers ($P>0.05$). Phytase increased outer breast meat ($P<0.05$) but the dietary treatments not influenced the others carcass yield of birds. Low dietary nutrient density significantly decreased tibia ash ($P<0.05$) but phytase supplementation improved tibia ash of birds similar to those fed a control diet. It could be concluded that phytase supplementation in low nutrient density diet improved the carcass yield and bone condition of broilers.

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INTRODUCTION

The increasing broiler production around the world has led to the application of nutritional strategies that improve nutrient utilization. The supplementation of exogenous enzymes to corn, soybean meal-based broiler diets allows supplying nutrient deficiencies and to reduce endogenous losses, thereby optimizing performance. Phytase is an enzyme that acts in the bonds of the phosphate group of phytate, releasing phosphorus and other minerals that are part of this molecule (Cromwell & Coffey, 1991). But the monogastric animals like poultry are unable to utilize this phytate phosphorus, as they lack endogenous phytase, and this results in the addition of inorganic feed phosphates to the poultry diets in order to meet the phosphorus requirements of poultry (Yu *et al*., 2004). The dietary supplementation of the exogenous phytase reduces nutrient variability of feedstuffs, and counteracts the antinutritional effects of phytate, increasing the accuracy of feed formulation.

Exogenous phytase is included in feed formulations not only to reduce phosphorus supplementation, but also to release minerals, particularly calcium, as well as amino acids and carbohydrates by the hydrolysis of phytate, improving nutrient utilization (Oluyinka*et al*., 2007; Slominski, 2011).Research has shown that the performance of broilers fed with different levels of inclusion of phytase and low levels of available P in the diets can improve with the addition of the enzyme (Santos *et al*., 2005; Fukayama*et al*., 2008). However, other researchers did not observe beneficial effect on broiler performance with the addition of phytase in the diets (Assuena*et al*., 2007). This study was conducted to evaluate the effects of phytase supplementation in diets on growth performance, carcass yield and bone condition of broilers, then determine the feasible addition of phytase in the diets of broilers for optimizing the feed formula.

MATERIALS AND METHODS

This study was conducted at Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. The experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Animals and management

A total 10 days old144 male ROSS 308 broiler chicks were used in this trial.

Chickens were randomly divided into three treatments and each treatment consisted of four pens (twelvebirds per pen). Birds fed a control diet or low nutrient density diet (phosphorus, calcium, protein, amino acids and dietary energy) with or without 500 FTU/kg phytase supplementation for 23days (Table 1).The feeding periods

were grower phase (10 - 24 days) and finisher phase (25 - 33 days). Feed and water were provided ad libitum, with water supplied by nipple drinkers. All chicks were inoculated with Newcastle disease vaccine and inactivated infectious bursal disease vaccine on start trial.

Parameters

Growth performance: The initial body weight of each chick was recorded at the beginning of feeding trial. The body weight (BW) and feed intake (FI) were measured onthe feed switching time basis which means evaluating on 10 (initial) , 24 and 33 days of age in order to calculation of average daily gain (ADG) and feed conversion ratio (FCR) and the mortality of broilers were observed.

Carcass quality: At the end of finisher periods (33 days), 2 birds per replicate (showing closest to the average body weight in the pen) were selected in order to determine the carcass quality as following; live weight, carcass weight, inner breast weight, outerbreast weight.

Bone condition: At the end of the experiment (33 days), 2 birds per replicate.The left tibia bone was collected, following; tibia weight and tibia ash, according to Gardiner *et al*.,(1961)

Statistical analysis

Data were analyzed as a completely randomized design using the ANOVA procedures of SAS (Statistical Analysis System, Version 9.0, 2002). The model used was as follows:

$Yij = \mu + \tau i + \varepsilon ij$

where

Yij= dependent variable

μ = overall mean effect

τi= fixed effect of treatments

I = control, with and without 500 FTU/kg phytase supplementation

εij= residual experimental error with $N(0, \sigma^2)$

The significance of the differences between the treatment group means for each parameter was evaluated using the Duncan's New Multiple Range Test (DMRT). Probabilities of $P < 0.05$ and $P < 0.01$ were taken to indicate significant differences. All statistical analyses were computed in accordance with the method of Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Growth performance

The growth performances of birds are shown in Table 3. All animals remained in good health throughout the trial. During the experiment the mortality rate was not found. The results indicated that low nutrient diet (negative control) resulted in reduce growth performances ($P<0.05$). However, at the end experiment, the feed intakes were not sinfluenced by the phytase supplementation.Similarly, Santos *et al*. (2005) and Guilherme *et al*. (2012) observed that feed intake and weight gain of broilers fed diets with nutritional levels reduced without addition of phytase were inferior to those from the positive control group. But, when diets were supplemented with phytase, birds presented feed intake and weight gain similar to those of the positive control. Chisato *et al*. (2003) reported that chicks receiving diets with phytase showed greater weight gain, feed intake feed efficiency, energy, phosphorus and nitrogen.

Phytase is an enzyme that acts in the bonds of the phosphate group of phytate, releasing phosphorus and other mineral that are part of this molecule (Cromwell & Coffey, 1991).This way, besides increasing phosphorus availability, the use of phytase also improves the availability of other minerals,such as magnesium, manganese and copper. Thus, its usein diets for broilers may provide positive responses on thedigestibility of feeds and broiler performance, having adirect effect on productive efficiency.

Carcass quality

The effects of phytase enzyme supplementation on carcass quality are shown in Table 4. The parts investigated were inner breast weight and outer breast weight and there were no significant difference in the other carcass yields. But, in this experiment, the diets supplemented phytase of broilers could improve the eviscerated carcass ration and outer breast muscleration. It was possible that phytase was effective to eviscerate ration carcass ration and outer breast muscle ration.These results agrees with previous findings of Angel *et al*., (2007) but opposite to those of Pillai *et al*., (2006) who showed that phytase supplementation significantly increased percentages of most of carcass merits compared to phosphorus deficient diets.

Bone condition

Effect of nutrient density and phytase supplementation in diets on bone condition of broiler during grower and finisher periods are show in table 5. Phytase in increase total ash in tibia($p<0.01$) and tibia ash ($p<0.05$) of broilerswhen reduced calcium, phosphorus, amino acid, and Metabolizable energy levels in feed. It is believed that phytase increases the release of phosphorus and other minerals from the feedstuffs. According to Nelson & Walker (1964) and Pereira *et al*. (2012), bone ash content is the most efficient parameter to estimate the amount of phosphorus released by phytase in corn and soybean meal-based diets.

CONCLUSION

In conclusion, supplementation with 500 FTU/kg phytase in low nutrient density diet improved the carcass yield and bone condition of broilergrower and finisher periods.

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KEYWORD：Phytase, Bone, Broiler

Table 1 Experimental diet composition

Item	Grower (10-24 days)			Finisher (25-33 days)		
	Control	Treatment	Negative	Control	Treatment	Negative
Corn	56.290	59.416	59.419	61.658	64.785	64.788
Rice bran oil	4.840	3.328	3.328	4.790	3.278	3.278
Soybean 48%	34.322	33.293	33.294	29.275	28.245	28.247
L-Lysine	0.161	0.173	0.173	0.137	0.149	0.149
DL-Methionine	0.311	0.311	0.311	0.272	0.271	0.271
L-Threonine	0.044	0.040	0.404	0.028	0.025	0.025
MDCP21	1.912	1.318	1.318	1.763	1.169	1.169
Calcium carbonate	1.248	1.241	1.241	1.215	1.207	1.207
Salt	0.267	0.275	0.275	0.274	0.282	0.282
Choline Choride 60%	0.250	0.250	0.250	0.233	0.233	0.233
PX[a]	0.025	0.025	0.025	0.025	0.025	0.025
Corn cob/Phytase	0.005	0.005	0.005	0.005	0.005	0.005
Antioxidant	0.050	0.050	0.050	0.050	0.050	0.050
Anticoccidial	0.050	0.050	0.050	0.050	0.050	0.050
Mold inhibitor	0.050	0.050	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000	100.000	100.000

[a]Premix: Vitamin and mineral premix content (per kilogram of feed): VitaminA 12,000,000 IU, Vit D 3,000,000 IU, Vit E 15,000 IU, Vit K1,500 mg, Thiamine 1,500 mg, Riboflavin 5,000 mg, Pyridoxine2,000 mg, Niacin 25,000 mg, Vit B 504 mg, Pantothenic acid8,000 mg, Folic acid 3,000 mg, Biotin 120 mg, Choline chloride160 mg, Antioxidant 30 g, Manganese 80 g, Zinc 60 g, Iron 40 g,Copper 8 g, Iodine 0.50 g, Selenium 100 mg, Cobalt 100 mg

Table 2 Calculated nutrient content of experimental diet

Item	Grower (10-24 days)			Finisher (25-33 days)		
	Control	Treatment	Negative	Control	Treatment	Negative
ME. for Poultry	3,150.000	3,150.000	3,097.155	3,200.000	3,200.000	3,147.157
Protein	21.000	21.000	20.776	19.000	19.000	18.776
Fat	7.190	5.786	5.787	7.283	5.879	5.880
Fiber	3.467	3.483	3.483	3.298	3.314	3.315
Calcium	0.900	0.900	0.800	0.850	0.850	0.750
Total Phosphorus	0.783	0.658	0.658	0.729	0.605	0.605
Avail. P for Poultry	0.450	0.450	0.350	0.420	0.420	0.320
Salt	0.320	0.320	0.320	0.320	0.320	0.320
Arginine	1.378	1.356	1.356	1.230	1.208	1.208
Isoleucine	0.918	0.911	0.905	0.823	0.815	0.809
Lysine	1.240	0.120	1.228	1.090	1.090	1.078
Methionine + Cystine	0.950	0.950	0.946	0.860	0.860	0.856
Methionine	0.631	0.631	0.630	0.569	0.569	0.568
Threonine	0.830	0.830	0.817	0.740	0.740	0.727
Tryptophan	0.256	0.254	0.251	0.228	0.226	0.223
Valine	0.951	0.941	0.941	0.864	0.854	0.854
Choline	1,500.000	1,500.000	1,500.075	1,400.000	1,400.000	1,400.070

Table 3 Effect of nutrient density and phytase supplementation in diets on growth performance of broiler during grower and finisher periods

Item	Control	Treatment	Negative	P-Value	SEM
Grower					
Initial BW (g)	273.88±16.67	276.13±8.59	265.42±11.27	0.48	3.58
BW (g)	1334.06±24.04	1348.94±9.34	1294.75±52.97	0.11	11.24
ADG (g)	99.35±1.85	100.49±0.72	96.32±4.07	0.12	0.86
FI (g)	1506.93±73.66^{B}	1327.94±148.81^{B}	1283.13±54.93^{A}	0.03	39.31
FCR (g/)	1.42±0.05	1.24±0.15	1.25±0.06	0.06	0.04
Finisher					
BW (g/)	2616.11±43.91	2608.09±36.50	2522.60±137.01	0.28	25.76
ADG (g/)	257.36±4.39	256.55±3.65	248.01±13.70	0.28	2.58
FI (g/)	1988.11±242.69	2058.33±28.92	1979.46±102.15	0.73	41.33
FCR (g/)	1.55±0.21	1.64±0.02	1.61±0.06	0.64	0.03
Grower-Finisher					
Initial BW (g)	273.88±16.67	276.13±8.59	265.42±11.27	0.48	3.58
BW (g/)	2616.11±43.91	2608.09±36.50	2522.60±137.01	0.28	25.76
ADG (g/)	77.99±1.33	77.75±1.11	75.15±4.15	0.28	0.78
FI (g/)	3784.43±272.25	3693.85±153.02	3543.46141.59	0.27	59.75
FCR (g/)	1.49±0.12	1.45±0.07	1.45±0.06	0.73	0.02

Mean ±SD, A and B means with different superscripts in the same row are significantly different (P<0.05).

Table 4 Effect of nutrient density and phytase supplementation in diets on carcass yield of broiler during grower and finisher periods

Item	Control	Treatment	Negative	P-Value	SEM
Body weight (g)	2526.00±83.32	2390.25±310.91	2431.88±134.59	0.53	29.01
Carcass(g)	2067.38±69.67	2066.88±101.06	1996.00±122.47	0.48	23.84
Inner Breast(g)	476.00±45.88	473.63±33.35	488.67±54.07	0.40	78.06
Outer Breast(g)	97.50±4.34	103.00±7.52	97.50±8.85	0.40	3.90

Table 5 Effect of nutrient density and phytase supplementation in diets on bone conditionof broiler during grower and finisher periods

Item	Control	Treatment	Negative	P-Value	SEM
Tibia (g)	12.81±1.22	12.70±0.42	12.18±0.74	0.31	0.18
TibiaAsh %	22.99±24.32^{A}	22.45±0.62^{A}	21.90±0.62^{B}	0.04	0.20
Total ash in tibia (g)	294.19±24.32^{a}	285.14±13.49^{a}	285.14±13.49^{b}	<0.01	4.05

Mean ±SD, a and b means with different superscripts in the same row are significantly different (P<0.01).
Mean ±SD, A and B means with different superscripts in the same row are significantly different (P<0.05).

REFERENCES

Angel, R., Saylor, W. W., Mitchell, A. D., Powers, W., and Applegate, T. J., 2007. Effect of dietary phosphorrous, phytase, and 25-hydroxycholecalc ciferol on broiler chicken bone mineralization, nutrient utilization, and excreta quality of broiler chickens, Poult. Sci. 87: 1200-1211

Assuena, V., Junqueira, O.M., Casartelli, E.M. 2007.Efeito da adição de diferentesníveis da enzimafitasesobre o desempenho de frangos de corte. In: Reuniãoanual da sociedadebrasileiradezootecni. Jaboticabal.Anais... Jaboticabal: SociedadeBrasileira de Zootecnia. 586.

Chisato, Y., Hirokazu, F. and Hisao, T. 2003. Effect of amino acid, enzyme mixture and phytase added to low protein and low phosphorus diet on growth performance and excretion of nitrogen and phosphorus in broilers. J. Poult. Sci. 40: 114-120.

Cromwell, G.L. and Coffey, R.D. 1991. Phosphorus - A key essential nutrient, yet a possible major polluant - its central role in animal nutrition. In: Alltech's annual symposium of biotechnology in the feed industry, 7., Nicholasville. Proceeding Nicholasville: Alltech Technical Publications.133-145.

Fukayama, E.H. Sakomura, N.K. DOURADO, L.R.B. 2008.Efeito da suplementação de fitasesobre o desempenhoe a digestibilidade dos nutrientesemfrangos de corte. RevistaBrasileira de Zootecnia, 37: 629-635.

Guilherme R.L, Luiz F.T.A., Arele A.C, Fernando D.C.T., Horacio S.R., Anastácia M.D.A.C., Wagner A.D. A., Valdir R.J. 2012. Diet supplementation with phytase on performance of broiler chickens.R. Bras. Zootec. 41:929-933.

Nelson TS, Walker AC. 1964. The biological evaluation of phosphorus compounds: A summary. Poul. Sci. 43:94-98.

Oluyinka AO, Cowieson AJ, Adeola O. 2007. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. Brit. J. Nutri. 99:682-690.

Pereira R, Menten JFM, Romano GG, Silva CLS, Zavarize KC, Barbosa NAA. 2012. Eficiência de umafitasebacteriananaliberação de fósforofíticoemrações de frangos de corte.Arq.Bras. Med. Vet. Zoo.64:137-144.

Pillai, P. B., Conner- Dennie, T. O., Owens C. M., and Emmert, J. L. 2006.Efficacy of an E. Coli Phytase in broiler fed adequate or reduced phosphorus diets and its effects on carcass characteristics. Poul.Sci. 85: 1200- 1211.

Santos, F.R., Sakomura, N.K., Mendonça, M.O. 2005. Efeito da suplementação comfitase emdietas de frangos de cortesobre a viabilidadeeconômica e desempenho. Revista Brasileira de CiênciaAvícolaSupl. 7:119

Slominski BA. 2011. Recent advances in research on enzymes for poultry diets (Review). Poul. Sci. 90: 2013-2023.

Steel, R.G.D., T.H. Torrie.(eds). 1980. Principles and Proce¬dures of Statistics: A Biometrical Approach. McGraw-Hill Book Company. New York. USA.

Yu B, Jan YC, Chung TK, Lee TT &Chiou PWS. 2004. Exogenous phytase activity in the gastrointestinal tract of broiler chickens. Anim. Feed Sci.Tect. 117: 295-303

O-12-10 Effect of Folic Acid and Docosahexaenoic Acid (DHA) Supplementation in diet and Incorporation in Egg of Laying Hens

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Introduction

Diet plays an important role in maintaining health. Among the different products delivering essential nutrients to the body, an egg has arguably a special place, being a rich and balanced source of essential amino and fatty acids as well some minerals and vitamins. This experiment focuses on the benefits to the consumer of improving the nutritional quality of eggs by enhancing levels of Folic acid and n-3 fattyacids such as Docosahexaenoic Acid (DHA). The advantages of simultaneous enrichment of eggs with Folic acid and DHA include better stability of polyunsaturated fatty acids (PUFA) during egg storage and cooking, high availability of such nutrients as folic acid and DHA prevent atherosclerosis and coronary heart disease of people consuming these eggs. Having reviewed the relevant literature it is concluded that ''designer eggs'' can be considered as a new type of functional food.

Folic acid play functional role to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reactions involving Folic acid (Bunchasak and Kachana, 2009). It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy (Pick *et al.*, 2005). In addition, DHA is the most abundant omega-3 fatty acid in the brain and retina. Dietary DHA may reduce the risk of heart disease by reducing the level of blood triglycerides in humans (Holub, 2009; Delgado-Lista *et al.*, 2012). Low levels of DHA have been associated with Alzheimer's disease. Several papers indicate the high efficiency of Folic acid and DHA retention in eggs. Accordingly, the efficiency of retention of total long chain n-3 fatty acid might be greater with marine algal source than Marine Fish source (Park *et al.*, 2015). Further, marine algal and/or vegetable source maintains consumer acceptability. Therefore, this experiment was performed to study the effect of dietary Folic acid and DHA supplementation and incorporation in yolk egg's laying hens.

Material and Methods

This study was conducted at Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. Experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals. An 8-week experiment was performed with a total of 96 KU-Leghorn laying hens. The hens were randomly divided into 2 groups and each group consisted with 4 replications of 12 each. An evaporative cooling system was used to control air ventilation and temperature. The hens were housed in wire cages with 4 birds per cage. The lighting program was set 16 hours from 05:00 to 21:00 daily. Feed and water were offered *ad libitum*. Two experimental groups were provided as following: 1) Control group and 2) Control group + DHA 10 g and Folic acid 10 mg/kg diet.

Throughout the experiment egg production and mortality were recorded daily whereas mean egg weight and feed consumption were determined at two weeks intervals. Based on these data daily egg mass (g/hen/d) and feed conversion ratio (kg feed/kg egg mass) were calculated for the entire experiment. Egg production, Folic acid and DHA concentration were measured on the 56th day of feeding. At the end of experiment, all eggs from each experimental unit was weighted, and 4 eggs from each replication that have weight close to the replication \'s mean was chosen to analyze egg qualities.

Folic acid in the egg yolk content was analyzed following method Bunchasak and Kachana (2009). Fatty acid composition of egg yolk content was determined according to the modified method of Kim *et al.* (2003). The data were significant difference between the mean of the group was separated by Student's t-test significant difference test at 5% probability level.

Results and Discussion

Egg production and Egg quality

Effect of dietary folic acid and DHA supplementation on egg production is presented in Table 1. Laying-hens

fed diets supplemented with Folic acid 10 mg/kg diet and DHA 10 g/kg not influenced body weight, hen-day egg production, egg weight, egg mass, feed intake and feed conversion ratio compared to the laying-hens fed the control diet (P>0.05). Therefore, this study well documented that dietary sources of folic acid and n-3 polyunsaturated fatty acid have no effect on egg production in layer (Hargis *et al.*, 1991; Mazalli *et al.*, 2004, Bunchasak and Kachana, 2009)

Effect of dietary folic acid and DHA supplementation on egg quality is presented in Table 2. Laying-hens fed diets supplemented with Folic acid 10 mg/kg diet and DHA 10 g/kg not influenced yolk, albumen, shell, albumen high and shell thickness compared to the laying-hens fed the control diet (P>0.05). This results of this study agree with the reports of Bunchasak and Kachana (2009) Hebert *et al.* (2011) Park *et al.* (2015) due to folic acid and DHA not effect on deposition mechanisms transport to eggs.

Fatty acid composition of egg yolk

Effect of dietary folic acid and DHA supplementation on egg yolk chemical composition is presented in Table 3. Laying-hens fed diets supplemented with Folic acid 10 mg/kg diet and DHA 10 g/kg had higher significantly levels of a-Linolenic acid (ALA, C18:3n-3), Docosapentaenoic acid (DPA, C22:5n-3), Docosahexaenoic acid (DHA, C22:6n-3) and Total omega 3 in egg yolk higher than compared to the laying-hens fed the control diet (P<0.01). However, dietary folic acid and DHA supplementation not influenced folic acid in the egg yolk (P>0.05). Supplementation DHA in diet significantly increase fatty acid composition such as a-Linolenic acid, Docosapentaenoic acid and Docosahexaenoic acid in egg yolk according same many report (Hargis *et al*., 1991; Mazalli *et al.*, 2004; Park *et al.*, 2015). However, the folic acid level in egg yolk not different with control group because the folic acid not deficiencies in diet as long as the feed intake was not significantly affected (Bunchasak and Kachana, 2009).

Conclusion

Supplemental folic acid and DHA can be enhanced DHA levels in egg yolk without the negative effect on egg production and egg quality and not influenced folic acid in egg yolk of laying hens.

KEYWORD : Folic acid, Docosahexaenoic acid (DHA), Laying hens

Table 1 Effect of dietary Folic acid and Docosahexaenoic Acid (DHA) supplementation on production of KU-Leghorn laying hens**.**

Item	Control group	Folic acid + DHA	p-value	SEM
Initial Body weight (g)	1,455.78	1,463.75	0.94	44.96
Final Body weight (g)	1,552.75	1,643.50	0.39	48.33
Hen-day egg production (%)	84.88	83.70	0.43	0.69
Egg weight (g)	50.36	51.19	0.08	0.24
Egg mass	42.74	42.85	0.90	0.38
Feed intake (g/h/day)	113.55	115.36	0.34	0.87
Feed conversion ratio (g/g egg)	2.66	2.69	0.70	0.04

Table 2 Effect of dietary Folic acid and Docosahexaenoic Acid (DHA) supplementation on egg qualities of KU-Leghorn laying hens**.**

Item	Control group	Folic acid + DHA	p-value	SEM
Yolk (%)	29.01	29.28	0.58	0.22
Albumen (%)	62.22	61.39	0.12	0.27
Shell (%)	8.77	9.33	0.10	0.21
Albumen high (mm)	9.28	7.86	0.06	0.31
Shell thickness (mm)	0.01	0.01	0.07	0.01

Table 3 Effect of dietary Folic acid and Docosahexaenoic Acid (DHA) supplementation on Omega 3 fraction and Folic acid deposition in yolk egg's KU-Leghorn laying hens.

Item	Control group	Folic acid + DHA	p-value	SEM
Omega 3 (g/100g)				
α-Linolenic acid (ALA, C18:3n-3)	0.05	0.07	<0.01	0.01
Stearidonic acid (SDA, C18:4n-3)	< 0.01	< 0.01	-	-
cis-11,14,17-Eicosatrienoic acid (ETE, C20:5n-3)	< 0.01	< 0.01	-	-
Eicosapentaenoic acid (EPA, C20:5n-3)	< 0.01	< 0.01	-	-
cis-13,16,19-Docosatrienoic acid (C22:3n-3)	< 0.01	< 0.01	-	-
Docosapentaenoic acid (DPA, C22:5n-3)	0.02	0.03	<0.01	<0.01
Docosahexaenoic acid (DHA, C22:6n-3)	0.12	0.70	<0.01	0.11
Total Omega 3	0.19	0.80	<0.01	0.12
Folic acid (μg/100g)	48.24	50.46	0.86	5.53

REFERENCES

Bunchasak, B. and S. Kachana. 2009. Dietary folate and vitamin B12 supplementation and consequent vitamin deposition in chicken eggs. Trop. Anim. Health Prod. 41: 11583-1589.

Delgado-Lista, J., P. Perez-Martinez, J. Lopez-Miranda, and F. Perez-Jimenez. 2012. Long chain omega-3 fatty acids and cardiovascular disease: A systematic review. Br. J. Nutr. 107 Suppl 2: S201-13.

Hargis, P. S., M. E. Van Elswyk, and B. M. Hargis. 1991. Dietary modification of yolk lipid with menhaden oil. Poult. Sci. 70:874-883.

Hebert, K., G. B. Tactacan, T. M. Dickson, W. Guenter, and J. D. House. 2011. The effect of cereal type and exogenous enzyme use on total folate content of eggs from laying hens consuming diets supplemented with folic acid. Appl. Poult. Res. 20 :303–312

Holub, B. J. 2009. Docosahexaenoic acid (DHA) and cardiovascular disease risk factors. Prostaglandins Leukot. Essent. Fatty Acids 81:199-204.

Kim, Y. J., K. W. Lee, S. Lee, H. Kim, and H. J. Lee. 2003. The production of high-purity conjugated linoleic acid (CLA) using two-step urea-inclusion crystallization and hydrophilic arginine-CLA complex. J. Food Sci. 68:1948-1951.

Mazalli, M. R., D. E. Faria, D. Salvador, and D. T. Ito. 2004. A Comparison of the feeding value of different sources of fats for laying hens: 1. Performance characteristics. J. Appl. Poult. Res. 13:274-279.

Park, J. H., S. D. Upadhaya and I. H. Kim. 2015. Effect of dietary marine microalgae (Schizochytrium) powder on egg Production, blood lipid profiles, egg quality, and fatty acid composition of egg yolk in layers. Asian Australas. J. Anim. Sci. 28:391-397

Pick, M.E., M. Edwards, D. Moreau, E. A. Ryan. 2005. Assessment of diet quality in pregnant women using the Healthy Eating Index. J. Am. Diet. Assoc. 105: 240–246.

O-13-3

Effect of long-term feeding of n-6 to n-3 fatty acid ratio in laying hen diets on yolk fatty acid accumulation

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Introduction

Fatty acid composition of egg yolks can be modified by using different dietary fat sources. However, all amount of fat in diet cannot accumulate in egg yolk. Some factor may influence on fat deposition such as breed, age and health of laying hens (Pardio et al., 2005; Wu et al., 2005). The n-6 fatty acid and n-3 fatty acid series are essential fatty acids that human must to be gotten from food. The n-6 fatty acids have linoleic acid (LA; C18:2n-6) as main precursor and the n-3 fatty acids have α -linolenic acid (ALA; C18:3n-3) as main precursor (Simopoulos, 2008). They can be metabolized to other long chain unsaturated n-6 and n-3 fatty acids by consecutive elongation and desaturation mechanism and substance for inflammatory, constrictive and aggregator precursor groups (Fraeye et al., 2012). Daily optimum gain of n-6 and n-3 can reduce risk to be cardiovascular disease, rheumatoid arthritis and neurological disease (Patterson et al., 2012; Whelan and Rust, 2006). The n-6 and n-3 fatty acids are not different with other fatty acid to modify fatty acid composition in egg yolk by feed laying hen for produce modified fatty acid egg at optimum n-6 to n-3 ratio. These eggs are healthy, inexpensive and high essential nutrition for human especially for infant (Oliveira et al., 2010)

Various kind of oils have used to feed laying hens for modify lipid composition in egg yolks, including linseed oil, chia seed or marine fish oil for n-3 source and soybean oil for omega-6 source. In this present work was carried out to evaluate effect of different n-6 to n-3 fatty acid ratios in laying hen diets by using soybean oil for n-6 source and tuna oil for n-3 source on egg yolk fatty acid accumulation when continue feed throughout a long-term period.

Materials and Methods

Birds and Experimental Diets

One hundred eighty 42-wk-old Isa brown laying hens were housed in cage (3 birds per cage) throughout 12 weeks of experimental period. Birds were randomly divided into 3 dietary treatments with 4 replicates (15 birds per replicate). All treatment diets were corn-soy-based, formulated to meet the recommendations for major nutrients (NRC, 1994) and to be equivalent in crude protein and metabolizable energy. Soybean oil and tuna oil were analyzed fatty acid composition and used to adjust n-6 and n-3 fatty acid ratios in experimental diets for 10:1, 5:1 and 1:1, respectively. The nutrient compositions of experimental diets are shown in Table 1.

Fatty acid composition Analysis

A total of 5 eggs per replication were collected randomly at wk 4, wk 8 and wk 12 of experimental period and separated yolk from albumen then mixed together within replicate and stored at -20°C. Egg yolk and sample diets were analyzed following the method of Folch et al. (1957) and Metcalfe et al. (1966). Lipids were extracted in methyl ester form and run through gas chromatography (Hewlett Packard, HP 6890 series GC system).

Statistical Analysis

The results were statistically evaluated by analysis of variance (ANOVA), experimental design by completely randomized design (CRD) and Duncan's new multiple range test with P=0.05. Trending of different n-6 to n-3 ratio evaluated by orthogonal polynomial using SPSS 13.0 (2004).

Results and Discussions

Fatty acid composition accumulation in egg yolk is shown in table 2. (10:1 of n-6/n-3 ratio in diet). LA, dihomo-γ -linoleic acid (DGLA; C20:3n-6), arachinodic acid (AA; C20:4n-6), Docosahexaenoic acid (DHA; C22:6n-3) composition decrease linearly. γ -linoleic acid (GLA; C18:3n-6) has quadratic effect (p=0.001). Effect of 5:1 ratio diet is shown in table 3. LA and DHA decrease linearly (P=0.026 and P=0.018 respectively) with increasing of GLA and AA (P=0.004 and PP<0.0001) with increasing of ALA and EPA has quadratic effect (P<0.0001), total n-3 increase linearly (P=0.014), total n-6 and n-6/n-3 decrease linearly (P=0.002 and P=0.003 respectively).

Fatty acid composition in egg yolks were related to content of n-6 and n-3 fatty acids found in laying hen diet.

Generally, lipids are absorbed through intestinal villi and be transported to liver before yolk precursor transport fatty acids to egg yolk while egg forming process that take time around 24 hours (Bauer et al., 2013). However, liver has activities to deform some fatty acid to usable form by enzyme that be synthesized by liver thus fatty acid composition in egg yolks will have major change then stable in 1 to 2 weeks after change feed formula (Cachaldora et al., 2008). In present study found trend of fatty acid accumulation in slightly different after 4 weeks of experimental according to Oliveira et al. (2010) found different age of laying hen influence fatty acid accumulation in egg yolk. Diet with 1:1 of n-6 to n-3 had trend in almost fatty acid composition that may from enzyme Δ-5 and Δ-6 desaturase have more efficiency to deform omega-3 series than omega-6 series around 4:1 (Patterson et al., 2012).

Conclusions

Egg yolk from laying hens were fed with long-term feeding had trends in n-6 and n-3 fatty acid. However, some fatty acid had no different was unclear but as mentioned above. Fatty acid composition in egg yolks were related diet, feed intake may be related on fatty acid accumulation that will be studied in future by evaluating the feed intake.

KEYWORD : Egg yolk, Laying hen, Long-term feeding, Omega-3 fatty acid, Omega-6 fatty acid

Table 1. Nutrient composition of experimental diets

Nutrient composition	n-6 : n-3 fatty acid ratio		
	10:1	5:1	1:1
Analyzed nutrient composition			
Dry matter (%)	90.52	90.07	90.71
Crude protein (%)	17.42	17.64	17.28
Crude fat (%)	8.47	8.53	8.76
Crude fiber (%)	3.92	4.11	4.07
Calcium (%)	4.13	4.03	3.94
Total n-6	59.05	50.47	26.96
Total n-3	5.07	9.62	20.35
n-6/n-3	11.65	5.25	1.32
Calculated nutrient composition			
ME (kcal/kg)	2,907	2,903	2,900
Available phosphorus (%)	0.38	0.38	0.38
Lysine (%)	0.92	0.92	0.92
Methionine + cystine (%)	0.66	0.66	0.66

Table 2. Effect of 10:1 of n-6 to n-3 fatty acid ratio in diet on egg yolk fatty acid accumulation (% of total fatty acid)

Item	4wk	8wk	12wk	SEM[1]	Contrast[2]
C18:2n-6	25.75[b]	25.69[b]	24.56[a]	0.187	L=0.006[3]
C18:3n-6	0.10[a]	0.12[b]	0.09[a]	0.003	Q=0.001[4]
C20:3n-6	0.22[b]	0.23[b]	0.19[a]	0.006	L=0.021[3]
C20:4n-6	2.11[c]	1.98[b]	1.81[a]	0.032	L=0.0001[3]
C18:3n-3	0.74	0.79	0.71	0.024	NS
C20:5n-3	n/a	n/a	n/a	n/a	n/a
C22:6n-3	1.98[b]	1.96[ab]	1.88[a]	0.017	L=0.032[3]
Total n-6	28.18[b]	28.03[b]	26.65[a]	0.202	L=0.007[3]
Total n-3	2.75	2.71	2.59	0.029	NS
n-6/n-3	10.30	10.20	10.29	0.083	NS

[a,b] Means within a row with different superscript letters differ significantly at $P<0.05$.
[1]Standard error of mean
[2] Refer to polynomials trend analysis
[3]Linear trend
[4]Quadratic trend

Table 3. Effect of 5:1 of n-6 to n-3 fatty acid ratio in diet on egg yolk fatty acid accumulation (% of total fatty acid)

Fatty acid	4wk	8wk	12wk	SEM[1]	Contrast[2]
C18:2n-6	22.5[b]	22.16[ab]	21.66[a]	0.155	L=0.026[3]
C18:3n-6	0.08[a]	0.08[a]	0.1[b]	0.003	L=0.004[3]
C20:3n-6	0.20	0.20	0.19	0.003	NS
C20:4n-6	1.52[a]	1.47[a]	1.81[b]	0.038	L<0.0001[3]
C18:3n-3	0.70	0.73	0.70	0.007	NS
C20:5n-3	n/a	0.11	n/a	0.004	n/a
C22:6n-3	4.08[b]	3.86[a]	3.88[a]	0.037	L=0.018[3]
Total n-6	24.30	23.91	23.77	0.155	NS
Total n-3	4.58	4.66	4.78	0.042	NS
n-6/n-3	5.08	5.14	5.19	0.031	NS

[a,b]Means within a row with different superscript letters differ significantly at $P<0.05$.
[1]Standard error of mean
[2]Refer to polynomials trend analysis
[3]Linear trend

Table 4. Effect of 1:1 of n-6 to n-3 fatty ratio in diet on egg yolk fatty acid accumulation (% of total fatty acid)

item	4wk	8wk	12wk	SEM[1]	Contrast[2]
C18:2n-6	13.92	13.93	13.99	0.086	NS
C18:3n-6	0.09[b]	0.05[a]	0.05[a]	0.004	L<0.0001[3]
C20:3n-6	0.18[c]	0.14[b]	0.12[a]	0.006	L<0.0001[3]
C20:4n-6	1.59[b]	0.84[a]	0.89[a]	0.079	L<0.0001[3]
C18:3n-3	0.25[a]	0.31[b]	0.36[b]	0.014	L<0.0001[3]
C20:5n-3	0.00[a]	0.41[b]	0.42[b]	0.041	Q<0.0001[4]
C22:6n-3	7.01	7.13	7.15	0.071	NS
Total n-6	15.78[b]	14.88[a]	14.97[a]	0.127	L=0.002[3]
Total n-3	7.40[a]	7.73[ab]	7.90[b]	0.084	L=0.014[3]
n-6/n-3	2.14[b]	1.94[a]	1.88[a]	0.036	L=0.003[3]

[a,b]Means within a row with different superscript letters differ significantly different at $P<0.05$.
[1]Standard error of mean
[2]Refer to polynomials trend analysis
[3]Linear trend
[4]Quadratic trend

REFERENCES

Bauer, R., J. A. Plieschnig, T. Finkes, B. Riegler, M. Hermann and W. J. Schneider. 2013. The developing chicken yolk sac acquires nutrient transport competence by an orchestrated differentiation process of its endodermal epithelial cells. Journal of Biological Chemistry 288:1088-1098.

Cachaldora, P., P. Garcia-Rebollar, C. Alvarez, J. De Blas and J. Mendez. 2008. Effect of type and level of basal fat and level of fish oil supplementation on yolk fat composition and n-3 fatty acids deposition efficiency in laying hens. Animal feed science and technology 141:104-114.

Folch, J., M. Lees and G. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497-509.

Fraeye, I., C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert and I. Foubert. 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Food Research International 48:961-969.

Metcalfe, L., A. A. Schmitz and J. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical chemistry 38:514-515.

Oliveira, D., N. Baião, S. Cançado, R. Grimaldi, M. Souza, L. Lara and A. Q. Lana. 2010. Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. Poultry science 89:2484-2490.

Pardio, V., L. Landin, K. Waliszewski, F. Pérez-Gil, L. Díaz and B. Hernández. 2005. The effect of soybean soapstock on the quality parameters and fatty acid composition of the hen egg yolk. Poultry science 84:148-157.

Patterson, E., R. Wall, G. Fitzgerald, R. Ross and C. Stanton. 2012. Health implications of high dietary omega-6 polyunsaturated fatty acids. Journal of nutrition and metabolism 2012.

Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental biology and medicine 233:674-688.

SPSS. 2004. User's Guide, Version 13.0 SPSS, Chicago, IL.

Whelan, J. and C. Rust. 2006. Innovative dietary sources of n-3 fatty acids. Annu. Rev. Nutr. 26:75-103.

Wu, G., M. Bryant, R. Voitle and D. Roland. 2005. Effect of dietary energy on performance and egg composition of Bovans White and Dekalb White hens during phase I. Poultry science 84:1610-1615.

O-13-4

EFFECTS OF SUPPLEMENTING PIG DIETS WITH DIETARY IRON LEVELS, TRYPTOPHAN AND PYRIDOXINE ON PERFORMANCE OF WEANING PIGLETS

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Introduction

Many animal nutritionists tried to improve feed intake of weaning pigs by supplementing spray dried porcine plasma (SDPP), a high quality protein source in diets for weaned piglets. SDPP can improve feed intake of weaning pigs has been shown in many reports (Hansen et al., 1993; Kates et al., 1994; Coffey and Cromwell, 1995; Kim et al., 2001; van Dijk et al., 2002). However, the inclusion of SDPP in the diet of weaning pigs improves feed intake, but increased feed cost.

According to Stein (1996); NRC (1998); Kerr et al. (2004) SDPP contains 78% protein with a relatively low concentration of methionine and isoleucine, but relatively high concentration of tryptophan (Trp). Trp is the precursor of serotonin which plays a role in the regulation of feed intake (Henrry and Sève, 1993). Henry et al. (1992) demonstrated that large neutral amino acids (LNAA) in pigs have been shown to compete with Trp for its passage through the blood-brain barrier, prior to serotonin synthesis in the brain. They reported that Trp/LNAA ratio was one of the important factors in pig appetite and then improve feed intake.

Vitamin B_6 is a collective term for pyridoxal, pyridoxine and pyridoxamine and their phosphorylated forms (Rall and Meydani, 1993). Pyridoxine plays a role in the conversion of Trp to niacin derivatives in rats, mice, and swine (Okada et al., 1997). Blodgett et al. (2002) reported that Trp can be converted to niacin. They suggested that Trp may function as a metabolic precursor of niacin. The metabolic pathway for Trp conversion to niacin involves the oxidative cleavage of Trp by the heme enzyme which requires Fe^{2+} (van Eys 1991; Oduho et al., 1994). Therefore, the purpose of this study was to examine whether supplemental Trp in combination with pyridoxine and iron could improve feed intake as well as SDPP.

Material Materials and Methods

Location

The trial was conducted in the environmental controlled chamber of the farm of the National Pingtung University of Science and Technology, Taiwan, Republic of China. The Animal Care Advisory Committee at the National Pingtung University of Science and Technology approved the experimental protocol.

Experimental pigs

Three-way crossbred (Landrace × Yorkshire × Duroc) females were used as experimental animals. A total of 24 pigs, which were weaned at approximately 28 days of age, were distributed into 4 treatments randomly. The pigs were housed in individual pens. There were 6 replicates per treatment. The experiment lasted for 8 weeks.

Experimental diets

Calculated composition of the experimental diets is presented in Table 1 to 2. Four experimental diets, which contained 0.22% total Trp were formulated to meet NRC (1998) recommendations, except for Trp supplementation in dietary treatment 3 and 4, which used synthetic Trp formulated to contain 2 times the NRC (1998) requirement. The diets were formulated to be isocaloric and isonitrogenic to meet the nutrient requirements of piglets.

Treatment 1: Iron (Fe), Trp content 0.22%.

Treatment 2: 5% SDPP + Fe, Trp content 0.22%.

Treatment 3: 0.30% synthetic Trp + pyridoxine + low Fe, Trp content 0.44%.

Treatment 4: 0.30% Synthetic Trp + pyridoxine + Fe, Trp content 0.44%.

Housing and management

Pigs were housed in partially slotted and solid concrete floor pens, with an automatic watering cup under continuous lighting. Feed and water were provided *ad libitum* during the entire experimental period of 8 weeks growth performance. Feed was provided in a mash form in the feeder. The feeders were checked twice daily at 0600 and 1800 hours to remove and weigh the residue in the feeder and also make sure feed had not empty. Feed refused and feed supplied were carefully weighed prior to feeding times. The amount of feed was about 0.5 to 1

kg more than the pigs could eat. Every evening, the residue in the feeders was collected into a plastic container and weighed. The daily feed consumption and weekly body weight were recorded for average daily feed intake (ADFI), average daily weight gain (ADWG), and feed efficiency (FE) calculations.

Blood analysis

Blood samples were also collected from 4 pigs randomly selected from each treatment the end of the experiment. Briefly, 10mL of blood was drawn from the jugular vein of each pig. Blood samples were then injected into collection tubes, centrifuged at 3000 rpm for 15 minutes and stored at -20 ℃ for subsequent amino acid analysis of HPLC.

Statistical analysis

For each animal, ADFI, ADWG, and FE ratios were calculated based on a weekly basis using the formulas: ADFI = Total weekly feed intake/7; ADWG = Total weekly WG/7; FE = ADWG/ADFI. The experimental data were analyzed as a randomized complete block design with one pen as the experimental unit. Pigs were blocked on the basis of initial weight, and analysis of variance was performed using the general linear model procedure of SAS software (2004). Differences among treatment means were determined using Duncan's New Multiple Range Test (DNMRT) at $P < 0.05$ significant level.

Results

The effects of dietary treatment on feed intake of pigs are shown in Table 3. There were significant differences observed in feed intake of piglets during the initial 4 weeks after weaning, pigs fed treatment 4 (Trp + pyridoxine + Fe) consumed more feed than other dietary treatments, followed by pigs fed treatment 1 and 2 (weeks 1-2, $P < 0.01$; weeks 3-1, $P < 0.05$). A similar trend was observed for the dietary treatments in which pigs supplemented with diet containing synthetic Trp combination with pyridoxine and low Fe (Treatment 3) recorded significantly ($P < 0.05$) poorer feed intake than the other treatments.

Weight gains of piglets fed the experimental diets are presented in Table 4. The results showed that during weeks 1-2 and week 1-4 of the trial, the highest weight gain was observed in pigs fed treatment 4 (Trp + pyridoxine + Fe) have better weight gain among the dietary treatments while the least ($P < 0.01$) value was recorded for pigs on treatment 3 (Trp + pyridoxine + low Fe).

The feed efficiency also showed the same trend as the trend in feed intake and weight gain. As shown in Table 5, during the initial 2 weeks after weaning (weeks 1-2), pigs fed diets containing synthetic Trp supplemented with pyridoxine and Fe had the greatest feed efficiency ($P < 0.05$).

The amino acid compositions of the blood in this study (8-wk) are presented in Table 6. Among the entire 18 amino acids detected, Trp and Arg were significantly different, the results showed that SDPP + Fe diet had greater Arg content than other dietary treatments, follow by synthetic Trp + pyridoxine + Fe ($P < 0.01$). The highest amino acid Trp contents was observed in pigs fed treatment 4 (synthetic Trp + pyridoxine + Fe) while the least value was recorded for pigs fed SDPP + Fe diet ($P < 0.05$).

Discussion

The results of the present study indicated that there was a tendency for the combination of Trp, pyridoxine and iron in low CP diet to improve feed intake. These findings are in concordance with Hsia (2005) who reported that feed intake of pigs improved when the total level of Trp is increased from 0.177% to 0.237% in a corn-soybean meal diets.

The finding in this study is contrary to those of Kerr et al. (1995), who reported that pigs fed the low crude protein diets without amino acid supplementation grew more slowly, and had less efficient in feed conversion.

Conclusions

In conclusion, the results of the present study demonstrated that supplement synthetic Trp with pyridoxine and iron tended to have greater feed intake and growth performance when compared to pigs fed diets with 5% SDPP. The SDPP can be replaced with synthetic Trp in combination with pyridoxine and iron without any negative effect on pig performance.

KEYWORD : Tryptophan, Iron, Pyridoxine, Weaning pigs, Performance

Table 1. The composition and calculated nutrient content of dietary treatments (% on as fed basis)

Ingredients	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe
Corn	83.34	87.10	83.80	83.80
Dehulled soybean meal	9.80	1.25	9.10	9.10
Soybean oil	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.50	1.10	1.50	1.50
Limestone	0.70	1.10	0.70	0.70
L-Lysine HCL	0.50	0.40	0.50	0.50
DL-Methionine	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30
Mineral premix	0.15	0.15	0.15	0.15
Vitamin premix	0.15	0.15	0.15	0.15
L-Threonine	0.20	0.10	0.20	0.20
L-Tryptophan	0.06	0.05	0.30	0.30
Spray dried porcine plasma	-	5.00	-	-
Calculated nutrient content, on as fed basis				
ME, kcal/kg[1]	3400	3400	3400	3400
Crude Protein,%	12.20	12.20	12.20	12.20
Calcium,%	0.70	0.70	0.70	0.70
Phosphorus,%	0.32	0.32	0.32	0.32
Lysine,%	1.00	1.00	1.00	1.00
Met + Cystein,%	0.58	0.58	0.58	0.58
Threonine,%	0.61	0.61	0.61	0.61
Tryptophan,%	0.22	0.22	0.44	0.44

[1] ME is calculated, whereas all other values are analyzed.

Table 2. Composition of vitamin premix[1] (gram, as-fed basis) and mineral premix[2] in experimental diets (mg per kg, as-fed basis).

Item	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe
Vitamin B_6 (pyridoxine)	0.153	0.153	0.306	0.306
Vitamin B_3 (niacin)	1.256	1.256	1.256	1.256
Vitamin K_3	0.100	0.100	0.100	0.100
Vitamin B_{12}	0.150	0.150	0.150	0.150
Calpan	0.918	0.918	0.918	0.918
Vitamin B_1	0.102	0.102	0.102	0.102
Vitamin B_2	0.375	0.375	0.375	0.375
Folic acid	0.031	0.031	0.031	0.031
Biotin	0.250	0.250	0.250	0.250
Choline	66.667	66.667	66.667	66.667
Vitamin E	20.000	20.000	20.000	20.000
Vitamin A/D_3	0.175	0.175	0.175	0.175
Copper	5.00	5.00	5.00	5.00
Iodine	0.14	0.14	0.14	0.14
Iron	80.00	80.00	40.00	80.00
Mn	3.00	3.00	3.00	3.00
Se	0.25	0.25	0.25	0.25
Zn	80.00	80.00	80.00	80.00

[1] Vitamin premix was supplied on a corn-based carrier.

[2] Mineral premix was supplied on a corn-based carrier.

Table 3. Effect of diet on feed intake of the experimental piglets (g/day)

Weeks	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe	SEM[1]	Sig.[2]
1-2	548.51^{ab}	451.49^{bc}	413.71^{c}	596.93^{a}	38.07	**
3-4	743.40^{ab}	639.85^{b}	646.88^{b}	856.73^{a}	54.98	*
5-6	942.95	899.06	826.83	982.35	51.64	ns
7-8	1100.90	1132.00	1079.50	1229.90	79.49	ns
1-4	645.95^{ab}	545.67^{b}	530.30^{b}	726.83^{a}	45.27	*
5-8	1021.91	1015.55	953.16	1106.11	59.94	ns
1-8	833.93	780.61	741.73	916.47	47.36	ns

^{a,b,c} Means with different superscripts in the same column differ significantly, $P < 0.05$; ns: not significant, $P > 0.05$

[1]SEM: Standard error of the mean.

[2]Probability of significance: ns, not significant, $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$.

Table 4. Effect of diet on weight gain of the experimental piglets (g/day)

Weeks	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe	SEM[1]	Sig.[2]
1-2	160.37^{b}	144.85^{b}	157.26^{b}	254.05^{a}	20.92	**
3-4	328.23	287.43	265.46	392.26	36.68	ns
5-6	371.20	357.84	377.84	369.64	35.24	ns
7-8	469.40	538.85	462.02	518.57	44.89	ns
1-4	244.30^{b}	216.14^{b}	211.36^{b}	323.15^{a}	24.31	**
5-8	420.30	448.35	419.93	444.10	29.18	ns
1-8	332.30	332.24	315.64	383.63	24.56	ns

^{a,b} Means with different superscripts in the same column differ significantly, $P < 0.05$; ns: not significant, $P > 0.05$

[1]SEM: Standard error of the mean.

[2]Probability of significance: ns, not significant, $P > 0.05$; **: $P < 0.01$.

Table 5. Effect of diet on feed efficiency of the experimental piglets (g feed/g)

Weeks	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe	SEM[1]	Sig.[2]
1-2	3.79[a]	3.42[ab]	2.84[ab]	2.49[b]	0.30	*
3-4	2.34	2.44	2.75	2.31	0.24	ns
5-6	2.66	2.83	2.29	2.87	0.23	ns
7-8	2.45	2.44	2.40	2.42	0.14	ns
1-4	3.07	2.93	2.80	2.40	0.18	ns
5-8	2.56	2.63	2.35	2.65	0.15	ns
1-8	2.81	2.78	2.57	2.53	0.12	ns

[a,b] Means with different superscripts in the same column differ significantly, $P < 0.05$; ns: not significant, $P > 0.05$

[1]SEM: Standard error of the mean.

[2]Probability of significance: ns, not significant, $P > 0.05$; *: $P < 0.05$.

Table 6. Effect of diet on amino acid contents of pigs blood (%)

Amino acids	Fe	SDPP + Fe	Trp + B_6 + low Fe	Trp + B_6 + Fe	SEM[1]	Sig.[2]
ASP	11.13	10.76	11.39	37.42	12.71	ns
GLU	9.10	9.06	9.19	9.74	0.22	ns
SER	62.70	61.52	61.82	69.07	1.98	ns
HIS	50.53	54.67	53.65	52.66	2.12	ns
GLY	7.94	7.46	7.88	7.65	0.45	ns
ARG	36.43^{c}	44.98^{a}	39.86^{bc}	41.85^{ab}	1.42	**
ALA	11.65	11.34	11.55	12.62	0.37	ns
TYR	16.69	20.01	17.03	18.60	1.04	ns
CYS	24.17	25.48	44.62	52.79	8.92	ns
VAL	7.42	8.75	8.29	7.54	0.40	ns
MET	10.41	11.62	8.15	8.09	1.36	ns
TRP	16.04^{b}	12.11^{b}	17.80^{ab}	29.08^{a}	3.72	*
PHE	55.05	49.60	52.74	42.99	5.55	ns
LEU	12.66	11.76	12.28	12.42	0.28	ns
LYS	7.45	7.15	7.72	7.80	0.28	ns
PRO	35.07	26.57	39.36	30.52	5.15	ns
THR	38.08	45.53	41.81	41.90	2.21	ns
ILE	9.87	11.98	11.45	12.03	1.30	ns

[a,b,c] Means with different superscripts in the same column differ significantly, $P < 0.05$; ns: not significant, $P > 0.05$.

[1]SEM: Standard error of the mean.

[2]Probability of significance: ns, not significant, $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$.

REFERENCES

Dong, G. Z. and J. R. Pluske, 2007. The low feed intake in newly-weaned pigs: Problems and possible solutions. Asian. Aust. J. Anim. Sci., 20: 440-452.

Ettle, T. and F. X. Roth, 2004. Specific dietary selection for tryptophan by the piglet. J. Anim. Sci., 82: 1115-1121.

Harper, A. E. and O. R. Roger, 1965. Amino acids imbalance. Proc. Nutr. Soc., 24: 173.

Henry, Y., B. Sève, P. Colleaux, P. Ganier, C. Saligaut and P. Jego, 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and serotonin. J. Anim. Sci., 70: 1873-1887.

Hsia, L. C., 2005. The effect of spray-dried porcine plasma and tryptophan on feed intake and performance of weanling pigs. Asian-Aust. J. Anim. Sci., 18: 75-79.

Kerr, B. K., F. K. McKeith and R. A. Easter, 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J. Anim. Sci., 73: 433-440.

Le Floc'h, N. and B. Sève, 2007. Biological roles of tryptophan and its metabolism: Potential implications in pig feeding. Livest. Sci., 112: 23-32.

Li, D. F., C. T. Xiao, J. H. Kim, W. T. Cho and I. K. Han, 1998. Effects of crystalline lysine, threonine and tryptophan supplementation of diets containing reduced protein levels on performance of growing pigs. Asian. Aust. J. Anim. Sci., 11: 43-50.

National Research Council, 1998. Nutrient requirements of swine. 10th rev ed. National Academy of Sciences, Washington, DC, USA.

Okada, M., E. Miyamoto, T. Nishida, T. Tomida and M. Shibuya, 1997. Effect of vitamin B6 nutrition and diabetes on vitamin B6 metabolism. J. Nutr. Biochem., 8: 44-48.

Rall, L. C. and S. N. Meydani, 1993. Vitamin B6 and immune competence. Nutrition Reviews., 51(8): 217-225.

SAS, 2004. SAS/STAT User's Guide. SAS Institute Cary, NC.

Sharda, D. P., D. C. Mahan and R. F. Wilson, 1976. Limiting amino acids in low-protein corn-soybean meal diets for growing-finishing swine. J. Anim. Sci., 42: 1175.

Voet, D. and J. G. Voet, 1995. Biochemistry (2nd Edition). John Wiley & Sons, Inc. New York. p. 759.

Wahlstrom, R. C., R. V. Merrill, L. J. Reiner and G. W. Libal, 1977. Mutant corns in young pig diets and amino acid supplementation of opaque-2 corn. J. Anim. Sci., 45: 747-753.

Woodworth, J. C., R. D. Goodband, J. L. Nelssen, M. D. Tokach and R. E. Musser, 2000. Added dietary pyridoxine, but not thiamin, improves weanling pig growth performance. J. Anim. Sci., 78: 88-93.

O-14-1

Characteristics of Egg Production, Fertility and Hatchability of F1 Local Village Chicken Cross in Southeast Sulawesi, Indonesia

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INTRODUCTION

One of the chicken ancestor in the world, *Gallus gallus* (red jungle fowl), was derived from Indonesia. They have been crossed with other chicken breeds and formed local village chicken which is distributed across Indonesian peninsula. Consequently, each region has specific village chicken and some of them have been characterized based on their morphological differences and claimed as a specific local village chicken of such region. There are 31 sub varieties of domestic chicken in Indonesia that have been identified based on their morphological characteristic differences. Among them are Nunukan chicken in Borneo island and Pelung chicken in West Java (Nataamidjaja, 2010). The rest have never been identified well, so they are generally called village chicken or local village chicken.

Generaly, the performance of village chicken is small and has low productivity both egg production and body weight gain. However, most of consumer prever village chicken to commercial broiler chicken which make the prize of village chicken become more expensive. Moreover, village chiken are more adaptive to environment (tropical environment) and resistant to desease compare to commercial broiler chicken.

One of the solutions to increase village chicken production is to cross village chicken with other breed which has high productivity especially in term of economic traits such as body weight and egg production. Several research reports have been assumed that good management skills in breeding (such as cross breeding), feeding and marketing were the keys factor to improve poultry production (Niranjan et al., 2008; Hidayat, 2012; Kperegbeyi et al., 2009; Allahyari et al., 2011). In previous study, we have reported that Bangkok crossbreed chicken was a good candidate for increasing the productivity of village chicken (Saili et al., 2015). Therefore, the objective of this study was to evaluate the characteristics of egg production, fertility and hatchability of F1 local village chicken cross.

METHODOLOGY

The research was conducted at Laboratory of Animal Production, Faculty of Animal Science, Universitas Halu Oleo, Indonesia. Twenty F1 of local village chicken cross (F1-LVC cross) hens aged ± 10 months and weighted 1977.30 ± 245.64g was used in this experiment. Those hens were produced by crossing local village chicken (LVC) with bangkok-crossed breed chicken (BCB) in previous reasearh. Two groups were made and each group consisted of 10 hens and 2 cocks of different breed for each group, and they all were kept individually in cages. Ten hens were performed backcrossing with LVC cock and 10 hens backcrossed with BCB cock. These four cocks aged ± 12 months, and weighted 2597.00 ± 232.77g. All chicken were fed by commercial feed which has approximately 2960 kcal/kg and 17.15% crude protein. Artificial insemination was applied 2 times a week during the experiment to produce fertile egg. Egg collection and weighting were conducted on daily basis and then incubated for 21 days using automatic incubator machine under the condition of 38.5°C and 70% of humidity. Candling was performed on day 7 of incubation to observed fertilized egg.

The variables measured included egg production, egg weight, egg fertility, egg hatchability and day old chick (DOC) weight. Egg production indicated the ratio between number of egg production and number of hens on daily basis, while egg weight was obtained by weighting each egg soon after egg collection on daily basis as well. The fertile egg was observed at day 7 of incubation using electric light assembled in incubator machine, whereas hatchability and DOC weight were calculated and measured using electrical balance, respectively, following 21 days of incubation. All data collected from each group were tabulated, presented as a mean and then compared the averages between groups using Orthogonal Contrasts Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Egg Production

There was no significant differences (P>0.05) in egg production between F1-LVC cross hens crossed by BCB

and LVC cocks. The F1-LVC hens crossed by BCB cocks could produced 8.33 ± 1.29 eggs per clutch while hens crossed by LVC cocks produced 7.94 ± 1.29 eggs per clutch. The average of eggs production obtained in this research was somewhat lower than egg production of village chicken reported by Sartika and Gunawan (2007) (53.44 eggs per six months or 8.9 eggs per clutch).

Egg Weight

The results showed that the average of egg weight obtained in this research was 41.43 ± 0.43 g. The average of egg weight of hens crossed by BCB was slightly heavier (41.73 ± 0.45g) compared to egg weight of hens crossed by LVC (41.13 ± 0.95g), but no significant differences was showed (P>0.05). The average egg weigth obtained in this research was higher than egg weight obtain from LVC crossed by both BCB (33.89g) and LVC (34.07g) reported in previous study (Saili et al., 2015).

Egg Fertility

All eggs in each clutch were incubated using automatic machine equipped with candling apparatus for 21 days under the condition of 38.5°C and 70% humidity. On day 7 of incubation eggs were performed candling to determine the fertile egg. Through candling, the condition of air cell, yolk, and albumen were possible being evaluated. It also can detect the bloody whites, blood spots, or meat spots, and enables observation of embryo development. The developing embryo indicated by the existence of a small reddish area with blood vessels extending away from it. This is the embryo floating around inside the egg, looking like a huge red spider. If the embryo dies, the blood draws away from the embryo and forms what is called a blood ring (Anonymous, 2013). The results of this observation were presented in Table 1.

Table 1. Egg fertility of F1-LVC cross hens crossed by different cock breeds

The data showed that egg fertility of hens crossed by BCB was lower (77.27%) than hens crossed by LVC (78.26%), but, none of them showed statistically differences (P>0.05). The results were lower than the average of egg fertility of village chicken reported by Muryanto *et al*. (2012) in which egg fertility of village chicken could reach 83.4% and its cross breed was 84.4%. The lower egg fertility obtained in this research might be caused by the technical problem during application of insemination and the quality of semen.

Hatchability

Hatchability represented hatching rates of egg following incubation either natural incubation or machinery incubation. Hatchability of F1-LVC cross hen crossed by different cock breeds were presented in Table 2.

The results showed that F1-LVC cross hens crossed by BCB cocks could produce egg with high hatchability (91.50 ± 6.97%) compared to hens crossed by LVC cock (90.26 ± 5.31), though no significant differences (P>0.05) were observed. The average of egg hatchability gained in this research (90.88%) was higher than village chicken hatchability (68.33%) reported by Bachari et al. (2006), and Sutiyono et al. (2006) in commercial egg layer crossed breed by village chicken (46.51%). King'ori (2011) reported that fertility and hatchability are trait that influenced by both genetic and environmental facrors such as good selection, proper post-lay handling of fertile eggs and the correct incubation process. While Abiola et al. (2008) reported that turning frequency during incubation could affect the hatchability of egg. In this research, the incubator machine was automatically turning the eggs frequently, so the embryo could develop well during incubation.

Table 2. Hatchability of F1-LVC cross hens crossed by different cock breeds

Day Old Chick (DOC) Weight

Day old chick weigh was measured a couple hours following hatching process in which the chick feathers had been dried. The averages of DOC weight obtained from this research were presented in Table 3.

Table 3. Egg and DOC weight of F1-LVC cross hens crossed by different cock breeds

The average of DOC weight obtained from BCB group was higher (27.64g) compare to LVC groups (27.19g), but no significant difference (P>0.05) was showed between groups. The average of DOC weight obtained in this research (26.44 ± 2.13 g) was lower to DOC weight reported by Bachari *et al.* (2006) in village chicken (35.51g).

CONCLUSSION

Based on the results, it was concluded that there was no significant differerences in egg weight, fertility and hatchability between F1-LVC cross hens crossed by BCB and crossed by LVC. It seems that production trait of F1-LVC cross hens has been stable, therefore in the future research, selection based on other economic trait such as feather color could be taken into consideration to produce more economic local village chicken.

KEYWORD : F1-LVC, BCB, Cross, fertility, hatchability, egg weight

Table 1. Egg fertility of F1-LVC cross hens crossed by different cock breeds

Cock breeds	No. of egg	No. of fertile egg	% Fertility
BCB	198	153	77.27±3.24
LVC	207	162	78.26±4.25
Averages		157.5	77.77±0.70

Table 2. Hatchability of F1-LVC cross hens crossed by different cock breeds

Cock breeds	No. of fertile egg	No. of hatched egg	Hatchability (%)
BCB	153	140	91.50±6.97
LVC	162	147	90.74±5.34
Averages	157.5	143.5	90.88±0.88

Table 3. Egg and DOC weight of F1-LVC cross hens crossed by different cock breeds

Cock breeds	Egg weights (g)	DOC weights (g)
BCB	41.73±0.45g	27.64 ± 1.19
LVC	41.13±0.95g	27.19 ± 1.40
Averages	41.43±0.43g	27.41 ± 0.32

REFERENCES

Abiola, SS., AO. Afolabi and OJ. Dosunmu. 2008. Hatchability of chicken eggs as influenced by turning frequently in hurricane lantern incubator. African journal of Biotechnology, 7(23):4310-4313.

Allahyari, MS., MS. Saburi and F. Keshavarz. 2011. Alalyzing farm management skills in poultry production enterprises in Iran. Life Science Journal, 8(1):61-67.

Anonymous. 2013. Incubation and Embryology: Candling Eggs. http://urbanext.illinois. edu/eggs/ res26-candling. html (June18, 2016).

Bachari, I., I. Sembiring dan DS. Tarigan. 2006. The effect of egg turning frequency on hatchability and DOC weight of free-range chicken. Agribisnis Peternakan, 2(3):101-105. In Indonesian.

Hidayat, C. 2012. Pengembangan produksi ayam local berbasis bahan pakan lokal. Wartazoa, 22(2):85-98. (in Indonesian).

King'ori, AM. 2011. Review of the factors that influence egg fertility and hatchability in poultry. International Journal of Poultry Science, 10(6):483-492.

Kperegbeyi, JI., JA. Meye and E. Ogboi. 2009. Local chicken production: strategy of household poultry development in coastal regions of Niger Delta, Nigeria. African Journal of General Agriculture, 5(1):17-20).

Nataamijaya, A.G., 2010. Development of local village chicken potency for supporting the increasing of farmer welfare. Jurnal Litbang Pertanian, 29(4):131-138. (In Indonesian).

Niranjan, M., RP. Sharma, U. Rajkumar, BLN. Reddy, RN. Chatterjee, and TK.Battacharya. 2008. Comparative evaluation of Production performance in improved chicken varieties for backyard farming. International Journal of Poultry Science, 7(11):1128-1131.

Saili, T., S. Rahadi, R. Badaruddin and LO. Nafiu. 2015. Improvement of Local Village Chicken Production in Indonesia: Case Study in South-East Sulawesi, Indonesia. Khon Kaen Journal of Agriculture, 43 Suppl.2:107-112.

Sartika, T. and B. Gunawan. 2007. Characteristic of Productivity Traits of Hen of Kampung Chicken at Base Selection Population. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor 2007. In Indonesian

Steel, R.G.D, J.H. Torrie. 1980. Principles and Procedures of Statistics. A biometric approach, 2nd ed, McGraw-Hill, New York.

Sutiyono, S. Riyadi dan S. Kismiyati. 2006. The fertility and hatchability of egg of layer artificially inseminated by some substances-diluted indigenous cock's semen. Journal of Indonesian Tropical Animal Agriculture, 31(1):36-40.

O-14-2

ALLELIC FREQUENCY OF 24-BP INDEL (INSERTION-DELETION) IN PROMOTER PROLACTIN GENE OF PAPUA LOCAL CHICKENS

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INTRODUCTION

Papua local chickens are non-commercial breed found in the Papua land, and they become one of Indonesia germplasms. These chickens have been considered as a primary commodity due to their contributions in welfare improvement of the local society in the region. Besides their high variability in body weight (Lumatauw et al., 1995; Haryani, 1999; Mu'in, 1999) and carcass percentage (Rahayu, 1990), the Papua local chickens also show high variability in the number and egg weight produced in every clutch (Lebang, 2002). This indicates that the production and reproduction performance of Papua local chickens are easy to improve through genetic selection.

Today, with the advance technology in molecular biology, genetic improvement of an animal population can be done through molecular approach. Polymorphism detection on the loci of protein coded gene associate with economically traits of animals is become possible. If a polymorphic loci associated with economically traits of animal is found, that polymorphic loci therefore can be used as molecular marker in selection program to improve a specific trait.

Prolactin is a peptide hormone secreted by the anterior pituitary gland and it has a wide role in the activities and biological function in all vertebrates. In birds, prolactin hormone (cPRL) has a crucial role in egg production and brooding behaviour due to the increase of prolactin secretion (Shimada et al., 1991) that results in regression of the ovary (Sharp et al., 1984) therefore, stop the egg production (Shimada et al., 1991).

The cPRL is coded by a gene located on chromosome 2 (Miao et al., 1999; Au and Leung, 2000), and become a candidate gene for brooding trait (Shimada et al., 1991; Dunn et al., 1998) and egg production (Cui et al*.,* 2006). Chicken prolactin gene is 9.536 bp in size, consists of 3 parts: promoter-1, promoter-2 and promoter-3 with 330 bp, 287 bp, and 314 bp, respectively (Kansaku, 2000), whereas the five exons of cPRL are exon-1 (81 bp), exon-2 (182 bp), exon-4 (180 bp) (Ohkubo et al., 1998), exon-3 (59 bp) (Miao et al., 1999), and exon-5 (418 bp) (Cui et al., 2004). The intron parts are: intron-1 (714 bp), intron-2 (406 bp), intron-4 (744 bp) (Dhara and Soller, 1999). The size of intron-3 remains unknown.

Promoter of cPRL is located on the start point and become crucial due to its early activation function for transcription of cPRL gene expression (Lewin, 1997). Mutation that occurs on the promoter region causes the cPRL gene fail to express its product and unable to express the brooding behaviour Therefore, the egg production will increase.

It has found several mutations of cPRL promoter region, and one is 24-bp (Cui et al., 2006; Liang et al., 2006; Rashidi et al., 2012). Indel on -358 site where Insertion (I) or Deletion (D) is presence. There are two alleles of this 24-bp locus: I and D alleles. In non-commercial breed of chickens, the I allele frequency vary from low to medium (Cui et al., 2006; Begli et al., 2010; Rashidi et al., 2012), while in the commercial layer, the I allele is commonly found. In their study, Cui et al (2006) did not find any other allele except the I allele in White Leghorn. In chicken, 24-bp Indel polymorphic is known significantly associated with egg production (Cui et al., 2006; Begli et al., 2010; Rashidi et al., 2012). These findings give an opportunity for breeders to form and develop a future layer type of the Papua local chickens.

The objective of this study is to get information regarding the allele frequency and genotype of 24-bp Indel in promoter cPRL gene of Papua local chickens. This information therefore, will be useful in the formation and development of layer type of the Papua local chicken in the future.

MATERIALS AND METHODS

Blood Samples

Sixty DNA samples isolated from blood samples of sixty Papua local chickens were used in this study. The experimental chickens that consisted of 25 males and 35 females were randomly collected from several farmers in Manokwari Regency of West Papua Province. About 1 ml of blood sample was collected from each bird via brachialis venous. The blood samples were carried to the Biochemistry Laboratory of Biotechnology Study Centre,

Gadjah Mada University for DNA isolation and DNA analysis.

DNA Isolation

DNA samples isolation of experimental birds was carried out by applying *phenol-chloroform* extraction method (Sambrook et al., 1989). The DNA samples obtained from the result of the isolation was measured for its concentration and purified (Muladno, 2002).

DNA Amplification and Genotyping

Specific DNA fragment amplification contained the studied loci was conducted with the *Polymerase Chain Reaction* (PCR) technic. The primer used to amplify the specific DNA fragment was *forward*: 5'-TTT-AAT-ATT-GGT-GGG-TGA-AGA-GAC-A-3', and *reverse*: 5'-ATG-CCA-CTG-ATC-CTC-GAA-AAC-TC-3' (Cui *et al*., 2006).

Genotype identification of 24-bp Indel/cPRLp was as follow: II genotype (*Insertion-Insertion*) characterized by one DNA fragment size 154 bp; ID genotype (*Insertion-Deletion*) characterized by two DNA fragments size 154 bp and 130 bp; and DD genotype (*Deletion-Deletion*) characterized by one DNA fragment size 130 bp (Cui et al., 2006).

Data Analysis

Data of genotypes and alleles of the studied locus (24-bp Indel/cPRLp) on Papua local chickens were estimated for their frequencies by using Nei and Kumar procedure (2000). Locus was polymorphic when the common allele frequency found in the population did not exceed 99%.

RESULTS AND DISCUSSION

Prolactin gene in chicken (cPRL) specifically on the promoter region is a candidate gene for brooding behaviour (Shimada et al., 1991; Dunn et al., 1998), and egg production (Cui et al., 2006). cPRL promoter gene is an important part that responsible in the expression or the function of cPRL. The position of promoter in cPRL gene is located at the *starting point* (Lewin, 1997) and has its role in activating the early transcription for the gene expression. If a mutation occurs in this promoter region, the cPRL gene will not function and fail to express its product, thus, the brooding behaviour will not appear.

Specific DNA fragment (size 130 bp and/or 154 bp) amplification located at the promoter region, contained 24-bp Indel on -358 site that flanked by a pair of specific primers (Cui et al., 2006) has demonstrated by using PCR (*Polymerase Chain Reaction*) in 60 DNA samples of Papua local chickens. The result of amplification showed three genotypes II, ID and DD (Figure 1). The chickens with II genotype were 4 birds, ID genotype were 29 birds and DD genotype were 27 birds.

The three genotypes found in this study were as the result of the presence of mutation on the promoter region. The polymorphism of 24-bp Indel on -358 site was due to the presence of insertion (I) and or deletion (D) as many as 24 bp (Cui et al., 2006; Liang et al., 2006; Rashidi et al., 2012). Sequence of DNA fragment size 154 bp (I allele) and size 130 bp (D allele), presented in Figure 2 and 3, respenctively.

Results for allele frequency and genotype calculations of 24-bp Indel/ cPRLp found that II, ID and DD genotype frequencies were 6,7%, 48.3%, and 45%, respectively while the I and D were 0.31 and 0.69.

The I frequency of 0.31 found in the Papua local chickens was considered medium. This is in accordance with Cui et al. (2006), Begli et al. (2010), and Rashidi et al. (2012) that the I frequency on non-commercial breed (local chickens) varied from low to medium, while on commercial breed (layer type), the I allele was common (common allele). In 2006, Cui et al did not find any other allele except the I allele on *White Leghorn*.

Based on the above research information, it was known that the presence of I allele (Insertion) in chickens gives positive effects on traits related to egg production. On the other hand, the presence of the D allele (Deletion) in chickens tends to give negative effects on egg production. Indel (Insertion-Deletion) is a term in molecular biology that has different definitions on several aspects. In the study of evolution, indels are used to bear a meaning for *an insertion* event (insert or interpolation) and or *deletion* (delete) (Kondrashov and Rogozin, 2004; Ogurtsov et al., 2004). In chickens, a 24-bp Indel (*Insertion-Deletion*) polymorphism is known significantly associated with egg production (Cui *et al.*, 2006; Begli et al., 2010; Rashidi *et al*., 2012). The presence of medium frequency of I allele found in Papua local chickens in this study indicated that the formation of Papua local chickens population that are high in egg production become easy to realized through a controlled mating application within the Papua local chickens with II and ID genotypes without chickens with DD genotypes involve. Therefore, the brooding trait in Papua local chickens will slowly disappeared and in turn a high egg production of Papua local chicken will be formed.

Several researches of 24-bp indel on the promoter region gene in cPRL of several breeds or genetic groups of chickens and its effects on quantitative traits had been demonstrated. Cui et al. (2006) carried out a research to study polymorphism on the promoter region gene in cPRL of several breeds of chickens and studied their effects on egg production. Genotyping of polymorphic 24-bp indel (insertion-deletion) loci on -358 site of cPRL gene was applied on 177 chickens that consisted of White Lenghorn, Yangshan, Taihe Silkies, White Rock, and Nongdahe. Results showed that the I allele (*Insertion*) and the D (*Deletion*) frequencies were 1 and 0; 0.05 and 0.95; 0.20 and 0.80; 0.22 and 0.78; 0.17 and 0.83, respectively. In the further analysis found that a polymorphic locus of 24-bp indel was significantly associated with the egg production where the presence of I allele gave positive effect on egg production. Jiang et al. (2005) informed their study that chickens with homozygote insertion (II genotype) of 24-bp on promoter region of cPRL could reduce the cPRL expression so that the chickens showed no brooding trait. It was concluded that the cPRL promoter could be used as genetic marker for brooding trait in chickens. Rashidi et al. (2012) studied the native chickens of Iranian and found that the allele frequencies of polymorphic 24-bp loci indel of cPRL were 0.59 for the I and 0.41 for the D allele. The frequencies of II, ID and DD genotypes were 0.39; 0.40; and 0.21, respectively. As comparison, result on polymorphic 24-bp Indel on this promoter region in Quail showed that the I and D alleles frequency was almost balance, 0.52 and 0.48 (Lotfi et al., 2013).

CONCLUSION

Polymorphism of 24-bp indel on the promoter region of prolactin gene was detected in Papua local chickens. The I allele frequency was 0.31 and categorized medium. This indicated that the brooding trait on hen population of Papua local chickens could be eliminated by increasing the I allele in the population through a controlled mating.

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KEYWORD : 24-bp Indel, Papua local chicken, polymorphism, promoter prolactin gene

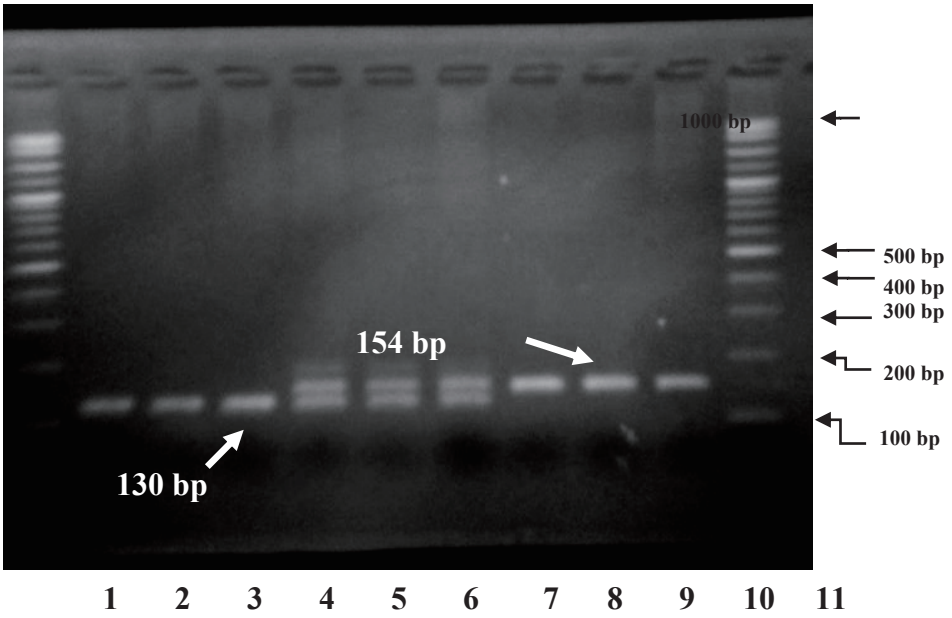

Figure 1. Genotypes of the 24-bp indel at np 358 by PCR with agarose gel electrophoresis in Papua local chickens. Lanes 1 and 11: DNA marker (100 – 3000 bp); lanes 2 – 4: DD (deletion-deletion, size:130 bp); lanes 5 – 7: ID (insertion-deletion, size: 130 bp and 154 bp); and lanes 8 – 10: II (insertion-insertion, size: 154 bp).

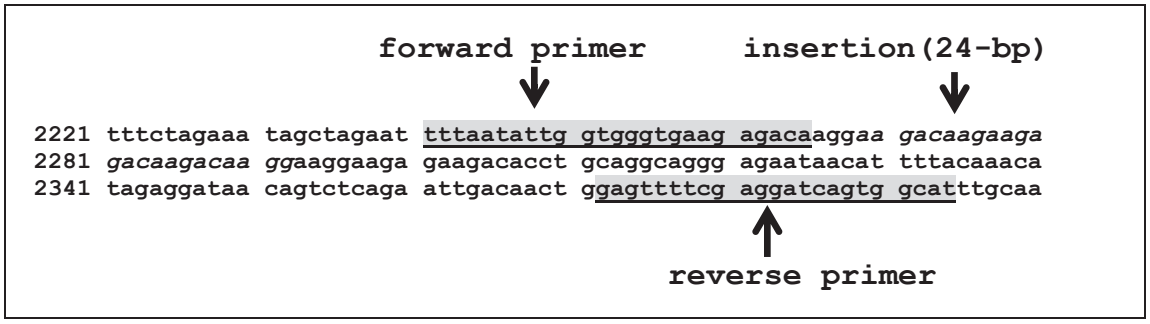

Figure 2. Sequent of DNA fragment in gallus gallus prolactin gen (promoter region), 154 bp (insertion allele). Source: GenBank: AB011438.2

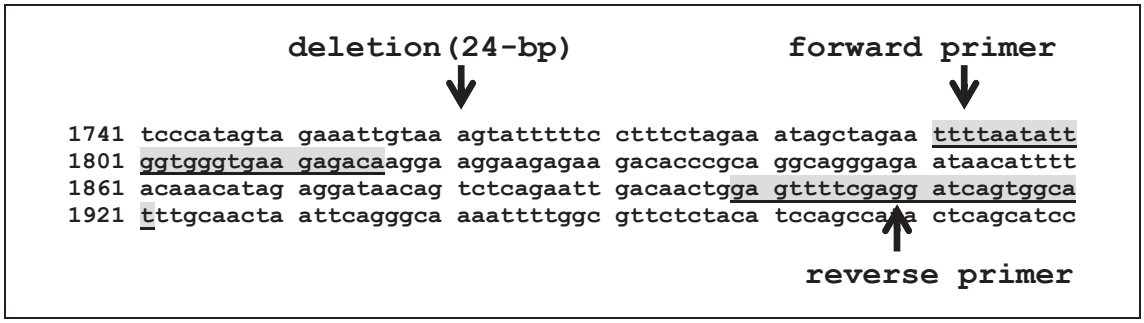

Figure 3. Sequent of DNA fragment in gallus gallus prolactin gen (promoter region), 130 bp (deletion allele). Source: GenBank: AF288765.2

REFERENCES

Au, W.L. & F.C. Leung. 2000. Genomic sequence of chicken prolactin gene. Departement of Zoology, The University of Hongkong, Pokfulam Road, Hongkong, SAR, China. www.ncbi.nlm.nih.gov/ entrez/viewer. fcgi?db-Nucleotide&dopt-GenBank [20 February 2014]. (Internet article)

Begli, H.E., S. Zerehdaran, S. Hassani, M.A. Abbasi, & A.K. Ahmadi. 2010. Polymorphism in prolactin and PEPCK-C genes and its association with economic traits in native fowl of Yazd province. *Iranian Journal of Biotech.*, 8:172-177. (*Journal*)

Cui, J.X., H.L. Du, Y. Liang, & X.Q. Zhang. 2004. Polymorphism of chicken prolactin gene. Departement of Animal Genetics, Breeding and Reproduction, College of Animal Science. South China Agricultural University, Wushan Street, Guangzhou, Guangdong, China. www.ncbi.nlm.nih.gov [24 Maret 2014]. (*Internet article*)

Cui, J.X., H.L. Du, Y. Liang, X.M. Deng, N. Li, & X.Q. Zhang. 2006. Association of polymorphism in the promoter region of chicken prolactin with egg produksi. *Poultry Science*, 85:26-31. (*Journal*)

Dhara, S.K. & M. Soller. 1999. Intragenic haplotypes in chicken prolactin in four diversed breeds. Hebrew Univ. of Jerusalem, Israel. www.ncbi.nlm.nih.gov [18 March 2014]. (*Internet article*)

Dunn, I.C., G. McEwan, T. Ohkubo, P.J. Sharp, I.R. Paton, & D.W. Burt. 1998. Genetic mapping of the chicken prolactin receptor gene: a candidate gene for the control of broodiness. *Br. Poul. Sc*i. 39: *suppl*.:S23-S24. (*Journal*)

Haryani, E., 1999. Keragaman Morfologi dan Morfogenetik Ayam Kampung di Kabupaten Manokwari. *Skripsi*. Fakultas Pertanian, Universitas Cenderawasih, Manokwari. (*Book*)

Jiang, R.S., G.Y. Xu, X.Q. Zhang, & N. Yang. 2005. Association of polymorphism for prolactin and prolactin receptor genes with broody traits in chickens. *Poultry Science*, 84:839-845. (*Journal*)

Kansaku, N. 2000. Genetic variation of chicken prolactin promoter. Database 2000. www.ncbi.nlm.nih.gov/ entrez/ viewr.fcgi?db-nucleotide&dopt-Genbank& val [19 March 2014]. (*Internet article*)

Kondrashov, A. S. & I. B. Rogozin. 2004. Context of deletions and insertions in human coding sequences. *Human*

Mutation. 23: 177-185. (*Journal*)
Lebang, D. 2002. Perbandingan Performans Produksi Telur Ayam Kampung di Dataran Tinggi Anggi dan Dataran Rendah Manokwari. *Skripsi*. Fakultas Pertanian, Peternakan dan Kehutanan, UNCEN, Manokwari. (*Book*)
Lewin, B. 1997. *Trancription is catalysed by RNA polymerase. In: Genes* VI. Oxford University Press, Inc, New York. pp. 289-334. (*Book*)
Liang, Y., J. Cui, G. Yang, F.C.C.Leung, & Z. Zhang. 2006. Review: polymorphism of 5'flanking region of chicken prolactin gene. *Domest. Anim. Endoc*., 30:1-16. (*Journal*)
Lotfi, E., S. Zerehdaran, M. Ahani, & E. Dehnavi. 2013. Genetic polymorphism in prolactin gene and its association with reproductive traits in Japanese quail (Coturnix-coturnix japonica). *Poul. Sci. Journal* 1(1):29-35. (*Journal*)
Lumatauw, S. A.L. Killian & A. Supriyantono. 1995. I*dentifikasi Sifat-sifat Morfogenetik Ayam Buras di Irian Jaya*. Laporan penelitian, Faperta Uncen, Manokwari. (*Book*)
Miao, Y., D.W. Burt, I.R. Paton, P.J. Sharp, & I.C. Dunn. 1999. Mapping of the prolactin gene to chicken chromosome 2. *Anim Genet*. 30: 473. (*Journal*)
Mu'in, M.A. 1999. Analisis Potensi Genetik Beberapa Ukuran Tubuh Ayam Kampung. *Jurnal Irian Jaya Agro* Vol. 7 No. 2. Desember 2000. Hal. 38- 42. (*Journal*)
Muladno. 2002. *Seputar Teknologi Rekayasa Genetika*. Pustaka Wirausaha Muda dan USESE Foundation, Bogor. (*Book*)
Nei, M. & D. Kumar. 2000. *Molecuar evolution and phylogenetics*. Oxford University Press, New York. (*Book*)
Ogurtsov, A. Y., S. Sunyaev, & A. S. Kondrashov. 2004. Indel-based evolutionary distance and mouse-human divergence. *Genome Research*. 14: 1610-1615. (*Journal*)
Ohkubo, T.M., Tanaka & K. Nakashima. 1998. Cloning and characterization of chicken prolactin gene. Kagawa University, Departement of Agriculture 2393 Ikenobe, Miki, Kita, Kagawa, Japan. www.ncbi.nlm.nih. gov [25 Maret 2014]. (*Internet article*)
Rahayu, B.W.I. 1990. Pengkajian Beberapa Sifat Phenotip Ayam Kampung di Kota Manokwari. *Skripsi*. Fakultas Pertanian, Universitas Cenderawasih, Manokwari. (*Book*)
Rashidi, H., G. Rahimi-Mianji, A. Farhadi, & M. Gholizadeh. 2012. Association of prolactin and prolactin receptor gene polymorphism with economic traits in breeder hens of indigenous chickens of Mazandaran province. *Iranian Journal of Biotechnology*, 10: 129-135. (*Journal*)
Sambrook, J., Fritcsh & T. Maniatis. 1989. *Molecular Cloning Laboratory Manual*. Second Edition, Vol. 3. Cold Spring Harbor Laboratory Press. USA. (*Book*)
Sharp, P.J., M.C. MacNamee, R.T. Talbot, R.J. Sterling, & T.R. Hall. 1984. Aspects of the neuroendocrine control of ovulation and broodiness in the domestic hen. *J. Exp. Zool*. 232.475-483. (***Journal***)
Shimada, K.I.H., K. Sato, H. Seo, & N. Matsui. 1991. Expression of prolactin gene in incubating hens. *Reprod. Fertil*.

O-14-4

The effect of *GH* gene and *IGF-I* gene on dominance effect of bodyweight of Korat chicken

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ABSTRACT

Normally, major genetic effect role in growth performance of broiler chicken is heterosis. Heterozygous genotypes cause to the effect, therefore, the highest magnitude effect should be found in the heterozygous genotype. Objectives of the study were investigate the association between genotypes of growth hormone gene (*GH*) and Insulin like growth factor I gene (*IGF-I*) and dominance effect of the bodyweight of Korat chicken (KR). Dominance effect of bodyweight at 0, 4, 6, 8 and 10 weeks of Korat chickens were estimated by BLUP method with Animal model with dominance effect. PCR-RFLP technique was used to genotype the *GH* and *IGF-I* gene. General linear model and ordinary least square were used as model and estimate the genotypes effect. The significant association was accepted at $\alpha \leq 0.05$. Highly significant association between genotypes of *GH* and *IGF-I* and the dominance effect in all weeks were found. Regard of *GH* gene, the highest magnitude of genotype effect, however, were not found in heterozygous genotypes. The results suggest that the *GH* and *IGF-I* genotypes have significant different effect on bodyweight in KR, but the effect of heterozygous genotypes were not the highest effect.

Key words: Growth performance, Dominance effect, Crossbred chicken, *GH* gene, *IGF-I* gene.

Introduction

Alternative meat chicken is needed for answering of new generation consumer requirement. Better texture, and flavor are desirable properties of chicken meat. KR is Thai indigenous crossbred chicken, it was established under the cooperation between Suranaree University of Technology, Thailand Research Fund, and Department of Livestock Development. The ultimate goal of the establishment of the chicken is to be the strong tool for small holder farmer's occupation, and to answer the need of consumer.

From previous studies Keambou et al. (2010); Williams et al. (2002); Youssao et al. (2009) reported that heterosis is main role for growth traits of chicken. Heterosis will be generated by heterozygous genotype (Hanafi and Iraqi, 2001; Keambou et al., 2010; William and Pollak, 1985). Therefore, heterozygous genotype of loci related with growth traits of chicken should be associated with the highest dominance effect, this is the hypothesis of the current study.

Growth hormone (*GH*) is a single polypeptide secreted by eosinophilic granulocytes of the anterior pituitary (Kato et al., 2002). It has many physiological functions in animals (Ma et al., 2012), such as promoting muscle growth (Ohlsson et al., 1998), bone formation (Millar et al., 2010). The *GH* gene is one of the most important genes that can affect chicken performance traits because of its important function in growth and metabolism (Vasilatos-Younken et al., 2000). The *GH* gene is a candidate gene for body weight secreted by the anterior pituitary gland under the hypothalamic control of two hormones, *GH*-releasing hormone (*GH*RH), which increases the secretion of *GH*, and somatotropin release-inhibiting factor (SRIF, also called somatostatin) which inhibits its secretion (Nicoll et al., 1986). It is known that *GH* is the main regulator of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis (Daughaday, 2000).

Insulin-like growth factor-I (*IGF-I*) secreted by liver which this liver region have *GH* receptor for get *GH* and adapted is *IGF-I* at liver regions (McMurtry et al., 1997). *IGF-I* is a highly conserved, 70 amino acid, single-chain polypeptide secreted by liver which that plays an important role in the control of growth and metabolism in chickens and mammals (Dawe et al., 1988; Florini et al., 1996). The *IGF-I* gene is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou et al., 2005).

Regard to the mentioned studied, the objective of the current study were investigate the association between genotypes of growth hormone *GH* and *IGF-I* genes and dominance effect of the bodyweight of KR.

Materials and Methods

Experimental design, animal and genotyping

Four hundred and seventy-eight Suranaree University of Technology (SUT) female chickens were mated with one hundred and four Leung Hang Khao (LK) male chickens to produce 644 KRs. All chickens were tagged with an ID for individual data collection.

All KRs were checked their genotype of *GH*, and *IGF-I* gene, and were separated into 6 groups follow their genotypes (A1A1, A1A3, A3A3 and AA, AC, CC respectively). CRD was applied for the experimental design, there were 4 replications in each genotype, and 30 KRs in each replication.

The protocol of Thakur et al.(2006) and Moe et al.(2009) were followed for genotyping the *GH*, and *IGF-I* gene, respectively.

Data and statistical Analysis

There were 3,220 data of body weight of the age at 0, 4, 6, 8, and 10 weeks which include with the genotyped KRs' body weight and 25,309 pedigree data were used to estimate parental dominance effect of body weight.

Parental dominance effect were estimated by animal model with permanent environment and parental dominance. Restricted maximum likelihood; REML were used to estimate variance component. BLUPF90 3.0 (Duangjinda et al., 2007) software was used for the estimation.

The association between genotype and dominance effect of body weight of KRs was analyzed by general linear model and ordinary least square and compare difference of genotype by Tukey's range test. Significant differences were accepted at $\alpha \leq 0.05$.

Result and Discussion

Significant different effect of different genotypes for both of genes were detected, the highest magnitude effect, however, were not the heterozygous genotypes.

In cased of *GH* gene were found highly significant association between genotypes and dominance effect of the body weight of KRs in all weeks (Table 1). The effect of genotype A3A3 was the lowest in all ages of KRs, while the effect of genotype A1A1, and A1A3 were the highest at the age of 0, 2, 4, 6, and 8 weeks. At the age of 10 weeks, the effect of A1A1 genotype was the highest. The result related with Feng et al. (1997); Kuhnlein et al. (1997); Nie et al. (2005) in issue of *GH* gene had to related with growth trait of chicken and A1A1 genotype were related with the best growth trait.

In the case of *IGF-I* gene, highly significant association between genotypes of *IGF-I* gene and the dominance effect of the bodyweight of KRs were found in all weeks (Table 1). Genotype AA had the highest effect at the age of 0 week, while genotype AC had the highest effect at 4, 6, and 8 weeks. And at the 10 weeks, genotype CC had the highest effect. The result related with Amills et al. (2003); Kadlec et al. (2011); Moe et al. (2009); Pandey et al. (2013); Tang et al. (2010); Wang et al. (2004); Zhou et al. (2005) found that *IGF-I* gene effect to growth performance in chicken.

Even the significant different association between different genotypes and dominance effect were found in both of genes, but the results were not accorded to the hypothesis of this study. Many factors, epistasis effect, environmental effect, for example, may cause to the effect of the genes. Expression of each genotypes of both of genes should be the further investigation.

Conclusions

The significant association between *GH*, and *IGF-I* gene and dominance effect of the body weight at 0, 4, 6, 8, and 10 weeks of KR were detected. However, the heterozygous genotypes of both of genes were not the highest effect. Expression of each genotypes of both of genes should be the further investigation for better understanding about the role of these genes on heterosis effect of body weight of chicken.

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KEYWORD : Bodyweight, Korat chicken, Dominant effect, Growth hormone gene, Insulin like growth factor I gene

Table 1 Dominance effect of *GH* gene and *IGF-I* gene in Korat chicken

Gene	Genotype	Dominance effect ± SE				
		1 d	4 wk	6 wk	8 wk	10 wk
GH gene	A_1A_1 (n=137)	1.13±0.23[a]	5.45±0.50[a]	11.65±1.20[a]	11.45±1.97[a]	11.67±1.28[a]
	A_1A_3 (n=98)	1.28±0.28[a]	3.75±0.61[a]	8.23±1.48[a]	4.86±2.42[a,b]	6.64±1.57[b]
	A_1A_3 (n=123)	0.04±0.25[b]	-0.321±0.54[b]	2.52±1.30[b]	0.60±2.13[b]	-3.68±1.39[c]
	P-Value	0.00	0.00	0.00	0.00	0.00
IGF-I gene	AA (n=35)	1.24±0.42[a]	-4.67±1.27[b]	-2.75±2.17[b]	-10.43±3.35[b]	-14.85±2.85[c]
	AC (n=127)	-0.58±0.24[b]	1.17±0.71[a]	3.57±1.21[a]	-0.82±1.87[a]	0.08±1.59[b]
	CC (n=124)	0.20±0.24[a,b]	2.93±0.72[a]	7.88±1.23[a]	3.29±1.90[a]	9.34±1.61[a]
	P-Value	0.00	0.00	0.00	0.00	0.00

REFERENCES

Amills, M., N. Jimenez, D. Villalba, M. Tor, E. Molina, D. Cubilo, C. Marcos, A. Francesch, A. Sanchez and J. Estany. 2003. Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. Poultry Science 82:1485-1493.

Daughaday, W. H. 2000. Growth hormone axis overview–somatomedin hypothesis. Pediatric Nephrology 14:537-540.

Dawe, S., G. Francis, P. McNamara, J. Wallace and F. Ballard. 1988. Purification, partial sequences and properties of chicken insulin-like growth factors. Journal of endocrinology 117:173-181.

Duangjinda, M., I. Misztal and S. Tsuruta. 2007. BLUPF90-DairyPAK 3.0 [computer programe]. Khon Kaen University, Thailand.

Feng, X., U. Kuhnlein, S. Aggrey, J. Gavora and D. Zadworny. 1997. Trait association of genetic markers in the growth hormone and the growth hormone receptor gene in a White Leghorn strain. Poultry Science 76:1770-1775.

Florini, J. R., D. Z. Ewton and S. A. Coolican. 1996. Growth Hormone and the Insulin-Like Growth Factor System in Myogenesis*. Endocrine reviews 17:481-517.

Hanafi, M. and M. Iraqi. 2001. Evaluation of purebreds, heterosis, combining abilities, maternal and sex-linked effects for some productive and reproductive traits in chickens. Second International Conference on Anim. Prod. Health in Semi-Arid Areas:4-6.

Kadlec, J., B. Hosnedlová, V. Řehout, J. Čítek, L. Večerek and L. Hanusová. 2011. Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. Journal of Agrobiology 28:157-163.

Kato, Y., Y. Murakami, M. Sohmiya and M. Nishiki. 2002. Regulation of human growth hormone secretion and its disorders. Internal medicine 41:7-13.

Keambou, T., Y. Manjeli, B. Boukila, S. Mboumba, T. M. Mezui and B. H. Touko. 2010. Heterosis and reciprocal effects of growth performances in F1 crosses generations of Local x Hubbard chicken in the Western Highlands of Cameroon. Livestock Research for Rural Development 22.

Kuhnlein, U., L. Ni, D. Zadworny and W. Fairfull. 1997. DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Animal genetics 28:116-123.

Ma, Q., S. Liu, Z. Zhuang, L. Lin, Z. Sun, C. Liu, H. Ma, Y. Su and Q. Tang. 2012. Genomic structure, polymorphism and expression analysis of the growth hormone (*GH*) gene in female and male Half-smooth tongue sole (Cynoglossus semilaevis). Gene 493:92-104.

McMurtry, J., G. Francis and Z. Upton. 1997. Insulin-like growth factors in poultry. Domestic animal endocrinology 14:199-229.

Millar, D. S., M. Horan, N. A. Chuzhanova and D. N. Cooper. 2010. Characterisation of a functional intronic polymorphism in the human growth hormone (*GH1*) gene. Human genomics 4:1.

Moe, H. H., T. Shimogiri, K. Kawabe, M. Nishibori, S. Okamoto, T. Hashiguchi and Y. Maeda. 2009. Genotypic Frequency in Asian Native Chicken Populations and Gene Expression Using Insulin-like Growth Factor 1 (IGF1) Gene Promoter Polymorphism. The Journal of Poultry Science 46:1-5.

Nicoll, C. S., G. L. Mayer and S. M. Russell. 1986. Structural Features of Prolactins and Growth Hormones That Can Be Related to Their Biological Properties*. Endocrine reviews 7:169-203.

Nie, Q., B. Sun, D. Zhang, C. Luo, N. Ishag, M. Lei, G. Yang and X. Zhang. 2005. High diversity of the chicken growth hormone gene and effects on growth and carcass traits. Journal of Heredity 96:698-703.

Ohlsson, C., B.-A. Bengtsson, O. G. Isaksson, T. T. Andreassen and M. C. Slootweg. 1998. Growth Hormone and Bone 1. Endocrine Reviews 19:55-79.

Pandey, N., R. Singh, V. Saxena, N. Shit, R. Singh, R. Sharma and K. Sastry. 2013. Effect of IGF1 gene polymorphism and expression levels on growth factors in Indian colored broilers. Livestock Science 155:157-164.

Tang, S., D. Sun, J. Ou, Y. Zhang, G. Xu and Y. Zhang. 2010. Evaluation of the IGFs (IGF1 and IGF2) genes as candidates for growth, body measurement, carcass, and reproduction traits in Beijing You and Silkie chickens. Animal biotechnology 21:104-113.

Thakur, M., S. Parmar, T. Tolenkhomba, P. Srivastava, C. Joshi, D. Rank, J. Solanki and P. Pillai. 2006. Growth hormone gene polymorphism in Kadaknath breed of poultry. Indian Journal of Biotechnology 5:189.

Vasilatos-Younken, R., Y. Zhou, X. Wang, J. McMurtry, R. Rosebrough, E. Decuypere, N. Buys, V. Darras, S. Van der Geyten and F. Tomas. 2000. Altered chicken thyroid hormone metabolism with chronic *GH* enhancement in vivo: consequences for skeletal muscle growth. Journal of Endocrinology 166:609-620.

Wang, W., K. Ouyang, J. Ouyang, H. Li, S. Lin and H. Sun. 2004. Polymorphism of insulin-like growth factor I gene in six chicken breeds and its relationship with growth traits. ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES 17:301-304.

William, R. and E. Pollak. 1985. Theory of heterosis. Journal of Dairy Science 68:2411-2417.

Williams, S., S. Price and P. Siegel. 2002. Heterosis of growth and reproductive traits in fowl. Poultry Science 81:1109-1112.

Youssao, I., M. Senou, M. Dahouda, M. Kpodékon, J. Djenontin, N. Idrissou, G. Bonou, U. Tougan, S. Ahounou and H. Assogba. 2009. Genetic improvement of local chickens by crossing with the Label Rouge (T55XSA51): growth performances and heterosis effects. International Journal of Poultry Science 8:536-544.

Zhou, H., A. Mitchell, J. McMurtry, C. Ashwell and S. J. Lamont. 2005. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. Poultry Science 84:212-219.

O-14-5

Variance Components and Heritability Estimates on Carcass Traits in Betong Chicken (KU Line)

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INTRODUCTION

Meat of native chicken is popularly eaten in South-East Asia. Meat of native chicken has a unique taste, texture, and low fat. Selling price of indigenous chicken meat is around two or three times higher than selling price of broiler meat. Moreover, native chicken is suitably managed under tropical climate. Indigenous chicken meat becomes more interesting channel in niche market. Betong chicken (KU Line) was developed from the close flock of Betong chicken breed which is a native chicken breed in the southern part of Thailand by Kasetsart University. Growth performances and reproductive traits have been remained to be an important criterion in this population. However, carcass traits also are important in selection program in poultry breeding. Carcass traits must be considered to a criterion for selection program in order to increase profitability. Carcass traits can be generally measured from their progeny and relative because these traits must be slaughtered before measured. Therefore, the objective in this study was to estimate variance components and heritabilities for some carcass traits in Betong chicken (KU Line) from its progeny.

METHODOLOGY

A pedigree population was constituted of 945 chickens from 7 generations deep. Performance data were collected from 526 chickens of the offspring in Betong chicken (KU Line) population. The offspring used in the present study were produced from 28 cocks and 97 hens. There were 2 progeny test flocks which were received same feeding, management and diets according to the recommendation of Putsakul et al. (2010). All animals were recorded pedigree and performance data.

All chickens in progeny test were weighed at 16 weeks old. Birds were randomly sampled within full-sib family in order to measure carcass traits. Chickens were slaughtered at 16 weeks old. Chickens were fasted an overnight and weighed LW before slaughtering. The process of slaughtering for ante-mortem and post-mortem according to the good manufacturing practices for poultry abattoir from Thai Agricultural Commodity and Food Standard (TACFS, 2006). Carcass was dissected into the different cutting parts according to standard of chicken meat (TACFS, 2005). Carcass, breast muscle and leg were weighed in this step.

The performance data were collected namely BW16, LW, CW, BMW and LEG. Descriptive statistics were calculated. Variance components and heritability estimates were estimated with the Average Information Restricted Maximum Likelihood (AI-REML) (Johnson and Thompson, 1995) by WOMBAT software program (Mayer, 2013). The information or prior values for estimation of variance in multiple trait analysis were obtained from single trait analyses in the same dataset. The animal model used in this analysis is shown in Figure 1.

RESULT & DISCUSSION

Descriptive statistics for BW16 and carcass traits (LW, CW, BMW, and LEG) are summarized in Table 1. The percentages of CW, BMW, and LEG were 81.26%, 14.41%, and 25.73%, respectively. The mean of BW16 and carcass percentages of Betong chicken (KU Line) were consistent with the previous reports in Betong chicken (KU Line) (Putsakul et al., 2010; Makchumpon et al., 2015).

Variance components and heritability estimates of BW16 and carcass traits (LW, CW, BMW, and LEG) are shown in Table 2. Heritability estimates of BW16 and carcass traits were ranged from 0.45 to 0.60. The heritability estimate of BW16 was high (0.47) which was supported in the earlier study result in Betong chicken (KU Line) by Wangtaweesukkamol et al. (2013). There was genetic variation in BW16 in this population. Selection of BW16 in parent stock can improve BW16 by genetic selection in the next generation. However, heritability estimate for BW16 has been found to be different from the report in native chicken breed in Ethiopia by Dana et al. (2011). Difference in heritability estimates might be attributed to method of estimation, breed, sample size, population

structure, and environmental effects.

Leg weight had the highest value of heritability estimate. The estimate of heritability of LEG was found to be higher than that of LEG by Gaya et al. (2006). Heritability estimate of BMW (0.56) was high in agreement with the other estimates which were reported by Chen et al. (2008) in Chinese native chicken, Le Bihan-Duval et al. (1999) in broilers and Zerehdaran et al. (2004) in broilers. The heritability estimates of LW and CW (0.50 and 0.45, respectively) were high. Moderate heritability estimate of CW was published by Zerehdaran et al. (2004) in broilers. Therefore, all of the estimates for carcass traits (LW, CW, BMW and LEG) were high heritable, genetic selection would be effective in improving these traits.

CONCLUSION

The results showed that genetic variation in BW16 and carcass traits (LW, CW, BMW, and LEG) were found in Betong chicken (KU Line) population. Heritability estimates of BW16 and carcass traits (LW, CW, BMW, and LEG) were high. Genetic selection of BW16, LW, CW, BMW, and LEG can improve genetic gain in these traits on this population. These carcass traits can be improved by genetic selection in Betong chicken (KU Line) population. Selection of carcass traits in parent stock can be effective in genetic response on these traits in their progeny.

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KEYWORD : heritability, carcass, Thai native chicken

$$\mathbf{y_i} = \mathbf{X_i b_i} + \mathbf{Z_i a_i} + \mathbf{e_i}; \quad \mathbf{var}\begin{bmatrix}\mathbf{a_i}\\ \mathbf{e_i}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\otimes\mathbf{A} & \mathbf{0}\\ \mathbf{0} & \mathbf{R}\otimes\mathbf{I}\end{bmatrix}$$

where $\mathbf{y_i}$ = the vector of the observations, $\mathbf{b_i}$ = the vector of fixed effects including hatching batch and sex, $\mathbf{a_i}$ = the vector of animal additive genetic effect, $\mathbf{X_i}$ = the incidence matrix of fixed effect associating elements of $\mathbf{b_i}$, $\mathbf{Z_i}$ = the incidence matrix of random effect associating elements of $\mathbf{a_i}$, $\mathbf{e_i}$ = the vector of residuals, **A** = the numerator relationship matrix, **I** = identity matrix, **G** and **R** = the (co)variance matrices of additive genetic and residual, respectively.

Figure 1 Animal model

Table 1 The number of records (N), mean ± standard deviation (SD), minimum (Min) and maximum (Max) of carcass traits

Trait[1]	N	Mean ± SD	Min	Max
BW16 (g)	526	1,931.58 ± 376.28	962	2,949
LW (g)	251	1,912.49 ± 374.65	1,197	2,763
CW (g)	252	1,554.14 ± 328.59	957	2,222
BMW (g)	252	275.55 ± 42.98	159	399
LEG (g)	252	492.03 ± 121.24	266	738

1 BW16 = body weight at 16 weeks old, LW = live body weight, CW= carcass weight, BMW = breast meat weight, and LEG = leg weight

Table 2 Variance components and heritability ± standard error ($h_a^2 \pm SE$) of body weight and carcass traits

Trait[1]	σ_a^2	σ_p^2	$h_a^2 \pm SE$
BW16	13,389	28,435	0.47 ± 0.12
LW	11,927	23,929	0.50 ± 0.13
CW	7,026	15,631	0.45 ± 0.13
BMW	532	945	0.56 ± 0.15
LEG	1,269	2,117	0.60 ± 0.14

1 BW16 = body weight at 16 weeks old, LW = live body weight, CW= carcass weight, BMW = breast meat weight, and LEG = leg weight, σ_a^2= additive genetic variance (g^2) and σ_p^2 = phenotypic variance (g^2)

REFERENCES

Chen, J.L., G.P. Zhao, M.Q. Zhao, J. Wen and N. Yang. 2008. Estimation of genetic parameters for contents of Intramuscular fat and inosine-5^'monophospahte and carcass Traits in Chinese Beijing-You chickens. Poult. Sci. 87: 1098-1104.

Dana, N., E.H. vander Waaij and J.A.M. van Aredonk. 2011. Genetic and phenotypic parameters for body weights and egg production in Horro chicken of Ethiopia.

Trop. Anim. Health Prod. 43(1): 21–28.

Gaya, L.G., J.B.S. Ferraz, F.M. Rezenda, G.B. Mourao, E.C. Mattos, J.P. Eler and

T. Michelan Filho. 2006. Heritability and genetic correlation estimates for performance and carcass and body composition traits in a male broiler line. Poult. Sci. 85: 837-843.

Johnson, D.L. and R. Thompson. 1995. Restricted maximum likelihood estimation of variance components for univariate animal models using sparse matrix techniques and average information. J. Dairy Sci. 78: 449-456.

Le Bihan-Duval, E., N. Millet and H. Remignon. 1999. Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. Poult. Sci. 78: 822-826.

Makchumpon, S., C. Bunchasak, T. Poeikampha, K. Poonthawee and C. Rakangthong. 2015. Comparison of meat quality between broiler and KU betong chickens. In Proceeding of 53rd Kasetsart University Annual Conference: Animals. 3-5 February 2015. Kasetsart University, Thailand.

Mayer, K. 2013. WOMBAT a Program for Mixed Model Analyses by Restricted Maximum Likelihood. Animal genetic and breeding unit, University of New England, Armidale, Australia.

Putsakul, A., C. Bunchsak, B. Chomtee, S. Kao-ian and P. Sopannarath. 2010. Effect of dietary protein and metabolizable energy levels on growth and carcass yields, pp. 158-166. In Proceeding of 48th Kasetsart University Annual Conference: Animals. 3-5 February 2010. Kasetsart University, Thailand.

Thai Agricultural Commodity and Food Standard. 2005. Chicken Meat (TACFS 6700-2005). Available Source: http://www.acfs.go.th/standard/download/eng/chicken_meat.pdf, August 27, 2014.

_____. 2006. Good Manufacturing Practices for Poultry Abattoir (TACFS 9008-2006). Available Source: http://www.acfs.go.th/standard/download/eng/poultry_abattoir.pdf, August 25, 2014.

Wangtaweesukkamol, N., W. Loongyai, B. Chomtee and P. Sopannarath. 2013. Genetic parameters for body weights in Betong chicken (KU Line). Agri. Sci. J. 44 (1) (Suppl.): 167-170.

Zerehdaran, S., A.L.J. Vereijken, J.A.M. van Arendonk and E.H.van der Waaij. 2004. Estimation of genetic parameters for fat deposition and carcass traits in broilers. Poult. Sci. 83: 521-525.

O-14-6

Relationship between eggshell strength and egg shape in White Leghorns

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ABSTRACT

【Objectives】 Shell strength is an important trait in the chicken egg industry, but the influence of egg shape on shell strength has not been investigated sufficiently. The main objective of this study was to clarify the relationship between egg shape and shell strength and to estimate the genetic parameters of traits related to shell strength. yMethods zWe assessed two populations of White Leghorns that were divergently selected for strong and weak shell strength according to non-destructive deformation of the eggshell (NDD) over 16 generations. We used the restricted maximum likelihood approach to estimate the genetic parameters of three traits—NDD, shell breaking strength, and shape index (100% × width/length). The phenotypic correlations between the shape index and breaking strength were calculated within and across generations.

【Results】 The heritability estimates for the three traits ranged from 0.371 to 0.534 for the strong line and 0.409 to 0.487 for the weak line. The phenotypic correlation between breaking strength and egg width across generations was positive in the weak line but negative in the strong line. However, in both lines, within-generation correlations between breaking strength and egg width were consistently positive. Similarly, the phenotypic correlation between breaking strength and egg length across generations was positive in the weak line but negative in the strong line, with the within-generation correlations of these traits consistently negative in both lines. Under the selection, the relationship between egg shape and shell strength was not obvious. However, analysis of the genetic parameters revealed that egg width had positive and length had negative genetic correlations with breaking strength. In conclusion, our results revealed that rounder chicken eggs tended to be more resistant to breakage than were more elongated eggs, for the range of values studied.

INTRODUCTION

Because it leads to substantial economic losses, eggshell breakage is a serious problem in the poultry industry. In addition, eggshell breakage poses a potential threat to food safety (Bain et al., 2006), such as salmonella contamination, and can be linked directly to poor eggshell quality. Therefore, considerable research effort has been directed toward reducing shell breakage and has largely focused on increasing eggshell thickness and breaking strength (Mertens et al., 2006). Traits including non-destructive deformation (NDD), eggshell thickness, and eggshell weight are generally considered to be reliable criteria for estimating eggshell strength. In addition, several studies have confirmed that eggshell strength is highly dependent on the egg shape index (Anderson et al., 2004; Altuntas and Şekeroğlu, 2008). In the present study, we furthered this line of reasoning by assessing how long-term selection for a single eggshell trait, NDD, affects the shape index in White Leghorns. Specifically, we clarified the relationships between egg shape and several traits related to eggshell strength.

MATERIALS AND METHODS

We used two populations of White Leghorns which were divergently selected from a single base population (*n* = 412) for 16 generations according to eggshell strength. Specifically, birds were selected for high or low NDD values, referred to hereafter as the weak line and the strong line, respectively. From generation 2 to generation 12, selection was based on individual performance; 80 female and 10 male chickens were selected per generation, by using the full-sib mean for male selection given that they did not have their own records. From generation 13 to generation 16, to prevent excessive inbreeding, a within-family selection procedure was used (Nirasawa, 2010). The restricted maximum likelihood approach (Patterson & Thompson, 1971) was applied to estimate genetic parameters for three traits—NDD, shell breaking strength, and egg shape index (that is, 100% × width/length)—in a multiple-trait animal model (Henderson, 1975). The strong line comprised 3843 birds (average, 240.1 per generation) in total, and the weak line comprised 3689 birds (average, 230.6 per generation). Each record represents the average of three measurements taken on three different eggs laid by a given hen between 36 and 38 weeks of age.

RESULTS AND DISCUSSION

Egg size and shape index

Over the 16 generations of selection, the generation average for egg width decreased from 4.19 to 4.04 cm in the weak line and from 4.22 to 4.06 cm in the strong line. Similarly, the average egg length decreased from 5.77 to 5.52 cm in the weak line and from 5.78 to 5.46 cm in the strong line. The size of eggs tended to be smaller after selection in both lines, consistent with a previous study in which single-trait selection (that is, NDD) reduced egg size (Murao, 2015). Regarding the egg shape index, the generation average increased from 72.7% to 73.2% in the weak line and from 73.1% to 74.4% in the strong line (Figure 1). However, none of these averages differed significantly between generations 1 and 16 ($P > 0.05$).

Correlations between egg shape and shell strength traits

Correlations between egg width and length, and breaking strength and non-destructive deformation were calculated using the averages of across generations (referred to as the overall correlations) as well as within each generation. Overall correlations using whole generations for weak and strong lines were as indicated in Table 1. Egg width and breaking strength showed the positive correlation, 0.871, in the weak line, however, it was negative, -0.280, in the strong line. Egg length and breaking strength showed the positive correlation, 0.630, in the weak line, but it was negative, -0.507, in the strong line. Therefore, these correlations between two lines were quite different.Regarding within-generation correlations (Table 2), egg width and breaking strength showed positive correlation in all generations in both lines. In contrast, correlations between egg length and breaking strength were nearly 0 or negative in both lines. Therefore, the sign of the overall correlation coefficients was quite different than that found within each generation.

Genetic parameters

The heritability estimates for three traits—NDD, breaking strength, and shape index—ranged from 0.409 to 0.487 for the weak line and from 0.371 to 0.534 for the strong line (Table 3). Phenotypic and genetic correlations between shape index and breaking strength were all positive in both lines. Therefore, our analysis of the genetic parameters revealed that rounder chicken eggs tended to be stronger than were more elongated eggs for the range of values examined. Together, our findings suggest that long-term selection on the sole trait of NDD decreased egg width and length in both lines of White Leghorns. Because the base population for this study had been selected by using multiple traits including egg weight, suspending selection on all traits except eggshell strength apparently decreased egg size. In support of this explanation, egg weight decreased in both lines selected according to eggshell strength only (Murao 2015). In addition, correlation values (Tables 1 and 2) were inconsistent depending on whether the overall population or that for the generation was analyzed. However, in both lines, within-generations correlations of breaking strength were positive with width and negative with length, and genetic parameters (Table 3) similarly showed the positive effect of width and negative effect of length on breaking strength.

To clarify the mechanisms underlying the changing sign of correlation values within compared with across generations, we present three populations—generations 2, 8, and 14 (G2, G8 and G14)—from the strong line as an example (Figure 2). Within each of these generations, breaking strength and width clearly were positively correlated, but when grouped into a single, overall population, the correlation between breaking strength and egg width was negative. We consider that two types of pressure served to 'hide' the positive within-generation correlation between width and shell strength when we estimated the overall correlation in the combined population. First, downward pressure was exerted leading to the selection of smaller eggs in both lines because of the suspension of selection on egg weight. The second pressure was caused by the intended selection on shell strength: the strong line was under pressure to move toward increased breaking strength, whereas the weak line was selected for decreased breaking strength. Therefore, the synchronized effect of these two pressures moved strong-line populations toward a decreased width and increased breaking strength. Therefore, even though the within-generation correlation was positive, the correlation for the overall population emerged as negative in the strong line. In contrast, pressure toward selection of decreased eggshell strength led to the overall positive correlation between breaking strength and width for the weak line.

In conclusion, the relationship between egg shape and shell strength was obscured under selection for NDD only. Our results ultimately revealed that rounder eggs tended to be more resistant to breakage than were more elongated eggs for the range of values studied.

KEYWORD : Chicken, Shell strength, Egg shape, Selection

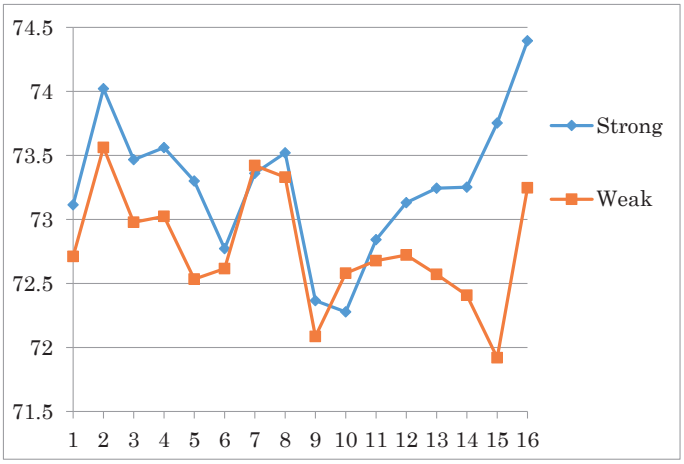

Figure 1. Generation average of shape index for weak and strong lines (Vertical axis: shape index %, Horizontal axis: generation number)

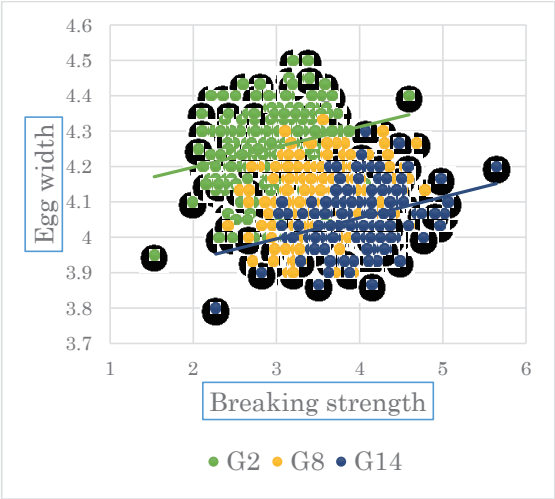

Figure 2. Breaking strength and egg width in the strong line

Table 1. Overall correlations between shape traits (width and length) and eggshell strength traits

	Weak line		Strong line	
	Width	Length	Width	Length
Breaking strength	0.871	0.630	–0.280	–0.507
Non-destructive deformation	–0.908	–0.829	0.594	0.699

Negative correlation values are in red.

Table 2. Within-generation correlations between shape traits and breaking strength

	Weak line		Strong line	
Generation	Width	Length	Width	Length
1	0.205	0.007	0.183	–0.097
2	0.095	–0.164	0.253	–0.135
3	0.316	0.041	0.128	–0.233
4	0.295	–0.085	0.162	–0.167
5	0.267	–0.136	0.116	–0.132
6	0.183	–0.217	0.089	–0.190
7	0.329	0.028	0.045	–0.197
8	0.251	–0.179	0.170	–0.241
9	0.193	–0.073	0.217	–0.084
10	0.271	–0.093	0.092	–0.280
11	0.278	–0.050	0.157	–0.257
12	0.015	–0.148	0.157	–0.257
13	0.368	–0.025	0.084	–0.196
14	0.155	–0.121	0.343	–0.217
15	0.114	–0.127	0.264	–0.049
16	0.092	–0.177	0.134	–0.081
Average	0.214	–0.095	0.162	–0.176

Negative correlation values are in red

Table 3. Heritabilities (diagonal) and phenotypic (upper triangle) and genetic (lower triangle) correlations between shape traits and eggshell strength traits

	Weak line				Strong line		
	NDD	BS	SID		NDD	BS	SID
NDD	0.409	–0.751	–0.076	NDD	0.457	–0.702	–0.059
BS	–0.832	0.487	0.250	BS	–0.824	0.371	0.287
SID	–0.086	0.315	0.479	SID	–0.049	0.311	0.534

NDD, non-destructive deformation; BS, breaking strength; SID, shape index

REFERENCES

Altuntas, E., & Şekeroğlu, A. (2008). Effect of egg shape index on mechanical properties of chicken eggs. *Journal of Food Engineering*, 85(4), 606-612.

Anderson, K. E., Tharrington, J. B., Curtis, P. A., & Jones, F. T. (2004). Shell characteristics of eggs from historic strains of single comb White Leghorn chickens and the relationship of egg shape to shell strength. *Int. J. Poult. Sci*, 3(1), 17-19.

Bain MM, Dunn IC, Wilson PW, Joseph N, De Ketelaere B, De Baerdemaeker J, Waddington D. 2006. Probability of an egg cracking during packing can be predicted using a simple non-destructive acoustic test. *British Poultry Science* 47, 462-469.

Henderson, C.R., 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics. 423-447.

Mertens, K., Bamelis, F., Kemps, B., Kamers, B., Verhoelst, E., De Ketelaere, B., ... & De Baerdemaeker, J. (2006). Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. *Poultry science*, 85(9), 1670-1677.

Murao, M.: Graduation study. Animal breeding laboratory, College of Bioresource Sciences, Nihon University. 2015

Nirasawa, K.: Studies on genetic improvement of eggshell strength by selection for nondestructive deformation. National Institute of Livestock and Grassland Science, 10:29-84. 2010

Patterson, H.D., Thompson, R., 1971. Recovery of interblock information when block sizes are unequal. Biometrika. 58, 545-554.

O-14-7 COMBINING ABILITY OF GROWTH TRAITS IN BLACK-BONE (BLACK-BONE HMONG, BLACK-BONE CHINESE) AND THAI NATIVE CHICKENS (PRADU-HANG DAM)

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INTRODUCTION

Black-bone chickens are more likely consumed in every year, as their meats have several good substances to healthy of consumer. For example, melanin is an antioxidant to increase immunology (Tian et al., 2007). Carnosine is a natural dipeptide consisting of β-alanine and histidine, which plays role for physiological buffer in muscle and anti-oxidant function (Rosick, 2006; Tu et al., 2009). In addition, their meat has many properties to body including reinforce immunity system, prevent emaciation and feebleness, treat diabetes and anemia, and cure women's diseases like menoxenia and postpartum complications (Qi et al., 2012; Tian et al., 2007). However, the growth rate of black-bone chicken breeds is slower than others Thai native chicken, which means they have not reached their full economic value yet. Therefore, combining the valuable meat quality of the black bone chickens with the efficiency of the Thai native chicken (Pradu-Hang dam: KKU55), as same as black characteristic, is interesting. However, all varieties of black chickens, genetic information for growth were scared. This information is necessary to bring the weight of the genetic parameters as a basis to consider to make progress in the breeding of growing the next great.

The aim of this research was to study general and specific combining abilities existing in growth traits of purebred and crossbred to selection improvement program.

MATERIALS AND METHODS

Data

Body weights at 0, 4, 8, 12, and 14 weeks of age were defined as growth traits form generations 1, a number of animals on **Table 2**. Black chicken breeds were three native chickens including Black-bone Chinese (BC), Black-bone Hmong (BH), Thai Native chicken (TN) and were bred by diallel mating system. This system produced nine mating pairs included BCxBC, BCxBH, BCxTN, BHxBC, BHxBH, BHxTN, TNxBC, TNxBH and TNxTN. Black chicken and crossbred Thai native chickens (Pradu Hang dam) was reared in an experimental farming of Research and Development Network Center for Animal Breeding (Native Chicken), Faculty of Agriculture, Khon Kaen University. Male to female ratio was 1:5. After hatching, all chickens were kept data from 0 till 14 weeks of age. Preparation of the data contained the number of the animal, hatch, sex, father number, mother number, generation, birth weight, body weight at 4, 8, 12, and 14 weeks of age, the pedigree file contains the serial number, father number, mother number and generations.

Statistical analysis

A diallel analysis was used to estimate the general and specific combining abilities and the specific combining ability, according to Griffing's (1956) method 1.

A diallel table was set up as follows:

Table 1 Mating system for the test diallel analysis.

where,
P= number of lines for crossing; Xi= grand total of breed i;
Xj= grand total of breed j; Xij= population mean;

General combining ability effects of crosses was calculated as:
gi= 1/2p (Xi. + X.j) - 1/p2 X..

Specific combining ability effects of crosses was calculated as:
sij = ½ (Xij + Xji) - 1/2p (Xi. + X.i + Xj. + X.j) + 1/2pX..
Reciprocal combining ability effects of crosses was calculated as:
rij= ½ (Xij+Xji)

RESULTS AND DISCUSSION

BODY WEIGHT

At 0 week, the body weight of S-BHxTN, G-TN and S-BCxTN were 33.97, 33.90 and 33.65 g, and were higher than other breeds (P <0.01; **Table 3**). Body weight at 4 weeks old was lower in G-BH, G-TN, S-BCxBH, S-BHxTN and R-TNxBH compared with G-BC and S-BCxTN (P <0.01). At 8 weeks old, G-BC body weight was higher than G-BH (794.03 vs 526.38 g;P <0.01), meanwhile the significant lowest body weights were recorded in S-BCxBH (612.59 g), S-BHxTN (613.78 g) and R-TNxBH (656.28 g) At 12 and 14 weeks, the body weight of S-BCxTN was the highest (P <0.01), while the body weight of G-BH was consistently the lowest.

COMBINING ABILITIES ON BODY WEIGHT

The crossbreeding genetic estimations for GCA, SCA and RCA on BW at day old to 14 weeks old are presented in **Table 4**. The crossbreeding genetic estimations for GCA were positive at 4, 8, 12 and 14 weeks old in a G-BC strain. However, the values of the G-TN strain were positive at 8, 12 and 14 weeks. The variation in GCA is due to additive genetic variance. G-BC and G-TN will be good for selection on genetic improvement program for body weight. The crossbreeding genetic estimations for SCA for BW were positive at day old to 14 weeks old only in the S-BHxTN strain. However, the values of the S-BCxTN strain were positive at 4, 8, 12 and 14 weeks. The variation in SCA is due to non-additive genetic variance; heterosis, dominance, over- dominance and epistasis (Singh and Kumar, 1994; Adebambo et.al., 2011). The crossbreeding genetic estimations for RCA were negative at day old to 14 weeks old only in the R-BHxBC strain, but the values were positive at day old to 14 weeks old only in the R-TNxBC strain.

CONCLUSIONS

BCxTN, using G-BC as a sire line and G-TN as a dam line, should be developed as crossbred due to good general combining ability for body weight. The diallel mating system was effective selection of growth traits to genetic progress.

ACKNOWLEDGEMENTS

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KEYWORD : Body weight, Growth performance, Thai indigenous chicken, Black-bone chicken, Combining ability

Table 1 Mating system for the test diallel analysis.

Sire Breeds[1/]	Dam Breeds		
	BC	BH	TN
BC	√	√	√
BH	√	√	√
TN	√	√	√

BC = Black-bone Chinese, BH = Black-bone Hmong, TN = Thai Native chicken.

Table 2 Number of data analysis.

Parameters	BW0	BW4	BW8	BW12	BW14
Purebred					
G-BC	72	71	67	67	67
G-BH	55	50	47	44	43
G-TN	108	103	100	99	96
Main crosses					
S-BCxBH	63	60	54	51	50
S-BCxTN	79	76	73	72	72
S-BHxTN	79	77	74	71	70
Reciprocal crosses					
R-BHxBC	86	80	80	76	74
R-TNxBC	103	100	94	89	88
R-TNxBH	54	51	43	42	41

Table 3 Least square means of body weight of Black bone chicken and crossbred (g/bird).

Parameters	BW0	BW4	BW8	BW12	BW14
Purebred					
G-BC	32.71^{ab}	344.08 ^{a}	794.03 ^{a}	1333.43 ^{a}	1580.75^{ab}
G-BH	29.87 ^{d}	208.20 ^{e}	526.38 ^{f}	926.59 ^{e}	1108.84 ^{d}
G-TN	33.90 ^{a}	268.05 ^{d}	666.70 ^{d}	1230.81 ^{b}	1522.19 ^{b}
Main crosses					
S-BCxBH	30.60 ^{d}	265.16 ^{d}	612.59 ^{e}	1093.14 ^{d}	1297.60 ^{c}
S-BCxTN	33.65 ^{a}	340.26 ^{a}	779.32^{ab}	1371.39 ^{a}	1635.97 ^{a}
S-BHxTN	33.97 ^{a}	255.71 ^{d}	613.78 ^{e}	1088.03 ^{d}	1324.71 ^{c}
Reciprocal crosses					
R-BHxBC	31.88^{bc}	287.87 ^{c}	711.13 ^{c}	1148.82^{cd}	1388.78 ^{c}
R-TNxBC	30.98^{cd}	317.38 ^{b}	748.19^{bc}	1355.51 ^{a}	1599.66^{ab}
R-TNxBH	31.85^{bc}	251.43 ^{d}	656.28^{de}	1171.90^{bc}	1376.34 ^{c}

^{abcdef} Means with different superscripts on the same column are significantly different; BW0, BW4, BW8, BW12 and BW14 mean body weights at 0, 4, 8, 12 and 14 weeks, respectively.

Table 4 Combining abilities for body weight at birth weight, 4, 8, 12 and 14 weeks old.

Parameters	N	BW0	4wk	8wk	12wk	14wk
General combining abilities (GCA)						
G-BC	344	-0.07	40.01	61.17	81.55	87.83
G-BH	239	-0.82	-30.37	-70.95	-131.89	-158.58
G-TN	506	0.89	-9.65	9.78	50.34	70.75
Specific combining abilities (SCA)						
S-BCxBH	278	-0.03	23.74	-7.07	-19.75	-12.15
S-BCxTN	372	-0.66	5.33	14.09	40.49	33.15
S-BHxTN	371	0.69	0.45	17.49	20.45	12.26
Reciprocal combining abilities (RCA)						
R-BHxBC	396	-0.64	-61.35	-49.27	-27.84	-45.59
R-TNxBC	474	1.34	11.44	15.56	7.94	18.16
R-TNxBH	231	-1.06	-2.14	21.25	41.94	25.81

G: General combining ability; S: Specific combining ability; R: Reciprocal combining ability.

REFERENCES

Adebambo, A.O., Ikeobi, C.O.N., Ozoje, M.O., Oduguwa, O.O. and Adebambo Olufunmilayo, A. 2011. Combining ability of growth traits among pure and crossbred meat type chickens. Arch. Zootec. 60 (232): 953-963.

Qi, R.L., C. Sun, H.L. Yang, X. Zhao and Z. Tao. 2012. The molecular mechanism of fat accumulation changes in Black-Bone chickens with different energy intakes. J. of Anim and Feed Sci., 21:361–371.

Rosick, and R. Edward. 2006. How Carnosine Protects Against Age-Related Disease. Available Online: http://www.lef.org/magazine/mag2006/jan2006_report_carnosine_02.htm. Accessed Oct. 29, 2013.

Singh, R.P. and Kumar, J. 1994. Biometrical methods in poultry breedings. Kalyani publishers. 240 pp.

Tian, Y., M. Xie, W. Wang, H. Wu, Z. Fu, and L. Lin. 2007. Determination of carnosine in Black-Bone Silky Fowl (Gallus gallus domesticus Brisson) and common chicken by HPLC. Eur Food Res Technol. 226:311–314.

O-14-8

Estimation of Growth Curve Parameters in Betong Chicken (KU Lines)

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Introduction

Success of improvement in commercial broiler production system has been achieved by selection for early growth and combined with improvement of environmental factors. The age at slaughter of commercial broiler chicken is reduced to 42 days or less than that and slaughter weight is higher than 2 kg. The production system and meat from broiler are similar all over the world. Native chicken meat in several countries in Asian countries is famous for unique taste and it can be sold with 2 or 3 times higher price than commercial broiler meat especially in niche market. However native chicken production system is different, takes long time to slaughter age and has high cost of production. Betong chicken is a renowned indigenous breed in the southern part of Thailand. Betong chicken (KU Line) has been developed by Kasetsart University from selection in a closed flock based on growth performance and general appearance to maintain some outstanding characteristics of original Betong, for example, slow feathering rate, golden brown feather color, red single comb and yellow skin. Dissimilarity of feather color (golden brown and white) was observed after selection for a few generations. Even they were developed from the same genetic origin; growth of them should be studied. Growth of animal is affected by genetic and non genetic factors. Non linear growth functions, logistic growth function, Richard, Gompertz and Von Bertalanffy, were usually applied in chicken and parameters describe body weight and age relationship (Grossman and Bohren, 1985; Rogers et al., 1987; Yang et al., 2006). The parameters interpret animal biology and body weight at hatching, age and body weight at inflection point and mature weight can be predicted. Several studies show that growth curve parameters were heritable and differ between male and female (Barboto, 1991; Mignon-Grasteaue et al., 1999; Yang et al., 2006). Therefore, the objective of this study was to investigate the different growth patterns due to feather color, sex and hatching batch of Betong chicken (KU Line).

Materials and Methods

Data originated from 3 hatching batches of golden brown and white Betong chiken (KU Line) flock at Kasetsart University. After hatching, each chick was identified by a wing band and weighted on the day of hatching and weighted weekly until 8 weeks of age and then every 2 weeks until 16 weeks of age. Chicks were reared in housing system and feed and water were supplied *ad libitum* acording to routine method . Sex of chick was classified by size of comb at 4 weeks old, and was confirmed at 8 weeks old. Standard routine vaccination rogram was provided for chickens in the flocks. Thus, total of 2,145 chickens with 20,221 records were included in this analysis. Two mathematical functions, Gompertz and Von Bertalanffy (Zheng, 1995; Mignon-Grasteaue et al., 1999) were chosen to fit the body weight data. The functions are shown as:

Gompertz function:

$y=A \times \exp(-B \times \exp(-K \times t))$

Von Bertalanffy

$y=A \times [1-[B \times \exp(-k \times t)]]^3$

where y is the body weight at age t; A is the upper asymptotic weight at infinity age or the estimation of mature weight; B is the integration constant; K is the rate of maturation; Five models with combined data (Model I), each main effect (color feather, sex and hatching batch; Model II, III and IV) and those 3 combination effects (Model V) were employed for each function to determine the importance of the effects. Marquardt algorithm of nonlinear estimation was carried out with the convergence criteria of 10^{-5} (SAS, 1990). Mean square error (MSE), coefficient of determination (R^2) and reduction sum square were computed for goodness of fit for each model. Predicted values of body weight at hatching and age and body weight at inflection point were calculated.

Results and Discussion

Estimates of growth curve parameter and predicted values for Gompertz and Von Beterlanffy function are shown in Table 1 and 2. Small differences of MSE and R^2 between Gompertz and Von Bertalanffy functions for every model were found and MSE of Von Bertalanffy was lower than that of Gompertz while R^2 of them was

high with similar values (0.96 to 0.98). Fitting parameters for each effects, feather color, sex and hatching batch, decreased MSE and reduction sum squares of Model II, III and IV compared with Model I were significant ($P<0.05$). Moreover differences of estimated parameters between male and female (Model III) agreed with several studies in commercial and native chicken (Mignon-Grasteau et al., 2000). Hatching batch in this study referred to the environment and management conditions which affected on estimates of growth curve. It is indicated that the combinations between those effects needed to be examined with Model V and the results revealed that interactions were found but not strong (Figure 1 and 2). However fitting the curve independently for each combination effects could explain the growth pattern of chicken. Estimates of "A" referred to mature weight from Von Bertalanffy (1,664.60 to 4,011.40 g) were higher than those from Gompertz (1,487.49 to 3,113.90 g) but values of "K" could not comparable but the magnitude of K was similar between those 2 functions. The higher values of K showed the younger age at inflection point which resulted in light body weight at that point. Estimates of growth curve parameters in the same feather color and sex but different hatching batch of Betong chiken (KU Line) were vary. Environmental effect from hatching batch 3 was poorer than that from hatching batch 2 and 1. The estimated mature weights of commercial broiler were higher than Betong chicken (KU Line) due to genetic origins and selection for rapid early growth rate (Knizetova et al., 1991; Hancock et al., 1995) Overestimated body weights at hatching from Gompertz were observed, on the other hand underestimated values were shown from Von Bertalanffy . Most of the estimated parameters at inflection point of Gompertz were higher than those of Bon Bertalanffy. Age and body weight at inflection point ranged from 43.42 to 72.19 days and 493.21 to 1,188.56 g and 47.64 to 69.45 days and 547.18 to 1,145.54 g for Gompertz and Von Bertalanffy, respectively. In Betong chicken (KU Line), female and light weight (white color) reached to the inflection point faster than male and heavy weight (golden brown color). The inflection point of commercial broiler strains in male was estimated to be 44.4 days of age (Golimytis, 2003). In contrast, the point in some purebred meat type chicken was later (Knizetova et al., 1991).

Conclusion

Gompertz and Von Bertalanffy functions were well fitted to estimate growth curve parameters. Von Bertalanffy seemed to be better than Gompetz for this Betong Chicken (KU Line). Models fitted with each combination between feather color, sex and hatching batch were the best according to less MSE. White feather color and female chicken reached to the inflection point earlier than golden brown feather color and male chicken. Due to the different of hatching batches, improvement of environment and management affected the growth of chicken.

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KEYWORD : Thai native chicken, Betong chicken (KU Line), growth curve, body weight

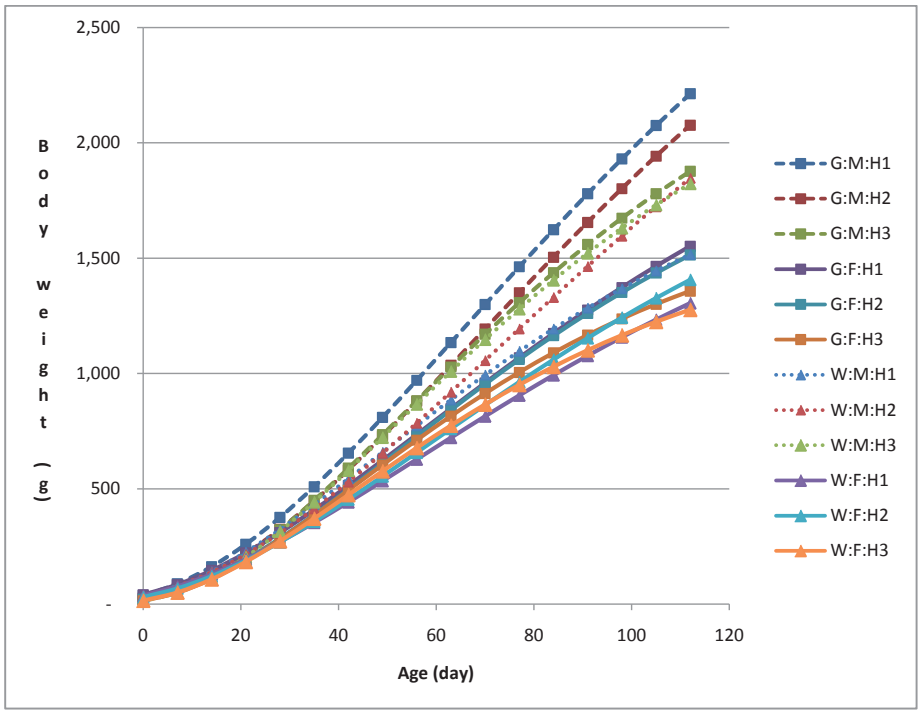

Figure 1 Prediction of growth curves from Gompertz function for each combination of feather color (G=golden brown, W=white), sex (M=male, F=female) and hatching batch (H1, H2 and H3 = hatching batch 1, 2 and 3)

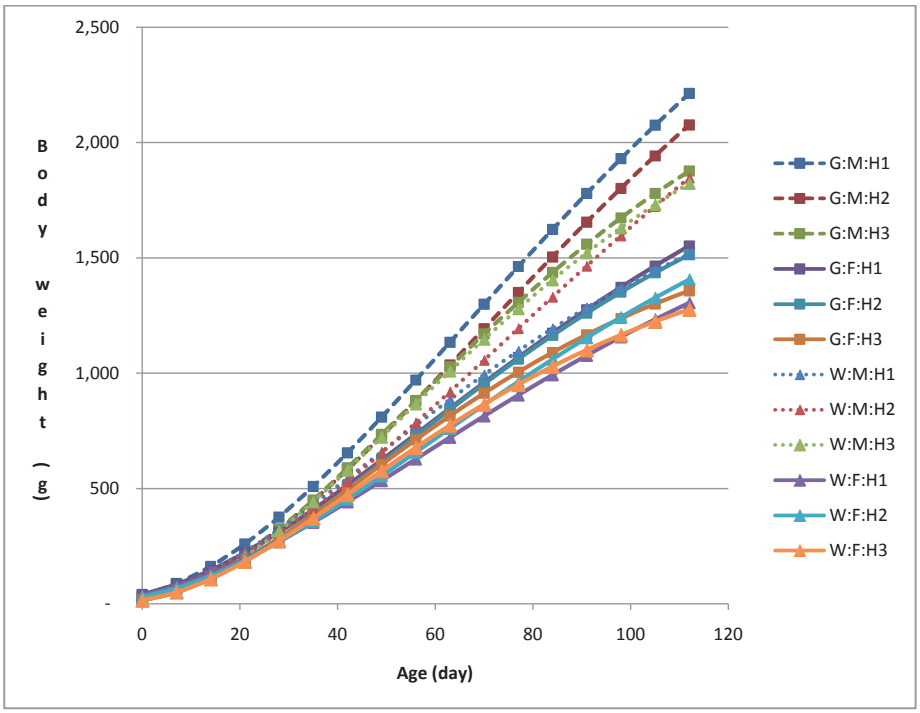

Figure 2 Prediction of growth curves from Von Bertalanffy function for each combination of feather color (G=golden brown, W=white), sex (M=male, F=female) and hatching batch (H1, H2 and H3 = hatching batch 1, 2 and 3)

Table 1 Estimates of growth curve parameters ±SE and predicted values of body weight at hatching (BW_0) and age and body weight at inflection point from Gompertz function

Item[1]	A (g)	B	k × 10^2 (d^{-1})	BW_0 (g)	At inflection point	
					Age (d)	BW(g)
Model I with combined data						
Combined data	2,226.00 ±17.53	3.82±0.02	2.33±0.02	48.78	57.53	818.90
MSE	35,410.00					
R^2	0.96					
Model II fitted feather color effects						
G	2,304.40 ±19.27	3.85±0.02	2.33±0.03	48.98	57.87	847.74
W	1,977.10 ±34.64	3.74±0.05	2.32±0.05	46.86	56.88	727.33
MSE	32,357.00					
R^2	0.96					
Model III fitted with sex effects						
M	2,694.90 ±21.60	3.96±0.02	2.26±0.02	51.43	60.88	991.40
F	1,887.40 ±16.08	3.66±0.02	2.34±0.03	48.68	55.42	694.34
MSE	18,572.70					
R^2	0.98					
Model IV fitted hatching batch effects						
H1	2,254.30 ±26.79	3.71±0.03	2.31±0.03	55.04	56.78	829.31
H2	2,432.00 ±39.39	3.87±0.04	2.17±0.04	50.90	62.32	894.68
H3	1,944.00 ±24.93	4.02±0.06	2.65±0.05	34.89	52.50	715.16
MSE	34,462.50					
R^2	0.96					
Model V fitted combinations of feather color, sex and hatching batch effects						
G:M:H1	3,113.90 ±38.72	3.93±0.03	2.16±0.03	61.15	63.37	1,145.54
G:M:H2	3,043.20 ±47.52	4.04±0.04	2.09±0.04	53.37	66.85	1,119.53
G:M:H3	2,367.70 ±29.94	4.12±0.05	2.54±0.05	38.35	55.77	871.03
G:F:H1	2,108.50 ±26.46	3.58±0.03	2.17±0.03	58.64	58.80	775.67
G:F:H2	1,951.30 ±29.77	3.79±0.05	2.39±0.05	43.96	55.78	717.84
G:F:H3	1,588.70 ±21.32	3.89±0.07	2.81±0.06	32.38	48.37	584.45
W:M:H1	1,874.80 ±44.64	3.64±0.08	2.50±0.09	49.44	51.63	689.70
W:M:H2	2,851.10 ±103.70	3.85±0.07	1.94±0.08	60.85	69.45	1,048.86
W:M:H3	2,258.20 ±49.41	4.18±0.10	2.62±0.08	34.41	54.63	830.75
W:F:H1	1,774.50 ±63.51	3.51±0.07	2.16±0.09	53.10	58.12	652.80
W:F:H2	1,939.70 ±59.03	3.64±0.07	2.15±0.08	50.92	60.09	713.58
W:F:H3	1,487.40 ±32.29	3.83±0.10	2.82±0.10	32.24	47.64	547.18
MSE	13,356.40					
R^2	0.98					

[1] A = upper asymptotic weight at infinity age or the estimation of mature weight; B = integration constant; K = rate of maturation; G = golden brown; W = white; M = male; F = female; H1, H2 and H3 = hatching batch 1, hatching batch 2 and hatching batch 3, respectively; MSE = mean square error; R^2 = coefficient of determination

Table 2 Estimates of growth curve parameters ±SE and predicted value of body weight at hatching (BW_0) and age and body weight at inflection point from Von Bertalanffy function

Item[1]	A (g)	B × 10^1	K × 10^2 (d^{-1})	BW_0 (g)	At inflection point	
					Age (d)	BW(g)
Model I with combined data						
Combined data	2,678.80 ±17.53	7.83±0.03	1.53±0.02	27.25	55.82	793.72
MSE	35,041.20					
R^2	0.96					
Model II fitted feather color effects						
G	2,774.80 ±35.44	7.87±0.03	1.53±0.02	27.02	56.15	822.16
W	2,350.20 ±61.74	7.74±0.07	1.54±0.05	27.09	54.70	696.36
MSE	32,270.00					
R^2	0.96					
Model III fitted sex effects						
M	3,316.40 ±41.38	7.99±0.03	1.45±0.02	27.08	60.29	982.64
F	2,232.30 ±28.32	7.63±0.03	1.57±0.02	29.64	52.75	661.42
MSE	18,442.60					
R^2	0.98					
Model IV fitted hatching batch effects						
H1	2,714.60 ±49.06	7.67±0.04	1.51±0.03	34.45	55.19	804.33
H2	2,972.80 ±73.99	7.93±0.06	1.41±0.04	26.29	61.47	880.83
H3	2,233.40 ±41.80	8.14±0.08	1.82±0.05	14.30	49.01	661.75
MSE	34,354.10					
R^2	0.96					
Model V fitted with combinations of feather color, sex and hatching batch effects						
G:M:H1	4,011.40 ±80.55	7.90±0.31	1.32±0.02	36.98	65.37	1,188.56
G:M:H2	3,863.00 ±96.01	8.11±0.49	1.31±0.03	25.93	67.87	1,144.59
G:M:H3	2,768.20 ±52.19	8.27±0.76	1.71±0.04	14.25	53.14	820.21
G:F:H1	2,580.80 ±26.46	7.50±0.34	1.40±0.03	40.35	57.92	764.68
G:F:H2	2,268.40 ±50.38	7.89±0.08	1.64±0.05	21.19	52.54	672.12
G:F:H3	1,783.50 ±33.69	8.01±0.09	1.98±0.06	14.13	44.28	528.44
W:M:H1	2,146.80 ±73.43	7.61±0.10	1.73±0.08	29.63	47.42	636.09
W:M:H2	3,716.60 ±217.80	7.87±0.08	1.19±0.07	35.92	72.19	1,101,21
W:M:H3	2,633.00 ±85.46	8.31±0.13	1.76±0.07	12.70	51.90	780.15
W:F:H1	2,144.50 ±116.10	7.40±0.09	1.41±0.08	37.66	56.56	635.41
W:F:H2	2,327.50 ±105.7	7.66±0.10	1.43±0.08	29.83	58.18	689.63
W:F:H3	1,664.60 ±50.60	7.91±0.15	1.99±0.09	15.02	43.42	493.21
MSE	13,210.30					
R^2	0.98					

[1] A = upper asymptotic weight at infinity age or the estimation of mature weight; B = integration constant; K = rate of maturation; G = golden brown; W = white; M = male; F = female; H1, H2 and H3 = hatching batch 1, hatching batch 2 and hatching batch 3, respectively; MSE = mean square error; R^2 = coefficient of determination

REFERENCES

Barbato, G.F. 1991. Genetic architecture of growth curve parameters in chickens. Theor. Appl. Genet. 83:24-32.

Goliomytis, M., E. Panopouloa and E. Rogdakis. 2003. Growth curves for body weight and major component parts, feed consumption and mortality of male broiler chickens raised to maturity. Poult. Sci. 82:1061-1068.

Grossman, M. and B.B. Bohren. 1985. Logistic growth curve of chickens: heritability of parameters. J. Hered. 76:459-462.

Hancock, C.E., G.D. Bradford, G.C. Emmans and R.M. Gous. 1995. The evaluation of the growth curve parameters of six strains of commercial broiler chicken s. Br. Poult. Sci. 36: 247-264.

Knizetova, H., J. Hyanek, B. Knize and J. Roubicek. 1991. Analysis of growth curves of fowl. I. Chickens. Br. Poult. Sci. 32:1027-1038.

Mignon-Grasteau, S., C. Beaumont, E. Le Bihan-Duval, J.P. Poivey, H. de Rochambeau and F.H. Ricard. 1999. Genetic parameters of growth curve parameters in male and female chickens. Br. Poult. Sci. 40:44-51.

Mignon-Grasteau, S., M. Piles, L.Varona.,H. de Rochambeau, J.P.Poivey, A. Blasco and C. Beaumont. 2000. Genetic analysis of growth curve parameters for male and female chickens resulting from selection on shape of growth curve. J. Anim. Sci. 78:2515-2542.

Rogers, S.R., G.M. Pesti and H.L. Marks. 1987. Comparison of three nonlinear regression models for describing broiler growth curves. Growth 51:229-239

SAS. 1990. User's Guide, Version 6. SAS Institute Inc. Cary, NC.

Yang, Y., D.M. Mekki, S.J. Lv, L.Y. Wang, J.H. Yu and J.Y. Wang. 2006. Analysis of fitting growth models in Jinghai mixed sex yellow chicken. International Journal of Poultry Science 5:517-521

O-14-9

Effect of housing system on expression of antioxidant enzyme and heat stress genes in commercial native chicken

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INTRODUCTION

In poultry industry of Thailand, manufacturers are interested in commercial native chicken production with an intensive and large-scale farm. The Commercial native chickens are originally crossbred native chicken developed for rapid growth, high meat quality and more stress tolerance (Tirawattanawanich et al, 2011). And then, the manufacturers can be responsive to their consumers who prefer high quality of the chicken meat such as tender meat, less fat and cholesterol. However, when the production of commercial native chicken has been changed to larger-scale or intensive farm, it may challenge some problem such as heat stress and oxidative stress which these factor affecting chicken productivity. Previous studies have identified that different factors in housing system, such as light, temperature, humidity, density and startle (Hester et al., 2005) ; have all been implicated in stress induction. An outdoor, free-confinement system might prevent stress, enhance comfort and chicken welfare, reduce abdominal fat (Wang et al., 2009) and increase meat flavor quality (Fanatico et al., 2006). All and all, housing system is an important environmental factor affecting the production and stressor stimuli in term of gene expression levels. Mostly, the stressors in poultry production at the cellular level are associated with oxidative stress due to an excess of free radical production or inadequate antioxidant protection (Surai, 2015). Being known as stress response indicator, heat shock protein 70 (HSP70) is capable of controlling cell balancing-condition and damage (Beloor et al., 2010) by the stress. In the condition of oxidative stress-induced, heat shock protein participates in detecting intracellular changes, protecting against protein misfolding and preventing activation of downstream events related to inflammation and apoptosis (Kalmar and Greensmith, 2009). On the other hand, oxidative stress can cause overproduction of free radicals (Uttara et al. 2009) and then the process of oxidative damage will express in biomolecules, (lipids, proteins, DNA) which leads to cell injury and death (McCord, 2000). The first level of antioxidant defense of the living cell is antioxidant enzymes and the important antioxidant enzymes consist of superoxide dismutase (SOD) and catalase (CAT). The function of SOD converts superoxide enzymatically into hydrogen peroxide and then, CAT has a function to transform hydrogen peroxide into water (Surai, 2016, Halliwell, 2012). Therefore, the study was conducted to analyze the effect of housing system, comparing between floor-housing and integrated chicken-fish farming system on the expression of genes involve antioxidant enzyme containing superoxide dismutase (SOD), catalase (CAT) and heat stress gene as heat shock protein 70 (HSP70) in commercial native chickens.

MATERIALS AND METHODS

Sample and extraction of Total RNA

Ten commercial native chickens from both housing systems were randomly selected for blood sampling. Blood collection was repeated on day 21, 45, 60 and 72 for total RNA extraction. Total RNA was extracted from white blood cell by GeneJET RNA Purification Kit (Thermo Scientific). The quantity was measured using a spectrophotometer (NanoDrop 2000 Thermo Scientific, Waltham, MA, USA) and store at -20℃ for use in quantitative Real-time PCR

Quantitative Real-Time PCR

One-step quantitative real-time PCR (qRT-PCR) was used to measure expression patterns of genes involved in antioxidant enzyme (SOD, CAT) and heat stress (HSP70). The primers of SOD and CAT genes followed Yarru et al. (2009); SOD gene (forward: 5'AGGGGGTCATCC ACTTCC3' and reverse: 5'CCCATTTGTGTTGTCTCCAA 3') and sized 122 bp, CAT gene (forward: 5'GGGGAGCTGTTTACTGCAAG 3'and reverse:5'TTTCCATTGGCTATG GCATT3'), and sized 139 bp. Documented from Mazzi et al. (2003), the primers of HSP70 gene were (forward: 5'AACCGCACCACA CCCAGCTATG 3'and reverse: 5'CTGGGAGTCGTTGAAGTAAGCG3') and sized 360 bp.

Quantitative real-time PCR was conducted using CFX96 real-time system (BIO-RAD). The total volume of the reaction was performed for 25 µl which containing 2 µl (20 ng/ µl) of total RNA, 12.5 µl of 2X SYBR green RT-PCR. Reaction Mix (BIO-RAD), 2 µl (3 µM/ µ l) of each primer, 1 µl of iScript reverse transcriptase for one-Step RT-PCR (BIO-RAD) and 6.5 µl of nuclease free water. Then, complete reaction mix was incubated in a real-time thermal detection system as follows: cDNA synthesis 10 min at 50℃ , iScript Reverse transcriptase inactivation 5 min at 95℃, PCR cycling and detection (40 cycles); 10 second at 95℃, 30 sec at 58℃, and ending with a melting curve analysis from 65℃ to 95℃. We considered 18s-ribosomal RNA used as the endogenous control gene in the qRT-PCR. For each sample, the experiment was performed in duplicate.

Statistical Analysis

The relative quantification of gene expression was recorded after normalizing for 18SrRNA gene expression computed by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen., 2001). Relative normalized expression *(*$2^{-\Delta\Delta Ct}$) data were analyzed by the GLM procedures at a significance based on the 0.05 level of probability. Type of housing systems and age of chicken at blood collected are used as fixed effects in the model. Then, means were compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The results showed that *the response ability in gene expression level of commercial native chicken, which gene related to heat stress and oxidative stress*. Commercial native chicken in two housing systems was no statistical difference in expression of SOD and CAT genes. However, the level of HSP70 gene expression demonstrated a significant *(P<0.05)* up-regulated at age 21 days ($2^{-\Delta\Delta Ct}$ = 1.38) and then down-regulated at age 60 days ($2^{-\Delta\Delta Ct}$ = 0.23) in the integrated chicken-fish farming system. Relative normalized expression *(*$2^{-\Delta\Delta Ct}$) data of SOD, CAT (Figure 1.) and HSP70 (Figure 2.) genes in commercial native chicken were fluctuated change at different ages of blood sampling. The results of the present study indicate that chicken from the integrated chicken-fish farming system*s was capable of cooling better than floor housing system.* As the result of beneficial linkages between fish farming and livestock production, in which, we can use fish culture water for cooling livestock housing (Little and Edward, 2003). *At the same tim*e, HSP70 gene set the stage for rapid induction of expression within minutes of cellular stress and then, exposed to stress, such as oxidative damage, physical injury or chemical stressors (Stetler et al., 2010). So, this is a reason to be possible that raising of commercial native chicken in floor housing system enables cooling less than the integrated chicken-fish farming system, *and* may be affected productivity interest due to heat stress.

CONCLUSION

The results from this study indicated that a different of the commercial housing system was effected on gene expression, the especially *gene related stress response ability*. So, the results of this study further confirm that the housing system will affect the physiology of the chicken, production and adversely affect the level of gene expression as well.

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KEYWORD : commercial native chicken, superoxide dismutase gene, catalase gene, heat shock protein 70 gene, housing system

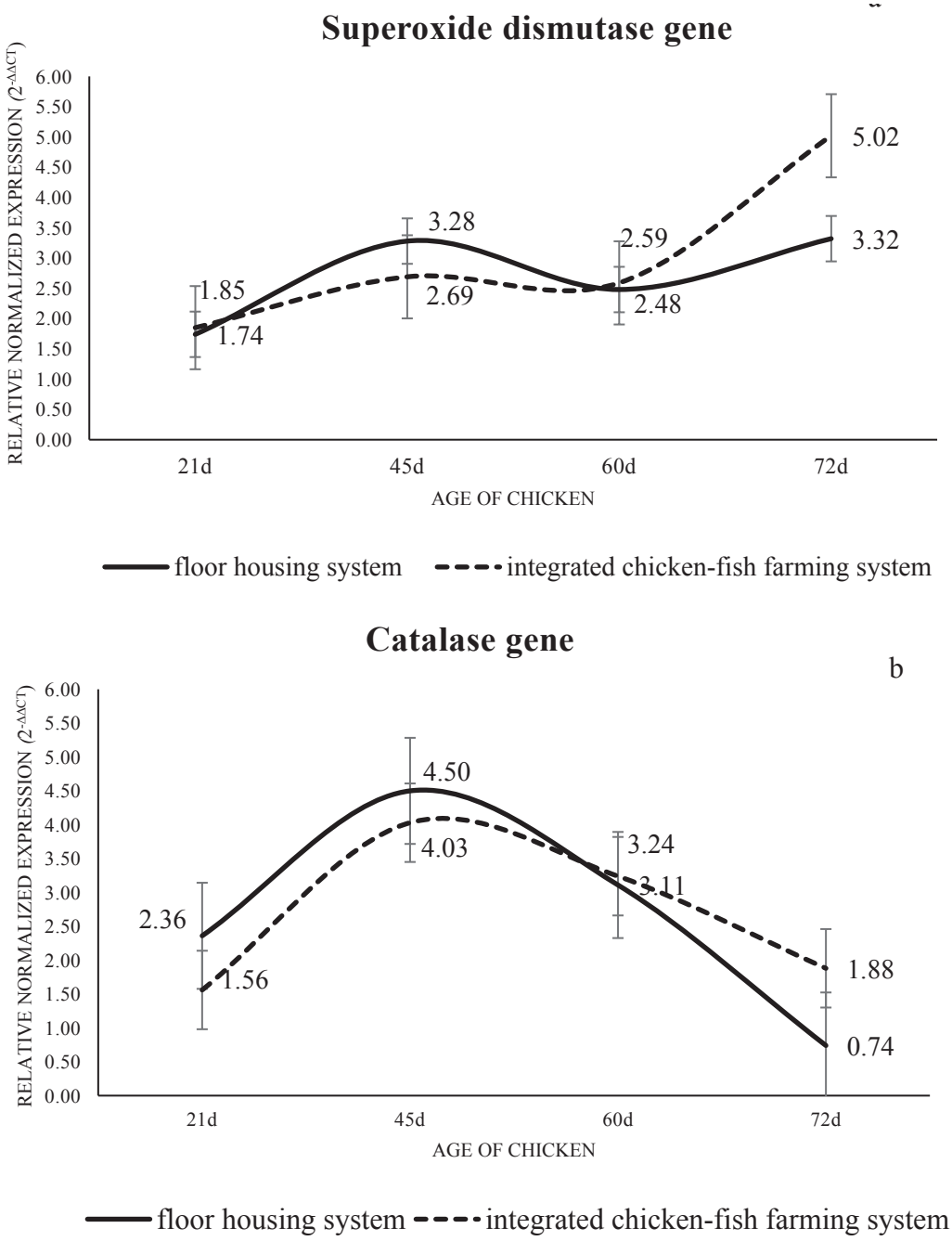

Figure 1. Relative normalized expression ($2^{-\Delta\Delta Ct}$) of antioxidant enzyme genes: a) superoxide dismutase (SOD), b) catalase (CAT) in different housing system; floor housing system and integrated chicken-fish farming system*s* of commercial native chickens at the age of chicken 21, 45, 60 and 72 days. Values represent the mean of 10 chickens per housing groups.

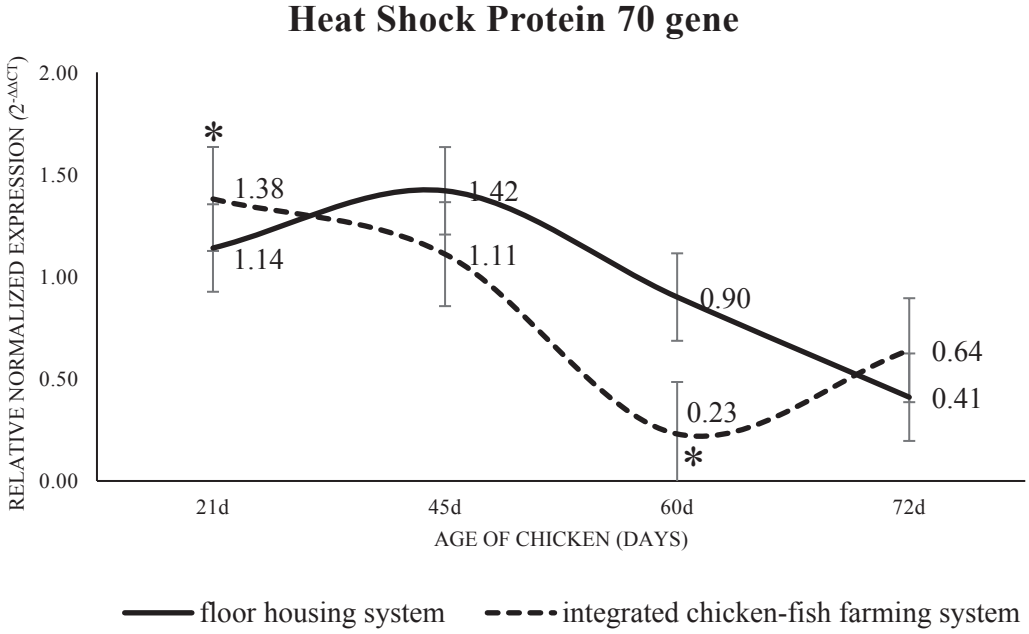

Figure 2. Relative normalized expression ($2^{-\Delta\Delta Ct}$) of heat stress gene: heat shock protein 70 (HSP70) genes in different housing systems of commercial native chickens at the age of chicken 21, 45, 60 and 72 days. Asterisk indicates a significant difference between housing groups; floor housing system and integrated chicken-fish farming system*s* at $P < 0.05$. Values represent the mean of 10 chickens per housing groups.

REFERRENCES

Beloor J., Kang H. K., Kim Y. J., Subramani V. K., Jang I. S. and Sohn S. H. 2010. The effect of stocking density on stress related genes and telomeric length in broiler chickens. Asian-australas. J. Anim. Sci. 23:437–443.

Fanatico, A. C., Pillai, P. B., Cavitt, L. C. and Emmert, J. L. 2006. Evaluation of slower- growing broiler genotypes grown with and without outdoor access: sensory attributes. Poult. Sci. 85: 337-343.

Halliwell, B. 2012. Free radicals and antioxidants: updating a personal view. Nutr. Rev. 70: 257-265.

Hester, P. Y., Alvarado, C. Z., Bilgili, S. F. and Denton, J. H. 2005. Planning our future: the Poultry Science Association strategic plan. Poult. Sci. 84:1814-1815.

Kalmar, B. and Greensmith, L. 2009. Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev, 61(4). 310-318.

Little, D. C. and Edwards, P. 2003. Integrated livestock-fish farming systems. Rome. FAO.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 −ΔΔ CT method. Methods 25: 402–408.

Mazzi, C.M., J.A. Ferro, M.I.T. Ferro, V.J.M. Savino, A.A.D. Coelho and M. Macari. 2003. Polymorphism analysis of the hsp70 stress gene in Broiler chickens (Gallus gallus) of different breeds. Gent. Mol. Biol. 26: 275-281.

McCord, J.M. 2000. The evolution of free radicals and oxidative stress. Am. J. Med. 2000. 108:652

Stetler, R. A., Gan, Y., Zhang, W., Liou, A., Gao, Y., Cao, G. and Chen, J. 2010. Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog Neurobiol, 92 (2), 184-211.

Surai, P. F. 2015. Antioxidant systems in poultry biology: heat shock proteins. Journal of Science. 5 (12): 1188-1222.

Surai, P. F. 2016. Antioxidant systems in poultry biology: superoxide dismutase. J. Animal Nutrition. Vol.1 1-8.

Tirawattanawanich, C., Chantakru, S., Nimitsantiwong, W. and Tongyai, S. 2011. The effect of tropical

environmental conditions on the stress and immune responses of commercial broilers, Thai indigenous chickens, and crossbred chickens. J. Appl. Poult. Res. 20 (4):409-420.

Uttara, B., Singh, A. V., Zamboni, P., and Mahajan, R. T. 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 7(1):65–74.

Wang, P. H., Ko, Y. H., Chin, H. J. and Hsu, C. 2009. The effect of feed restriction on expression of hepatic lipogenic genes in broiler chickens and the function of SREBP1. Comp. Biochem. Physiol. Biochem. Mol. Biol. 153: 327-331.

Yarru, L. P., Settivari, R. S., Gowda, N. K., Antoniou, E., Ledoux, D. R., and Rottinghaus, G. E. 2009. Effects of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poult. Sci. 88(12): 2620-2627.

O-14-10

Cholesterol of Black-Bone, Thai Native and Their Crossbreds Chickens

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INTRODUCTION

Currently, human in the 21st century are concern about their health due to increasing presence of chemicals in foods. Furthermore, public sectors in developed countries have realise and support on use of "original life or slow life". On the contrary, in Thailand, use of antibodies, pesticides and other chemicals have been commonly used in livestock sector. Therefore, we need to explore on possible resources to be used as functional food in Thailand. Especially livestock products, Thai native chickens has good meat quality in terms of taste and firmness (Jaturasitha et al., 2008), low cholesterol level than broiler (Charoensin et al., 2013; Wattanachant et al., 2005). As a result, demand for Thai native chicken meat has been increasing fast. There is also a report on the presence of antioxidants in Black-bone chicken (Chen et al., 2008) that protect against cancer (Vinarov et al., 2002). Moreover, traditional Chinese believed that the uterus will recover quickly at postpartum if Black-bone chicken meat is given to women after delivery (Ho et al., 2011). Hence, Thai native black chickens may also contain essential antioxidants. Recently, Khon Kaen University-Research and Development Network Center for Animal Breeding (Native Chicken) (KKU-TRF) has developed Thai native chicken crossbred chickens to increase poultry products. Therefore, this study aims to determine cholesterol identity in Thai native and their crossbred chickens and compare with Black-bone populations in order to suggest improvement for functional food in the future for Thailand poultry industry.

MATERIALS AND METHODS

Chicken population

We used Complete Randomized Design (CRD) in the present experiment. Black-bone (Black Chinese, BC; Black Hmong, BM), Thai native (Pradu Hang Dam, PD) and crossbreds (PDxBC, PDxBM, BCxBM, BCxPD, BMxPD and BMxBC) were fed broiler feed *ad libitum* based on age. Birds at week 0-4, a diet consisting as crude protein 21%, metabolisable energy 3,150 Kcal/kg were provided, while at week 4-16 ration containing crude protein 19% and metabolisable energy 3,150 Kcal/kg were fed.

Data collection

Body weight of individual birds were weighed at 0, 4, 8, 10, 12 and 14 weeks of age using electronic spring balance. Cholesterol in blood plasma of each population was measured using in-house method (AOAC, 2012). At 12 weeks of age, all birds were not provided with feed and water for at least 8-10 hr prior to blood collection, but birds 97 were selected randomly. About 1.0 cc blood was collected from wing vein using syringe and placed in a microtube 1.5 ml, which was stored in refrigerator with 4 ºC. At 12 weeks of age, birds were slaughtered to evaluate breast, thigh and drumstick weight.

Data analysis

General linear model (GLM) was used to compare cholesterol level among breeds and LSMEANS was used to separate the means at ($P<0.05$) by SAS ver 9.0. Model was represent in terms of cholesterol level (mg%) which is cholesterol level (mg/100g), is overall, is fixed effect as male and female,is fixed effect as 2.1 and 2.2,is fixed effect as BC, BM, PD, PDxBC, PDxBM, BCxBM, BCxPD, BMxPD and BMxBC. In term of carcass trait, breast, thigh and drumstick was expressed as percentage of live weight.

RESULTS AND DISCUSSION

Cholesterol level

The effect of sex and population on cholesterol level is presented in Table 1. Sex tentatively affected on cholesterol level ($P<0.10$). Female birds blood contained slightly lower cholesterol (93.23 mg%) than the male (99.87 mg%), which is in line to the earlier report for Thai native chicken female (Sanchai et al., 2000; Charoensin et al., 2013). Similarly, Chinese native female chicken contains lower cholesterol in serum than male (Musa et al., 2007).

On the other hand, there was highly significant on cholesterol level among populations (P<0.01). Black-bone BM (88.50 mg%) and crossbred PDxBM (74.50 mg%) contained lower cholesterol than the other groups. In addition, both black-bone BM and crossbred PDxBM weighed heavier body weight age at week 12 than the other groups (Table 1). Hence, our results infer that the Black-bone BM and crossbred PDxBM are appropriate to improve for functional food in the future. We further suggest that the Back-bone BM and crossbred PDxBM could be included for chicken stewed with Chinese medicine. We also measured the heaviest body weight for Black-bone BC(1333 g) and crossbred BCxPD (1371 g) at the marketing size and the cholesterol level was close to Black-bone BM. Therefore, Black-bone BC and crossbred BCxPD may also be suitable to improve for functional food in Thailand.

Skin color

Consumers strongly believed that the black skin color is associated with high melanin content in chicken meat. In Figure 1, Black-bone BC represented dark-black skin color compared to other chickens, and BCxBM and BMxBC were close to Black-bone BC. Therefore, we recommend to use Black-bone BC as sire and dam line to improve black skin color in population. This results further confirms that Black-bone BC is appropriate to be used as for functional food in the future.

Carcass percentage

Breast, thigh and drumstick percentage among breeds did not differ significantly (P>0.05) show in Table 2.

CONCLUSION AND SUGGESTION

We classified 9 chicken populations into 2 groups to be used as functional food in the future for Thailand poultry industry. The first chicken group consisted of Black-Bone BM and crossbred PDxBM, and these population contains low cholesterol and the lightest body weight at the marketable size were. The second groups were Black-bone BC and BCxPD and these birds had low cholesterol, the heaviest body weight at 12 weeks of age with dark-black skin color.

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KEYWORD：Cholesterol Identity, Black-Bone chickens, Thai Native chickens, Crossbreds Chickens

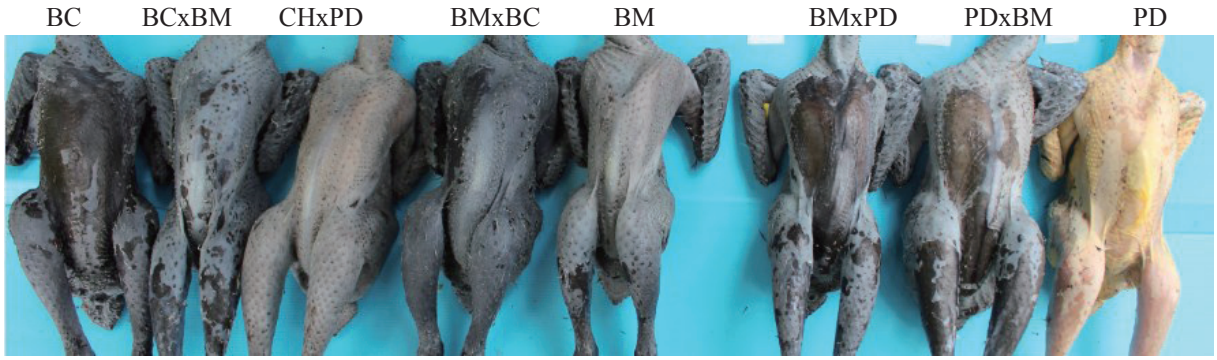

Figure 1 Skin color of Black-Bone, Thai native and their crossbreds chickens

Table 1 Means±SE cholesterol level of Black-Bone, Thai native and their crossbreds chickens

Traits	Breeds								
	BC	BCxBM	BCxPD	BMxBC	BM	BMxPD	PDxBC	PDxBM	PD
Cholesterol level (mg%)									
Mixed	97.92± 5.26^{BC}	104.42± 6.00^{BC}	93.82± 4.07^{BC}	96.12± 3.83^{BC}	88.50± 5.57^{AB}	104.50± 4.55^{BC}	108.75± 7.88^{C}	74.50± 11.14^{A}	100.44± 4.07^{BC}
Female	99.60± 3.37	101.80± 3.14	91.13± 5.88	82.85± 7.89	77.50± 8.25	108.83± 10.26	102.50± 3.50	107.67± 5.86	107.67± 5.86
Male	95.00± 3.76	106.00± 11.00	96.43± 4.80	106.40± 2.34	99.50± 6.43	100.17± 10.83	115.00± 5.00	122.83± 5.86	122.83± 5.86
Marketing size (g) of age at 12 weeks									
Mixed	1333± 25.9^{A}	1102± 23.7^{DE}	1371± 25.5^{A}	1148± 25.3^{CD}	951± 28.2^{F}	1086± 25.6^{E}	1357± 19.6^{A}	1188± 24.8^{BC}	1235± 23.7^{B}
Female	1211	1145	1165	1012	872	1023	1240	1137	1142
Male	1534	1038	1217	1298	976	1269	1454	1325	1500
	----------Mixed breeds----------								
Female	93.51±2.35^{a}								
Male	99.84±2.40^{b}								

BC = Black Chinese x Black Chinese, BCxBM = Black Chinese x Black Hmong, BCxPD = Black Chinese x Pradu Hang Dam, BMxBC = Black Hmong x Black Chinese, BM = Black Hmong x Black Hmong, BMxPD = Black Hmong x Pradu Hang Dam, PDxBC = Pradu Hang Dam x Black Chinese, PDxBM = Pradu Hang Dam x Black Hmong, PD = Pradu Hang Dam x Pradu Hang Dam. $^{A, B}$ in the same row indicate significant difference among groups ($P<0.01$); $^{a, b}$ in the same row indicate significant difference among groups ($P<0.10$).

Table 2 Carcass of Black-Bone, Thai Native and Their Crossbreds Chickens

Traits	Breeds					
	BC	BCxBM	BMxBC	BMxPD	PDxBM	PD
Breast, %	14.36±0.8	15.41±1.9	11.80±1.9	14.34±0.8	12.91±1.9	14.14±0.8
Thigh, %	13.52±0.4	14.65±1.0	13.19±1.0	13.70±0.4	11.68±1.0	13.58±0.4
Drumstick, %	11.57±0.4	11.86±0.9	11.15±0.9	10.97±0.4	12.31±0.9	11.69±0.3

BC = Black Chinese x Black Chinese, BCxBM = Black Chinese x Black Hmong, BCxPD = Black Chinese x Pradu Hang Dam, BMxBC = Black Hmong x Black Chinese, BM = Black Hmong x Black Hmong, BMxPD = Black Hmong x Pradu Hang Dam, PDxBC = Pradu Hang Dam x Black Chinese, PDxBM = Pradu Hang Dam x Black Hmong, PD = Pradu Hang Dam x Pradu Hang Dam.

REFERENCES

Charoensin S., M. Duangjinda, B. Laopaiboon, W. Boonkum, S. Kunhareang and N.Promwatee. 2013. A comparative study on cholesterol, triglycerides and uric acid level in plasma of fraction native chicken. Agricultural Sci. J. 44: 1 (Suppl.): 351-354.

Chen, S. R., Jiang, B., Zheng, J. X., Xu, G. Y., Li, J. Y., & Yang, N. 2008. Isolation and characterization of natural melanin derived from silky fowl (Gallus gallus domesticus Brisson). Food Chemistry. 111: 745-749.

Ho, M., Li, T. C., & Su, S. Y. 2011. The association between traditional Chinese dietary and herbal therapies and uterine involution in postpartum women. Evidence-Based Complementary and Alternative Medicine. 1-9.

Jaturasitha, S., T. Srikanchai, M. Kreuzer, and M. Wicke. 2008a. Differences in Carcass and Meat Characteristics between Chicken Indigenous to Northern Thailand (Black-Boned and Thai Native) and Imported Extensive Breeds (Bresse and Rhode Island Red). Poult. Sci. 87:160–169.

Musa, H.H., G.H. Chen, J.H. Cheng, and G.M. Yousif. 2007. Relation between abdominal fat and serum cholesterol, triglycerides, and lipoprotein concentrations in chicken breeds. Turkish journal veterinary animal sciences 31: 375-379

Vinarov, A. U., Robysheva, Z. N., Sidorenko, T. E., & Dirina, E. N. 2002. Biotechnology of Pigment Melanin. In Proceedings of the 1st international congress "biotechnology-state of the art and prospects of development". p96.

Wattanachant, S., S. Benjakul, and D. A. Ledward. 2005. Microstructure and thermal characteristics of Thai indigenous and broiler chicken muscles. Poult. Sci. 84:328–336.

O-15-1

The performance of livestock extension agent in empowerment of farmers at Pinrang Regency, South Sulawesi, Indonesia

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INTRODUCTION

The existence of extension agent is needed to increase effectiveness of extension activities. A good performance is the main thing in extension agent. This only allows when the socialization program is hosted by the institutional system extension that clear and its implementation supported by competent of extension agent .

To increase a competency and a good performance of extension agent, the government make every effort to revitalize an extension activities through play a role, function themselves and organize back agricultural extension that realized unity understanding, and unity of the policies. One milestone to revitalization of the extension by implemented the act of system agricultural extension, fishery and forestry (SP3K) No 16 Tahun 2006 on 18 October 2006. The act is a starting point in empowering farmers through the improvement of the human resources and institutional the agricultural extension agent.

The Act No 18 year 2009 mention that the extension of animal health is an effort to empower farmers that aims to improve knowledge, skills, and change attitude and behaviour that conducted through non formal education. Extension was a non-formal education institution that emphasized the behavioral changes of the farmers and their family to more defenseless into a better direction, have a challenge own in doing their functions and roles. The agricultural extension agent who will educate the farmer and family in order to get a satisfaction and increase they income. Hence, this policy is important due to revitalization of extension agents, that is a front line for agricultural development. Harianto, *et al*. (2014) stated that extension agents play an important role in the development of farms in a region, because they are agent of change and as technical service in the community, therefore, it is a requirement of extension agents having performance that can be as an empowerment and empower the farmers.

Performance is an implementation of the plan that had been developed. The implementation of performance is conducted by human resources with the ability, competence, motivation and interests. When the organization respect and treats human resources nicely, it will affect attitudes and his behavior in running performance. According to Mardikanto (2008), performance as notes of outcome resulting from certain function of extension agent. A member of a contribution to core can be measured with the assessment of the performance of work.

Performance of extension agent is an assessment for overall work activities that have already been done and then to be compared with conformity target to be achieved through the set indicators. According to the minister of agriculture Act number: 91/PERMENTAN/ OT.140 /9/2013, performance of agricultural extension agent can be assessed through three main indicators such as, extension preparation, the implementation of the extension and evaluation. These three indicators are able to give an overview of extension agent performance and provide input about point-point that a weakness of agricultural extension agent.

Considering the importance of assessing the performance of extension agents as this assessment can be used for them improve their works, empower the farmers, and subsequently, the performance of extension agents become much better,and finally the farmers will be defenseless and independent. Extension agents with a good performance can be seen from the results of extension works at the farmers level (Abdullah and Ibrahim, 2014). Farmers who have been empowered and independent will be able to increase their welfare by increasing cattle production and finally affecting their income. The aim of this study was to examine the performance of extension agents at Pinrang Regency, South Sulawesi.

MATERIALS AND METHODS

This study was conducted in Pinrang Regency, South Sulawesi Province, Indonesia, during a period of three months (February - April 2016). The method of this study was descriptive that aimed to explain a base line condition of event.The sample was collected using census method, in which this method is a technique in doing sampling that covering the total members of the population (Sugiyono, 2011). The reason using this method due to that the number of population was not too large as well as to avoid error. The number of respondent in the present study was all the extension agents; 101 persons. The data were collected through survey with the

help of a questionnaire and then followed by conducting focus group discussion. All measurement of extension agent performance was conducted using scoring method. Indicators of measurements for this study was using an indicator that based upon the agriculture minister Act number : 91/PERMENTAN/OT.140/9/2013. The assessment of extension agent performance were based on by three main indicators such as, extension preparation, the implementation of the information, and evaluation and reporting. Indicators performance of extension agent were: a). Extension agents performance in preparation of extension, variables for this factor were how to make data for assessing a potential areas and agro ecosystem, how to guide farmers in drafting a group farmers plan, how to establish a extension programs, how to make an annual work plan. b) Extension agents performance in the implementation of the extension, variables for this factor including conducting desimination of extension materials based on the need of the farmers, implementing demonstration method, implementing field visits, implementing course method, increasing farmers capacity in access to the information, creating a group farmers, increasing level of group farmers, developing farmers institution, increasing commodity production. c) Performance of extension agents in evaluating extension, variables for this parameter were to evaluate the performance of extension, make a report on the implementation of extension. Data obtained in the study was analyzed descriptively. The measurement of each questions was scoring with the lowest score was one and the highest score was five.

RESULTS AND DISCUSSION

Performance has a correlation with capability of the farmer in an institution. Extension agents as a human resources in an organization of extension have the potential that serves to achieve a goal of organization. The performance of extension agents itself was one of a reflection on human resources potential, while human resources was an essential factor and the engine of organization. The expected of extension agents performance could be an assistance and consultant for the farmers. The results of this study showed that the majority of extension agents performance at Pinrang Regency was categorized as a good performance. Assessment for each indicators elaborated as follows.

Performance of livestock extension agent in preparation of extension

The performance of the extension agents in preparation of extension account for 87.1% of extension agents. This percentage included making data about the potential of the region and agro ecosystem which included work area map, potential of the work area, a monograph of the work area, and the plan of extension activities. This indicated that preparation extension activities has been done by extension agent in regency Pinrang. At the preparatory stage of extension, the extension agents knowledge is needed. Van Den Ban and Hawkins (1999) stated that education has a function as the basic knowledge for the extension agents to unites the various center science to farmers in accordance with the need. At this stage, the largest score was on the parameter for creating data about potency of the region and agro ecosystem.This results showed that almost all the extension agents have carried out the creating of data about potency of the areas that contains maps the work area, potential map of the work area, monoghraphy areas and plans for activities extension village (RKPD). The ability of the extension agents to assess the potency of area due to most of the extension agent live in this work area, made easier for them to make this work.

Data of the potential regions prior to conduct extension was required, considering characteristic of the farmers was varied not only individual characteristic but also social and physical environment. Furthermore, extension agent have to understand and capable to determine a characteristic of the farmers in changing their behavior. This finding was consistent with Abdullah (2012), stated that extension can change attitude of the farmers when extension agent conducting a preparation before doing an extension activities. Performance extension agents in guiding and assisting of plan drafting on farmer group (RDKK) account for 51.5%. A total of 75,2% extension agents have performed the preparation of programa extension that including the development of programa extension village, recapitulation village programa, problems ranking, the drafting of programa and synchronization of extension activities. While performance extension agents to the annual work plan account for 91.1%. This indicated that performance of extension agent in preparing extension at Pinrang Regency has shown a good performance.

Performance of livestock extension agent in implementing an extension activities

Implementing of extension has a strong correlation with extension activities that performed by extension agent. The results showed that there was 46.5% of extension agent have conducted dissemination of extension materials based on farmers need covering more than 8 topic of extension materials in one year. A total of 92.0% of extension

agents have performed an extension using field visit method with average of visit was 45 per year. Implementing of extension method that was conducted by extension agent more than 3 times a year was demonstration method was 56.4%, field visit method was 53.5% and course method was 56.4%, respectively.

This finding showed that the existence of extension agent was important due to the capability of extension agent in changing farmer behavior. Van den Bans and Hawkins (1999) stated that human behavior has a relation to their past experiences, likewise farmers. The response of farmer to extension relate to their past experience. When extension agent capable to assiss a farmer in increasing knowledge, motivating a farmer and helping a farmer in facilitating to find a resources in terms of finance, infrastructure and marketing, the farmer will keep connecting to the extension agent.

Performance of extension agent in increasing access to information in developing farmer business account for 60.4%. The performance of the extension agents in the development of farmers economic institutional was 48.5%. While the performance of the extension agents in improving the level of farmer group/Gapoktan was low, account for 29.7 %. Meanwhile, performance of extension agent in developing non formal insititutional of farmers business to be a legal institutional farmer bussiness was 49.5%. This finding showed that performance of extension agent in implementing extension activities was quite well except for the aspect of developing institutional business is still require to be increased.

Performance of livestock extension agent in evaluating and reporting of extension activities

Each program of extension activity that have been planned should be ended by evaluation and should be started by the evaluation of previous activity. The purpose of evaluation is to examine whether a program or activity has been implemented fit with a planning and match with a goal of extension activity. Hornby and Parnwell (1972) stated in Mardikanto (2008) that the word evaluation in daily life often interpreted as the same terms of assessment, which is a the act of decision-making to judge an object, the state of, event or certain activity.

While reporting was a technique of management in collecting data in line with the implementation of the socialization program and the problems faced by. Reporting activities was an important to monitore and observe on the process, activity, the results and the impact of a particular activity in order to keep the activities in the right track.

The result research, 73.3% extension agents evaluated the performance of extension for 4 times. An activity to evaluate and report an extension activities was conducted using an instrument made by working unit management and organizers of extension in each level of administration and was conducted for period of once. While performance extension agents in making report on the implementation of extension per month, per quarter , and per year was 89.1%. This finding showed that an activity to evaluate and report the works of extension agents in Pinrang Regency have been conducted based on the Act of number 91/PERMENTAN /ot.140/9/2013. Thus, it can be said that performance of extension agents in Regency of Pinrang in general has run well, just need to improving the role of extension agents in the development of farmers or the farmers group to be institution of economic people in rural areas.

CONCLUSION AND RECOMMENDATIONS

The performance of extension agent in preparation extension account for 88.71%, with parameter of variables were making the data area and the potential for agro-ecosystems covering the work area map, a map of potential areas of work, monographic work area, and plan activities of extension. A total of 51.5% of extension agents did to guide the farmers in preparation of the business plan. Performance of extension agent in the implementation extension account for 46.5% through conducting dissemination of extension materials according to the needs of farmers over 8 topics extension in a year. A total of 92.0% extension agent performed the method of extension in the face-to-face, which was more than 45 visits/year. Application of the method of extension was more than 3 times/year through demonstration method (56.4%), visit days (53.5%), and the course (56.4%). Performance of extension agent in the economic institutional development; the development of farmer groups into a limited liability company was still low (4.0%), on the other hand, development of farmers groups into farmers cooperation was high (49.5%). In the extension evaluation, 89.1% extension agents reported the implementation of extension per month, per quarter, and per year. Thus, it can be concluded that the performance of extension agents have generally been going well, and the need of the role of extension agent in the development of farmer groups to become an economic institution in rural communities.

KEYWORD : livestock extension agent, performance, farmers, empowerment

REFERENCES

Abdullah , A and Ibrahim, H. 2014 .Perception farmers extension of the performance in development process technology rice straw and waste cattle production . The Journal Jitro.Vol.1.No.1 September 2014 .

Abdullah , 2012 .Performance extension agents in improving adoption technology Feed support the development of the beef cattle .Proceeding Seminar on National Farm . Medan , May 19 2012 . It .193-196

Harianto , E . , Surahmanto , Putu Arimbawa .2014 .Performance agricultural extension agent as fuel information facilitators and escort in the development of Bali cattle (bos sondaicus in Muna Southeast Sulawesi . Agriplus , vol .24: 232-239

Mardikanto , T .2008. Extension Agricultural Development , Sebelas Maret University Press Surakarta

Sugiyono , 2011 .The Methodology Quantitative and Qualitative ; Bandung; Alfabeta

The Minister for Agriculture no .91 in 2013 . The Assessment Extension

Van Den Ban and H.S. Hawkins.1999 . Agricultural Extension .Yogyakarta Kanisius .

Undang-Undang Republic of Indonesia No.18 .2009 .Undang-Undang Republic of Indonesia No.18 Thn 2009 about Livestock and Their Health .The Indonesian , Jakarta

Undang-Undang Republic of Indonesia No 16 .2006 .Undang-Undang No. 16 Extension Information System , Fisheries , and Forestry .The Indonesian , Jakarta

O-15-2 PRODUCTION SYSTEMS AND INCOME GENERATION FROM THE BLIGON GOAT FARMING IN THE RURAL COMMUNITY IN GUNUNGKIDUL OF YOGYAKARTA PROVINCE, INDONESIA

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ABSTRACT

This study was conducted to Investigate the potentials of Bligon Goat Farming System for income generation in the rural community of Girimulyo Village, Gunungkidul Regency of Yogyakarta Province.Data was collected from 30 Bligon goat farmers which taken purposively as the respondents with semi structural interview. Enterprise budgeting of Bligon goat farming business made to analyze profitability and feasibility that could be used for further planning for farmers to develop their business. The result of this study showed that the Bligon goat farming was a potential alternative to be developed for income generation that can be developed in rural communities to generate of income. Nevertheless, government policy was urgently needed such as provide loans with low interest rate below 6%. Furthermore, the production system in the term of kidding interval and mortality in the breeding system were needed to be improved. kidding interval should be lower than 8 months and mortality of kid until 12 months age should be lower than 6% would give higher farm income value. It was a challenge for scientists to develop technology that supports the improvement in those two aspects.

INTRODUCTION

Indonesia is an agricultural country because around 85% of the population lived in the rural and had livelihood as farmers. Nevertheless,the average ownership of arable land was small, around 0.1 - 0.5ha especially in Java, including in Yogyakarta. Small farmers generally had low income so that they kept animal, such as goat, to increase their income although it was only complementary and supplementary to their farming system. Murjito, et al. (2011) stated that almost all goats in Indonesia were kept by small farmers in the rural in a small scale around 2-7 heads because they only had small capital. Smallholder farmers' resource were limited and weaken by varied agricultural conditions, such as land, water and soil fertility and types of crops and livestock (Verschelde et al. 2013). However it was believed that animals may kept by smallholder farmers to eliminate poverty, especially in the poor countries and developing countries (Pica-Ciamarra, et al., 2015). Goats were small ruminants that do not require plentiful resources and much capital as cows do. Goats grew faster than cows and their meat were an excellent source of red meat similar to beef. Therefore, development of Bligon goat farming business would support the red meat consumption of Indonesian society as well as provide employment opportunities in rural communities.

Maart-Noelck and Musshoff (2013) stated that in regard to decide investment for expanding the farming business, farmers had a tendency to learn previous investments and assessed the gained value from time to time. They would decide to invest if the investment was proven profitable. However, in order to do so, the smallholder farmers needed to understand the characteristics of the production systems to develop a profitable Bligon goat farming business plan.

Enterprise Budgeting was an accounting technique which was used to handle problems of scale economies, replacement of durable inputs, inflation and technological change. Along with proper and correct analysis, it could also help farmers make the most appropriate decision to develop business plan for their profits (Bradford and Debertin, 1985; Kay, et al., 2008; Paudel et al., 2013). The objective of this study was to investigate the potentials of Bligon Goat Farming System for income generation in the rural community of Girimulyo Village, Gunungkidul Regency of Yogyakarta Province.

MATERIALS AND METHODS

Study areas

The study was conducted in the village of Girimulyo, Gunungkidul regency in Yogyakarta province, which were source of Bligon goats. In 2015, the total population of goat in Indonesia were 18,879,596 heads, whereas in Yoyakarta Province were 411,209 heads. The increase rate of national goat population in 2010-2015 period was

an average of 1.29%/year. In the same period, Yogyakarta Province had greater growth rate than the national's which was 6.68%/year (Directorate General of Livestock and Animal Health, 2015). This indicates that Yogyakarta had the potential for the development of goat farming including Bligon goat in that study area.

Data collection

The data were collected from 30 respondents of Bligon goat farmers in the study area taken with purposive sampling method. The basis for respondents selection to extract the data of production was the minimum ownership of one goat of productive female and the minimum goat-raising experience of one year. The data was collected by survey method through deep interviews to farmers using a complete structured questionnaire. Technical data was collected in July to September 2013 and assumed that these data related technology had not changed until 2015. Meanwhile, the economic data in the form price of input and output were taken in December 2015.

Data Analysis

Data from the completed questionnaires were tabulated and edited, then used as a basis for further analysis. The analysis was performed descriptively for demographics characteristic and livestock production system.Enterprise Budgeting (EB) of Bligon goat farming business usedfor the analysis of income generation was used as a basis for developing a profitable business plan in the future (Paudel et al., 2013; Kay et al., 2008).

RESULT AND DISCUSSION

Identity of Respondent

Table 1 showed that the number of goat keeping remained small with an average of 5 heads. This showed that they kept the goat as a side job only, supplementary to their farming, that cause low productivity and marginally profitable. The average of agricultural land ownership was 0, 74 ha/farmer. This was quite wide compared to farmers in the irrigated areas especially in Java which was only about 0.10 ha (Widiati, 2006).

Bligon Goat Farming Production Systems

In general, farmers in developing and poor countries including in the research area, raised goats together with crop farming, utilizing the family labors. Goats could graze forages from existing plants outside the crop field , in addition they were also given some leguminous plants, cassava and corn from the crop field. In return, they produced manure that could be used as fertilizer for the soil in the field. Nugent and Yotopoulus (1976) stated that technology could improve production and productivity, but it cost while most farmers have only limited capital. Therefore, developing the smallholder goat farming needed financial aid of cheap credit to access technology such as feed technology which can increase productivity.

Animal feeding. Cost of feed took the highest percentage (60-70%) of all operational cost (Murjito, et al*.,* 2011). Farmers in research location gave forage such as by-products, field grass and legume from their own crop field, thus feeding cost was almost reduced but it needed time from family labours to collect the forage. Feeding cost could not be completely reduced because farmers still need to buy concentrate such as rice brand and pollard as feed supplement for the goats. The average concentrate feeds given to the goats in the study area was 0.93 kg/day/farmer. As the capital was limited, they could only afford a few, depending on the daily cash availability (Widiati, 2006 ; Murjito, 2011). For the goat that is in a period of growing and the male goat at least needed concentrate feed ± 1 kg / day /head, depending on the weight of goats (Murjito, 2011; Ginting and Simon, 2009). Therefore, concentrate feed for goats in the research location amounting to 0.93 kg / day / farmer for 5 heads was not yet meet the needs.

Animal breeding. The main product of the goat farming was kids that was born to raised to adulthood and then to be sold for revenue, thus the female goat reproduction quality should be better managed. Generally, natural mating was implemented using superior males in the study area as the breeder mate. The technical parameters of reproduction would affect the cost and revenue such as service per conception (S/C), kidding Interval, litter size or the number of kids per birth and kid mortality up to 1 year age. Result of this study for the technical parameters respectively were 1.44 times, 8.83 months, 1.56 heads and 6.7%. Meanwhile, same study in different village showed that the result for those parameters respectively 1.23 times, 8,53 months and 1.74 heads, but no data on kid mortality (Murjito et al., 2011).

Marketing of goat production. The results of this study showed that the goat farmers usually sold the kid at 8-12 months age to the middleman of village. Direct selling the markets were considered time consuming and more costly as there was a "marketing cost". As they were utilizing the family labours, time spent in the market was considered a waste because those particular time could be better be used to work in the crop field.

Enterprise Budget Analysis for Bligon goat

Technical and economical practice in the Bligon goat production system were used for EB. In general, non-specific Bligon goat farming was a commercial business. In technical parameters, the use of forage was from grazing only, therefore the cost of forage feed was equal to the use of labor. In goat production system, farmers generally produce kids, maintained until about 1 years old age and then it sold to receive a extra income. However, some farmers kept animals as a savings in a sense that they could sell them at any immediate time they need cash. Enterprise Budgeting of Bligon goat farming in the study area was shown in Table 2.

Table 2 showed that the Bligon goat farming generates positive of farm income although small. If it was calculated based on income derived from management and family labor, it was IDR 2,256,330 ($1 = IDR 13,500) which was beneficial for farmers to support their daily life. In rural area where job opportunities were limited, animal farming activities were remain in practice, even though the marginal value of labor was low but it showed positive merit (Widiati, 2012). In the cultivation activities, the lack of government policy or less appropriate with the farmer conditions would result in lower income value connected to the input costs (Stür et al., 2013). Based on EB for Bligon goat farming, some aspects that could be improved to increase profits were : shortening kidding Interval was below 8 months and reduce kid mortality to below 6%. In addition, the government policy was urgently needed to provide cheap credit with low interest rate below 6% in order to develop of the farming business.

CONCLUSION

Bligon goat farming was an alternative that could be developed in rural communities. Although farm incomes were small but farming practice still lasted, showing the scarcity of employment opportunities in the rural area. To increase the farm income, government policy was urgently needed such as provide loans with low interest rate. Adoption of technology in Bligon goat production system should be pursued, especially of kidding interval and mortality of goat until 1 year age must be lower than the current practices.

KEYWORD : Income generation, bligon goat farming, enterprise budgeting, Gunung Kidul

Table 1. Bligon goat farmers identity as respondents in the study area (n=30)

No.	Item	Average	Deviation standard
1.	Age of farmers (year)	43.53	5.37
2.	Formal education of household head (years)	9,33	2.62
3.	Experience on raising of goat (years)	18.40	5.50
4.	Goat ownership (head)	5.93	2.26
	kid (heads)	1,23	
	young goat (heads)	1,67	
	mature male (heads)	1	
	mature female (heads)	2,03	
5	Agricultural land size(hectare)	0.74	0,39
6.	The main occupation /persons (%)		
	farmer	27 (90)	
	off farm	1 (3,33)	
	civil servant	2 (6,67)	

Table 2. *Enterprise Budgeting* for Bligon goat farming*(*IDR/farm/year*)*

Item	Technical and economic parameters	*(*IDR/Farmer/year*)*	
Revenue			
Selling of kid(s) from maintenance 2 female goat	kidding interval 9 month, litter size : 1.56, price of goat in 1 year age = IDR 1.200,000, mortality of kid until 1 year age (6,7%)	2,652,000	
Price increase from maintenance 1 male fattening	Average of fattened goat prices = IDR 1,750,000	550,000	
Total revenue			3,202,000
Cost			
Fixed cost			
Interest rate of capital to buy 2 female goat and 1 male goat	Average of female goat prices = IDR 1,180,000, male goat aged of 1 year = IDR 1,200,000	213,600	
Depreciation of goat cage	Price of making a cage = IDR 500,000/m^2	375,000	
Total fixed cost (A)			588,600
Variabel cost			
Forage equated with the use of labor = 0.8 hour x 365 day **(D)**	Daily wage of labor =IDR 40,000 for 8 hours of work per day	1,460,000	
Concentrate = 0.43 kg x 365 day	Average of concentrate prices = IDR 2,600/kg	408,070	
Equipment	1 unit/year	81,000	
Health	There are subsidies	47,000	
Total variabel cost**(B)**			1,996,070
Total cost **(C)**			2,584,670
Estimated farm income **(E) = A-C**			617,330
Estimates of return to management and family labour = **E + D**			2,256,330

REFERENCES

Bradford, G. L. & Debertin D. L.1985. Establishing Linkages Between Economic Theory and Enterprise Budgeting for Teaching and Extension Programs. Southern Journal of Agricultural Economics 17 (02), 67-76.

Directorate General of Livestock and Animal Health. 2015. Statistics livestock and animal health. Ministry of Agriculture of the Republic of Indonesia, Jakarta.

Ginting and P. Simon. 2009. Technical Guidelines for Management of Feeding Goat In The Business. Bogor: Center for Research and Development of Animal Husbandry Agency for Agricultural Research and Development Department of Agriculture.

Kay,R. D.,W.M. Edwards & P.A.Duffy. 2008. *Farm Management*. Sixth Edition. McGraw-Hill Companies Inc. 1221 Avenue, New York.

Maart-Noelck, SC. and O. Musshoff. 2013.Investing Today or Tomorrow?An Experimental Approach to Farmers' Decision Behaviour. Journal of Agricultural Economics 64 (2), 295–318.

Murdjito,G., IGS. Budisatria, Panjono, N. Ngadiyono & E. Baliarti. 2011. Performances of Bligon goats kept by farmers at Girisekar village, Panggang, Gunungkidul Bulletin of Animal Science , 35(2): 86-9.

Nugent, J.B. and P.A. Yotopoulus. 1976. Economic of Development Empirical Investigations. Harper International Edition. Harper & Row Publisher, New York and London.

Paudel, K.P., N.R. Martin, G.Wehtje & T. Grey. 2013. Economic Decision making Using Enterprise Budgeting and Statistical Analysis : An Illustration in Weed Control Practices in Peanut Production.Journal of Production Agriculture11 (1), 48-52.

Pica-Ciamarra, U., L. Tasciotti, J. Otte, A. Zezza. 2015. Livestock in the Household Economy: Cross-Country Evidence from Microeconomic Data. J. Dev. Policy Review. 33 (1) , 61–81.

Stür, W., TT. Khanh, A. Duncan. 2013. Transformation of smallholder beef cattle production in Vietnam. Int. Journal of Agricultural Sustainability 11(4), 363-381.

Verschelde, M., M. D'Haese, G. Rayp and E. Vandamme. 2013. Challenging Small-Scale Farming: A Non-Parametric Analysis of the (Inverse) Relationship Between Farm Productivity and Farm Size in Burundi. Journal of Agricultural Economics64(2),319–342.

Widiati, R. 2006. The integration of beef cattle farming system on the farm household in Merapi Volcanic Slope, Sleman Yogyakarta (The Application of Linear Programming Analysis Model Toward Sustainable Livestock Development). The 4th Inter-national Seminar on Tropical Animal Production. Faculty of Animal Science, Gadjah Mada University, Yogyakarta Indonesia. Pp. 536-541.

Widiati, R. 2012. Financialfeasibilityof beef cattle breeding withvariouscapitalaidsinrural area of GunungKidul District of Yogyakarta. Bulletin of Animal Science 36(2), 1-7.

O-15-4 Estimating Consumer fs Response to Livestock Products Food Quality: Evidence from Households in Indonesia

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Introduction

Consumer faces constraint on budget when they make consumption decision to maximize his/her utility. Allocating budget among commodity consumed is a must.

The economic variables, income and prices, and non-economic variable are the most important factors that determine food consumption. Cross-section household data provide household composition, expenditure, quantity consumed of food and nonfood, and other economic, social, and regional differences among households. As income increase, consumption of foods moves to higher quantity and quality food following their taste and preference change.

This paper attempts to study consumers' response to quality in livestock products foods and estimates quality elasticity with respect to income over time

Methods

This study was used raw household data record from household expenditure data in urban and rural area of D.I Yogyakarta Province (here after DIY Province). The data used come from the detailed expenditure and consumption on food and non-food. The 2011 and 2013 Household Expenditure Survey (SUSENAS) was conducted by Central Bureau of Statistics (CBS) involving sampled-households.

Study of Hicks and Johnson (1968), Gale and Huang (2007) presented methodology to capture effect of quality through a nonlinear Engel relationship. According to their model, Engel curve expresses the relationship between household expenditure and income, as given in equation (1).

$$e(Y) = pq(Y) \quad (1)$$

Deaton 1988 and Deaton 1997 noted that Engel's curves using expenditure and quantity approach have always assume a log-linear function. But recent studies (Banks *et al.,* 1997; Tey *et al*, 2009; Huang and Gale, 2009) have shown that a linear Engel curve cannot describe the real individual behavior.

Expenditure e_j and quantity demanded q_j of food items depends on economic and non-economic factor such as household's demographic variables z.

In this study, we define expenditure equation $e_j = f(y,z)$ and quantity equation $q_j = f(y,z)$ using non-linear or quadratic Engel relationship as

$$\ln q = \alpha + \beta\, q\,(1/Y) + \gamma_q \ln Y + \mu \quad (2)$$

Similarly, for expenditure (e) and income (Y) relationship, equation (2) can be modified as:

$$\ln e = \alpha + \beta\, e\,(1/Y) + \gamma_e \ln Y_y + \mu \quad (3)$$

Estimation of equations (2) and (3) would give values of parameters α , β , γ . If both β and γ are not equal to zero, then elasticities would be worked out, as follows:

$$\eta = -\beta q(1/Y) + \gamma q \quad (4)$$

$$\varepsilon = -\beta e(1/Y) + \gamma e \quad (5)$$

To analyse consumers' response to quality over time, we modify equations (2) & (3), incorporating dummy-variable (D) for time differences, as follows.

$$\ln q = \alpha_1 + \beta q_1(1/Y) + \gamma q_1 \ln Y + \alpha_2 D + \beta q_2(D/y) + \gamma q_2 \ln(DY) + \mu \quad (6)$$

$$\ln e = \alpha^*_1 + \beta^* e_1(1/Y) + \gamma^* e_1 \ln Y + \alpha^* D + \beta^* e_2\,(D/Y) + \gamma^* e_2 \ln(DY) + \mu^* \quad (7)$$

Equations (6) & (7) incorporate dummy-variable (D = 0 for base period; D = 1 for new period) in both of its differential intercept.

Results and Discussion

Using data of 7105 households from SUSENAS Household Survey data for DIY Province, conducted during 2011 and 2013, we estimated equations (6) and (7) for some livestock products. The results of the estimated equations along with diagnostic statistics (t-ratio, F statistic and R2) are provided in Table 1 (equation 6) and Table 2

(equation 7), and discussed and interpreted, as follows.
The differential intercept and differential slope, in both equations, have turned out to be statistically significant in these livestock products, indicating that significant changes have occurred in quantity and expenditure elasticities during 2013 compared to the base yearThe coefficients β q and β e, explained in equation (2) & (3), have turned out to be statistically significant in beef, chicken, egg and milk products, suggesting that log-log-inverse (LLI) formulation of the model validate the non-linear behavior of Engel curve for livestock products food in DIY Province. All selected livestock products foods appear to give similar results, with exception of milk (where β q is statistically insignificant) and egg (where β e is statistically insignificant).Quantity elasticity of demand for these livestock products with respect to consumer income is estimated for year 2011 and 2013 (Table 3). Expenditure elasticity with respect to income is estimated for 2011 and 2 The quality elasticity is thus positive and is estimated for 2001 and 2005.

Conclusions

The quantity and expenditure elasticities with respect to consumers' income for livestock products foods have substantial changes during 2013 compared to base period of year 2011. The quality elasticity with respect to consumer's income turns out to be positive for all livestock products foods. However, it declined in magnitude in almost all livestock products food except for milk products from year 2011 to 2013.

KEYWORD：livestock products foods, expenditure elasticity, quantity elasticity, quality elasticity, Indonesia

Table 1. Empirical Result of Quantity Model

	Empirical Result	F-Ratio	R^2
Beef	Ln q = -3.277 - 60632.871 (1/Y) + 0.199 (LnY) -0.773 (D) (-3.732) (-3.511) (2.959) (-4.311)	16.176	0.108
Chicken	Ln q = -4.878 -33611.288 (1/Y) +0. 377 (LnY) -1.151 (D) (-16.909) (-6.335) (16.993) (-22.121)	221.128	0.201
Egg	Ln q = -4.686 -7978.866 (1/Y) + 0.333 (LnY) -.910 (D) (-24.201) (-3.409) (21.659) (-24.308)	268.419	0.139
Milk	Ln q = -2.085 -1588.066 (1/Y) + 0.204 (LnY) -.558 (D) (-4.282) (-1.038) (5.088) (-4.898)	15.114	0.015

(Figures in parenthesis represent t-ratios)

Table 2. Empirical Result of Expenditure Model

	Empirical Result	F-Ratio	R^2
Beef	Ln e = 6.927 - 62600.977 (1/Y) + 0.261 (LnY) - 0.561 (D) (7.687) (-3.528) (3.773) (-3.041)	39.173	0.228
Chicken	Ln e = 3.482 - 25914.287 (1/Y) + 0. 502 (LnY) - 1.214 (D) (11.945) (-4.833) (22.386) (-23.085)	365.436	0.294
Egg	Ln e = 3.370 -2936.291 (1/Y) + 0.446 (LnY) - 0.980 (D) (18.561) (-1.338) (30.978) (-27.940)	546.566	0.248
Milk	Ln e =0.198 + 7337.349 (1/Y) + 0.772 (LnY) - 1.947 (D) (0.566) (6.662) (26.739) (-23.709)	269.610	0.213

(Figures in parenthesis represent t-ratios)

Table 3. Quantity, Expenditure & Quality Elasticity (2011 and 2013)

	Quantity Elasticity		Expenditure elasticity		Quality elasticity	
	2011	2013	2011	2013	2011	2013
Beef	-0.435	-0.599	-0.126	-0.598	0.309	0.001
Chicken	-0.197	-0.073	0.098	0.12	0.295	0.193
Egg	-0.066	0.055	0.191	0.19	0.257	0.135
Milk	-0.034	-0.091	0.181	0.731	0.215	0.822

REFERENCES

Bank, J., r. Blundell, a. Lewbell. 1997. Quadratic Engel Curves and Consumer Demand. *The Review of Economics Statistics*, 79: 527-539.

Deaton, A. 1998. Quality, Quantity and Spatial Variation of Price. *American Economic Review*, 78: 418-430.

Deaton, A. 1997. *The Analysis of Household Surveys: A Micro Econometric Approach to Development Policy*. Baltimore USA: John Hopkins University press

Gale, F. and K. Huang. (2007). Demand for Food Quantity and Quality in China. U.S. Department of Agriculture, Economic Research Service, ERR-32. Washington D.C.

Hicks, W. W. and S.R. Johnson (1968). Quantity and Quality Components for Income Elasticities of Demand for Food. American Journal of Agricultural Economics 50:1512-1517.

Huang, K.S, and F. Gale. 2009. Food Demand in China: Income, Quality, and Nutrient Effects. *China Agricultural Economic Review*, 1: 395-409.

Tey, T. S., S. Madnasir, M. Zainalabidin, S. Jinap, R. A. Gariff, 2009. Demand for Quality Vegetables in Malaysia". *International Food Research Journal*, 168: 313-327.

O-15-5

The Analysis of Cost and Benefit of the Managerial Accounting on the Dairy Goat Farms in Taiwan(2012,10~2013,9)

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INTRODUCTION

Taiwan's accession to the World Trade Organization (WTO) will deliver an immediate impact to Taiwan's dairy farmers. The government wishes to reduce the production cost of milk. The main purpose of the study was to analyze the structure of cost and to compare the profits of different scales, districts of dairy farms and to identify the key factors affecting their costs and profits margins. We also make suggestions for dairy farmers how to reduce their production cost.

MATERIALS AND METHODS

I. Material

The experiment was conducted on 9 dairy goat farms, but it has complete data of one year only 6 farms. These samples were guided by the editor to record the managerial data on dairy goat farms.

II. Methods

The samples of the dairy farms were determined by purposive sampling. The dairy goat farms recorded daily managerial production costs and revenue since October, 2012 to September 2013. We use the method of farm management to analyze the managerial profits and to compare the benefits of different scales and districts on dairy goat farms in Taiwan.

RESULTS AND DISCUSSION

I. The Analysis of Average Managerial costs and Profits on Dairy Goat Farms.

1.the production cost and revenue per head of dairy goat

The materials mainly based on managerially recording accounts of 6 dairy goat farms. The production cost including home labor and capital interests of dairy goat per head was about US$704.0 (Table 1) one year. The main components of cost structure were feed cost (57.1%), labor cost(19.0%), depreciation cost of ewe (26.4%), depreciation and repair of building (13.2%). The gross revenue of dairy goat per head was about US$734.4, mainly including the net revenue of selling milk (80.2%), sale of dairy goat (12.9%), estimate of ewe hogget self-produced (3.5%). The net revenue and farm earnings were about US$30.5, US$121.8 respectively of dairy goat per head.

2.the managerial cost and revenue per farm

The average heads on feeding per dairy goat farm was about 428.7 heads. If we calculated the raising heads per year with animal unit, the average raised dairy goat was about 252.1 heads per farm. The percent of milking goat was 43.4% per farm. The production cost including capital interests was about US$189.8 thousands per farm. The gross revenue was about US$204.0 thousands and the net revenue was us$14.2 thousands per farm.

3.the production profits of milk per kg in view of the farm management

If we analyze the production cost and revenue in view of the farm management, the gross production cost including capital interest was US$1.5. The main components of gross revenue included sale of milk and by-products. The by-products included the sale of dairy goat, culled goat, pregnant ewe, ewe hogget, baby rams, goat waste, estimate of forage self-produced, estimate of ewe hogget self-produced. The net production cost excluded the value of by products of the goat milk per kg was US$1.47. The gross revenue and the net revenue of milk per kg was about US$1.51, US$0.04 respectively. If the estimate of home labor was excluded from the production costs, the farmer can get the farm earnings about US$0.24.

4.the production costs and revenue of milk per 100 kg in view of the milking goat

If we analyze the production costs in view of the milking goat, the costs was about US$106.7 per 100 kg of milk. The gross revenue and net revenue of milk per 100 kg were about US$110.68, US$4.39 respectively.

The Comparison of the managerial profits for the different district of dairy goat farms in Taiwan.

1.the comparison of production profits per head of dairy goat

The production cost of the dairy goat per head at the middle Taiwan was more than that at the southern Taiwan about US$58.75 (8.7%), owing to the feed cost of the dairy goat per head at the middle Taiwan also more than that at the southern Taiwan about US$4.75 (1.2%). Because the production of milk per head of milking goat at the middle Taiwan was about 630.37 kg less than that at the southern Taiwan about 65.21 kg one year. Thus the net revenue per head of dairy goat at the middle Taiwan was US$-60.73 less than that at the southern Taiwan about US$182.36 one year.

2.the comparison of the production profits of the milk per kg

In view of the farm management, owing to the production of the milking goat on the middle Taiwan farms was less than that at the southern Taiwan about 65.21 kg, the production cost of milk per kg was about US$1.62 more than that at the southern Taiwan about US$0.31. The net revenue of the milk per kg at the middle and the southern Taiwan were about US$-0.12, US$2.32 respectively. Thus the managerial efficiency for the dairy goat farm on different district have great difference.

III. The Comparison of the managerial profits on different scales of dairy goat farms

In general, the average production cost and the farm sizes has intimate relationship. We differentiated the samples into 2 groups based on feeding heads of dairy goats: 200~400, 401~700.

1.the comparison of the production costs and revenue per head of dairy goat

The least production costs per head of dairy goat among the 2 sizes was the size: 200~400. Its cost was about US$692.2. Next was the farm size 401~700, its cost was about US$715.82. Factors affecting the great difference between the two sizes of the production costs were feed cost. If the farmers did not produce forage especially using imported forage, they would pay more feed cost than others, because the imported price of forage, corn and soybean increased dramatically in recent year.

The gross revenue is intimated with the production of milking goat. The gross revenue of the size 200~400 was less than that of the size 401~700. The net revenue were positive excepting for the first size. The net revenue per head of the size 200~400 was about US$-3.30, but the size 401~700 was about US$64.21. If the home labor was excluded from production cost, the farm earnings per head of the size 200~400 was US$106.24 and that of the size 401~700 was US$137.45.

2.the comparison of the production costs and revenue of goat milk per kg in view of farm management

In view of farm management, the size of the least production cost among the 2 sizes was 200~400, its cost was about US$1.53 per kg of milk more than that of the size 401~700 about US$0.11. The size of the most net revenue among the 2 sizes was the size: 401~700 heads on feeding, its net revenue was about US$0.12 per kg of milk. Next was the farm size: 200~400 heads on feeding, its net revenue was about US$-0.04.

3.the comparison of the production costs and revenue per 100 kg of milk in view of milking goats

If we compare the production cost and revenue of the milking goats, the size of the least production cost among the 2 sizes was 401~700 heads on feeding, owing to the production of milking goat having great difference. The production of milking goats for the first size was 636.16 kg less than that on the second size about 53.63 kg (7.77%).

Although the production of the milking goats per head for the first size lower than the second size about 53.63 kg, the higher ratio of milking goats (43.66%) only more than that of the second size just 0.37%, the production cost more than that for the second size about US$5.03. As the result of the net revenue for the two sizes were about US$-0.52, US$9.3 respectively. In conclusion, if the smaller farm size enhance the pure technical efficiency such as productivity, the ratio of milking goat, it can compensate for the fault of diseconomy of dairy goat farms.

CONCLUSIONS

In view of the net revenue per head of dairy goat and per kg of milk, the size of the dairy goat 401~700 was the highest managerial efficiency among the 2 farm sizes. Owing to the high feed price and low milk purchased price, the dairy goat farmer can get low profits. As the results, the dairy goat farmers always raise meat goats from ram to increase profits. Under the condition of high feed price and lower purchared price of goat milk, the larger size you have the more profits you get for the dairy goat farm. If the farmer is not lack of capital and has enough home labor, the farm size 401~700 was suggested. The smaller size of dairy goat farms can be enhenced of the pure technical efficiency to make up for the fault of diseconomy and increase the ratio of milking goats. Under the condition of the increasing price of imported forage, it was suggested that the farmer using domestic forage to substitute for imported forage would be favorable.

KEYWORD：Dairy Goat, Goat Milk, Production Costs

Table 1 The comparison of the production cost for different scale of dairy goat farms in Taiwan(Oct.2012~Sep.2013)

unit:US$ / head

item	heads on feeding					
	200-400		401-700		weighted average	
	US$	%	US$	%	US$	%
feed cost	390.13	56.37	413.76	57.80	401.94	57.10
forage	138.11	19.95	124.74	17.43	131.43	18.67
concentrate	207.21	29.94	180.01	25.15	193.61	27.50
TMR	44.81	6.47	109.01	15.23	76.91	10.92
labor cost	133.97	19.36	134.10	18.73	134.03	19.04
medicine cost	6.07	0.88	5.68	0.79	5.87	0.83
other cost	42.91	6.20	33.85	4.73	38.38	5.45
direct cost (1)	573.08	82.80	587.38	82.06	580.23	82.42
the depreciation of ewe	57.99	8.38	51.83	7.24	54.91	7.80
deperciation and repair of building	20.91	6.20	34.13	4.24	27.52	3.91
deperciation and repair of machine	26.38	1.82	25.30	2.59	25.84	3.67
indirect cost (2)	105.28	15.21	111.26	15.54	108.27	15.38
1st production cost (1) + (2)	678.36	98.01	698.64	97.60	688.50	97.80
land rent (c)	0.00	0.00	2.59	0.36	1.29	0.18
debt interest (a)	0.00		13.41		6.71	
capital interest (b)	13.77	1.99	14.60	2.04	14.18	2.01
2nd production cost (I) (A)	678.36		714.64		696.50	
2nd production cost (I) (B)	692.14	100.00	715.82	100.00	703.98	100.00
sample farms	3		3		6	

*: 1.US$1=NT$29.60

2. 2nd production cost (I) (A) = 1st production cost + (c) + (a)
2nd production cost (I) (B) = 1st production cost + (c) + (b)

3.capita interest = (1st production cost x 4.5%) / 2

*data recorded by 6 dairy farm within period from Oct.1, 2012 to Sep,30 2013.

REFERENCES

S.YLeu.2001.The analysis of the production cost and revenue for the milk in Taiwan. The annual year book of the agricultural management. 7:1-34.

S.YLeu.1986. The production profits ofthe dairy famers in Taiwan. The monthly journal of food grains and livestock. 152:38-45.

O-15-7 Reproductive Performance and Expression of Mucin in the Lower Segment of Oviduct Between Indonesian Native Naked-Neck and Normal Feathered Chicken

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Abstract

Lower segment of oviduct is the primary tissue where microorganisms may invade from external environment colonizing in the vagina. Mucin composed of glycoproteins play significant roles in the barrier against infection on the mucosal surface. The aim of this study was to determine reproductive performance and expression of mucin in the lower segment of oviduct between Indonesian native naked neck and normal feathered chicken. Six males and 30 females of Indonesian native chicken were paired with a ratio of a male and 5 females Indonesian native naked neck and normal feathered chicken, respectively. Egg production was recorded daily and were hatched every week. The lower segment of oviduct (isthmus, uterus, and vagina) of Indonesian native naked neck and normal feathered chickens were collected. The expression of mucin mRNA in the mucosal oviduct was analyzed by reverse-transcription-polymerase chain reaction (RT-PCR), whereas mucin polysaccharides was localized by Alcian blue (AB) staining. The result of this research shows that the egg production (20.2%), egg fertility (67.8%), egg hatchability (54.4%), embryonic mortality (13.3%), egg weight (44.5g), and egg index (77.3%) of Indonesian naked neck chicken do not show significant differences compared to that of normal feathered chicken, namely (29.9%), (53.3%), (48.8%), (8.9%), (44.3%), (78.3%), respectively. Mucin mRNA was expressed in the mucosal oviduct of isthmus, uterus, and vagina in both naked neck and normal feathered chicken. The Alcian blue positive substances were localized on surface of mucosal epithelium in the vagina, whereas it was negligible in the uterus and isthmus in both naked neck and normal feathered chicken. These results suggest that the Indonesian native chicken had a lower productivity, higher embryonic mortality and lower hatchability. Moreover, mucin covers the surface of mucosal oviduct in both naked neck and normal feathered chicken, probably to form mucosal barrier.

INTRODUCTION

In general, mucosal barrier systems formed by mucus gel, epithelial cell junctional structures, and leukocyte activity, play important role to prevent infection by pathogenic agents in mucosal tissues. Mucins have the ability to form a physical barrier and act as adhesion decoys to invading agents (Linden et al., 2008a), and they may prevent pathogen penetrance by inhibiting bacterial adhesion to the mucosal epithelium surface (Berry et al., 2002). Mucins either have direct antimicrobial activity or carry other antimicrobial molecules (Linden et al., 2008b). Cell surface mucins may also initiate intracellular signaling in response to bacteria, and thus they have both a barrier and reporting function on the apical surface of mucosal epithelial cells (Linden et al., 2008a). If microorganisms cross an epithelial barrier and begin to replicate in the mucosal tissues, phagocytic cells including the monocytes or macrophages, or polymorphonuclear leukocytes (PMNs) recognize, ingest, and destroy them (Murphy et al., 2007; Macia et al., 2012).

Glycoprotein sugar-residues could be identified and characterized by lectins. Lectins bind to a specific sugar residue of glycoprotein with high affinity. WGA, a lectin from wheat germ agglutinin (*Triticum vulgaris*), binds specifically to N-acetylglucosamine (GlcNAc) and N-acetylneuraminic acid (sialic acid). Jacalin lectin, the major protein from jackfruit (*Artocarpus heterophylus*) seeds, shows highly specific binding to galactose (Gal) and N-acetylgalactosamine (GalNAc) (Kabir, 1998; Tatsuzuki et al., 2009; Fallis et al., 2010).

Reports of mucin glycoprotein expression in the oviduct of Indonesian naked neck and normal feathered chickens were very limited. Thus, it is of great importance to determine reproductive performance and expression of mucin in the lower segment of oviduct between Indonesian native naked neck and normal feathered chicken.

MATERIALS AND METHODS

Experimental birds. Indonesian native naked neck and normal fethered chicken with the age and weight of the relatively uniform were used in this study. All chickens were identified according to non feather distribution,

namely naked neck and normal feathered chickens. The lower segment of oviduct (isthmus, uterus, and vagina) of Indonesian indigenous naked neck and normal chickens were collected. The expression of mucin gene in the mucosa was analyzed by reverse-transcription polymerase chain reaction (RT-PCR). Localization of mucin polysaccharide was analyzed by alcian blue (AB) staining.

PCR analysis for expression of mucin. Total RNA was extracted from the mucosal tissues of isthmus, uterus, and vagina using Sepasol RNA I Super. They were treated with DNase to remove genomic DNA and were reverse-transcribed using ReverTra Ace according to the manufacturer's instructions. PCR was performed using Takara Ex Taq. Primers used for mucin analysis were as follows (forward: 5'-TCT TCC GCT ACC CTG GGC TCT GTAA-3'; reverse: 5'-CTC ATG CAG TTC TAG CAA GAT ACT-3'). PCR products of mucin were separated by electrophoresis. PCR was performed as described by Ariyadi et al. (2012).

Alcian Blue Staining. Tissue samples of the lower segment of oviduct (isthmus, uterus, and vagina) were fixed with 10% (vol/vol) formalin in PBS, dehydrated, and embedded in paraffin. The sections (4 μ m in thickness) were air dried on slides. Histochemical localization of mucin polysaccharide was performed by Alcian blue (**AB**) staining. Sections were deparaffinized and immersed into 3% (vol/vol) acetic acid for 1 min, followed by staining with AB dissolved in 3% (vol/vol) acetic acid for 1 h. After being washed in water, the sections were dehydrated and mounted.

RESULTS AND DISCUSSION

The result of this research shows that the egg production (20.2%), egg fertility (67.8%), egg hatchability (54.4%), embryonic mortality (13.3%), egg weight (44.5g), and egg index (77.3%) of Indonesian naked neck chicken do not show significant differences compared to that of normal feathered chicken, namely (29.9%), (53.3%), (48.8%), (8.9%), (44.3%), (78.3%), respectively. These results suggest that the Indonesian native chicken had a lower productivity, higher embryonic mortality and lower hatchability. It was supported by Yakubu et al. (2008) that Nigerian indigenous naked neck chickens had hatchability of 71.49% and embryonic mortality of 28.66%. Islam and Nishibori (2009) showed that Bangladeshi indigenous naked neck chickens had embryonic mortality of 16%. Caratachea et al. (2010) reported that Mexican naked neck chickens had egg production of 54% and egg weight of 51.0 g. Sharifi et al. (2010) reported that Na genes might induced embryonic mortality in the naked neck broiler dams. Moreover, Yakubu et al. (2008) reported that Nigerian indigenous naked neck chickens had egg weight of 43.04 g and egg index of 74.68%. Islam and Nishibori (2009) showed that Bangladeshi indigenous naked neck chickens had egg weight of 39.99 g and egg index of 82.22%.

Figure 1 shows the expression of mucin gene in the isthmus, uterus, and vagina of both Indonesian indigenous naked neck and normal feathered chickens. Mucin gene was expressed in the isthmus, uterus, and vagina of both Indonesian indigenous naked neck and normal feathered chickens. Electrophoresis of PCR product showed that mucin gene was expressed at 317 bp, where the band of mucin was denser in the naked neck than of the normal feathered chickens.

Mucin mRNA was expressed in the mucosal oviduct of isthmus, uterus, and vagina in both naked neck and normal feathered chicken. The Alcian blue positive substances were localized on surface of mucosal epithelium in the vagina, whereas it was negligible in the uterus and isthmus in both naked neck and normal feathered chicken. These results suggest that mucin covers the surface of mucosal oviduct in both naked neck and normal feathered chicken, probably to form mucosal barrier. It was supported by Smirnov et al. (2005) that the mucin glycoprotein was expressed in the chicken jejenum and ileum. Rajkumar et al. (2010) revealed that the immune competence was higher in the naked neck chickens than of the normal feathered chickens. Ariyadi et al (2012) showed that expression of mucin mRNA was higher and immunoreactive mucin5AC on the surface of mucosal epithelium was denser in laying than molting hens. Ariyadi et al (2013) showed that estrogen upregulated mucin expression and increased AB-positive polysaccharide in the oviduct of laying hens.

Conclusions. These results suggest that mucin gene as well as mucin glycoprotein covers the surface of mucosal lower oviductal tract in both naked neck and normal feathered chickens, probably to form mucosal barrier.

Explanations of figure

Figure 1.Expression of mucin gene in the isthmus, uterus, dan vagina of both Indonesian indigenous naked neck and normal feathered chickens. Electrophoresis of PCR product showed that mucin gene was expressed.

KEYWORD : Native chicken, Reproduction, Mucosal oviduct, Mucin

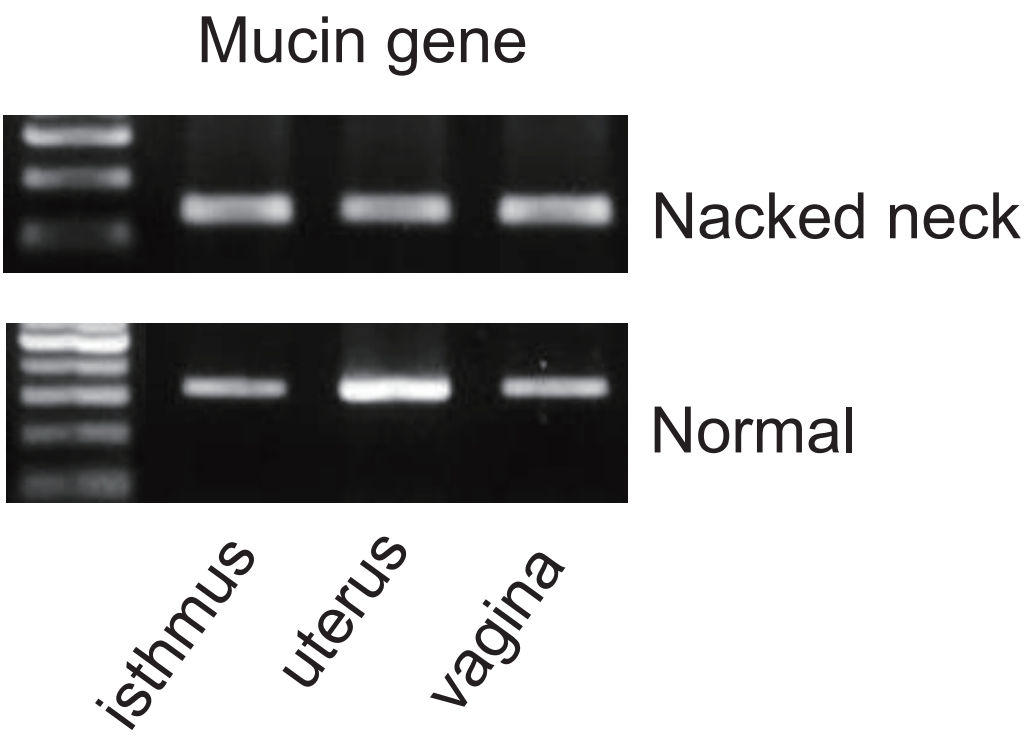

figure1

REFERENCES

Ariyadi, B., N. Isobe, and Y. Yoshimura. 2012. Differences in the mucosal surface barrier formed by mucin in the lower oviductal segments between laying and molting hens. Poult. Sci. 91:1173-1178.

Ariyadi, B., N. Isobe, and Y. Yoshimura. 2013. Induction of mucin expression by estrogen and lipopolysaccharide in the lower oviductal segments in hens. Poult. Sci. 92: 3205-3213.

Berry, M., A. Harris, R. Lumb, and K. Powell. 2002. Commensal ocular bacteria degrade mucins. British J. Ophthal., 86:1412-1416.

Caratachea, J., E.G. Vazquez, R. Garciduenas-Pina and G. Salas-Razo, 2010. Egg production in naked-neck creole (Nana) hens and in those with normal plumage (nana) in the region of the mexican plateau. C.J. Agric. Sci., 44: 281-284.

Fallis, L.C., K.K. Stein, J.W. Lynn, and M.J. Misamore. 2010. Identification and role of carbohydrates on the surface of gametes in the zebra mussel, Dreissena polymorpha. Biol. Bul., 218:61-74.

Islam, M.A. and M. Nishibori, 2009. Indigenous naked neck chicken: a valuable genetic resource for Bangladesh. W. Poult. Sci. J., 65: 125-138.

Kabir, S. 1998. Jacalin: a jackfruit (*Artocarpus heterophyllus*) seed-derived lectin of versatile applications in immunobiological research. J. Immunol. Methods, 212:193-211.

Linden, S.K., T.H.J. Florin, and M.A McGuckin. 2008a. Mucin dynamics in intestinal bacterial infection. PLoS One 3:1-14.

Linden, S.K., P. Sutton, N.G. Karlsson, V. Korolik, and M.A. McGuckin. 2008b. Mucin in the mucosal barrier to infection. Mucosal Immun., 1:183-197.

Macia, L., A.N. Thornburn, L.C. Binge, E. Marino, K.E. Rogers, K.M. Maslowski, A.T. Vieira, J. Kranich, and C.R. Mackay. 2012. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immun. Rev., 245:164-176.

Murphy, K, P. Travers, and M. Walport. 2007. Janeway's immunobiology. 7th ed. Garland Science, Taylor and Francis Group, New York and London.

Rajkumar, U., B.L.N. Reddy, K.S. Rajaravindra, M. Niranjan, T.K. Bhattacharya, R.N. Chatterjee, A.K. Panda, M.R. Reddy and R.P. Sharma. 2010. Effect of naked neck gene on immune competence, serum biochemical and carcass traits in chickens under a tropical climate. Asian-Aust. J. Anim. Sci., 23: 867–872.

Sharifi, A.R., P. Horst and H. Simianer, 2010. The effect of naked neck gene and ambient temperature and their interaction on reproductive traits of heavy broiler dams. Poult. Sci., 89: 1360-1371.

Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan and Z. Uni. 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. J. Nutr. 135: 187-192.

Tatsuzuki, A., T. Ezaki, Y. Makino, Y. Matsuda, and H. Ohta. 2009. Characterization of the sugar chain expression of normal term human placental villi using lectin histochemistry combined with immunohistochemistry. Arch. Histol. Cytol., 72:35-49.

Yakubu, A., D.M. Ogah and R.E. Barde, 2008. Productivity and egg quality characteristics of free range naked neck and normal feathered Nigerian indigenous chickens. Int. J. Poult. Sci., 7: 579-585.

O-16-1

The Effect of Sexed Semen Methods Toward Motility and Ratio of X and Y Sperm Filial Ongole Cattle

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INTRODUCTION

Effort to increase the efficiency of using AI in cattle is how to obtain the effectiveness of sperm separation (sexed semen) technology to separate X and Y sperm chromosomes. There are various methods of sexed semen has been found including the sedimentation method using albumin column and percoll density gradient centrifugation (Hafez and Hafez 2008). Motility is an important indicator for characterization of sperm quality after processing. Sexing methods easily applied is egg white sedimentation and percoll density gradient centrifugation method (SGDP) because after having been frozen more than 30% motility and more than 80% Y sperm proportion (Susilawati 2014). It necessary to determine the effect of sexed semen methods using egg white sedimentation and percoll density gradient centrifugation toward motility and ratio of X and Y sperm Filial Ongole Cattle.

MATERIAL AND METHODS

Semen was collected using AV from five Filial Ongole Cattle in Beef Cattle Research Station, Pasuruan, Indonesia. After collection, fresh semen was evaluated macroscopically (colour, pH, volume) and microscopically (concentration, mass motility, individual motility, life sperm and abnormal sperm). Only fresh semen with a minimum of 70% individu motile sperm and 2+ mass motility used in this study. Semen collection was regularly conducted twice a week.

The selected semen separated using egg white sedimentation and percoll density gradient centrifugation technique. X and Y sperm separation method using egg whites sedimentation with 3 densities (10%, 30% and 50%) made from highest to lowest density (Purwoistri et al. 2013), and incubated for 20 minutes. Separation method using 10 density percoll with 10 arranged from highest to lowest density and centrifuged at 2250 rpm for 5 minutes (Susilawati 2014). Andromed as a based extender was diluted using aquabidest with 1:4 ratio. The experiment was designed using completely random design with 3 treatment (non sexing, percoll density gradient centrifugation, egg white sedimentation methods) and 10 replication .

The parameters observed, percentage of X and Y sperm based on morphometry of sperm wide head, percentage of motility before and after separation of the sexed semen. The obtained data were analyze with analysis of variance (ANOVA) and continued by Duncan test if there was significant or very significant different.

RESULTS AND DISCUSSION

Characteristic of Fresh Filial Ongole Cattle Semen

The semen samples from Filial Ongole Cattle used in this study were evaluated both macroscopic and microscopically. The mean characteristics of ten ejaculates used in this study such as volume, color, pH, consistency, mass motility, progressive motility, viability, abnormality, consentration were 4,4 ± 2,18 ml, creamy, 7 ± 0, thick, 2+, 70 ± 0%, 95,12 ± 0,98%, 0,92 ± 0,27%, 1.758 ± 137,66 x 10^6/ml, respectively. The semen used in this study was normal (Garner and Hafez 2008; Ax et al. 2008; Pineda 2003). The morphometry observation of the fresh semen, showed that the percentage of X and Y sperm approaching 50%:50% (X : Y: 50,4 ± 1,17% : 49,6 ± 1,17%), this is in accordance with the general state of the X and Y sperm ratio in the fresh semen is 50%:50%, and after fertilization, 50% the embrio should be males, and 50% should be females (Pineda 2003). Research results Susilawati et al. (2015) show by using sperm without sexing, it produced 59,25% male calves.

Motility of Filial Ongole Cattle Sexed Semen

Motility percentage of Filial Ongole Cattle sexed semen with different methods is shown in Table 1.

Table 1. Motility percentage of Filial Ongole Cattle sexed semen with different methods

Sexing Methods	Motility (%)
Non Sexing (control)	64 ± 3.94[b]
SGDP (Upper Fraction)	48 ± 8.28[a]
SGDP (Under Fraction)	53 ± 7.93[a]
Egg white sedimentation (Upper Fraction)	57 ± 8.23[b]
Egg white sedimentation (Under Fraction)	50 ± 8.19[a]

[a, b] different superscripts in column indicate highly significant different (P<0,01)

The result in Table 1. shows that the sperm motility was highly significant different (P<0,01) after sexing between sexing methods. The sperm motility of egg white sedimentation (Upper Fraction) sexed semen was better than other treatments and no different from non sexing method (control). The sperm motility of percoll density gradient centrifugation is lower than egg white sedimentation. This is because the centrifugation cause the more damage the sperm membrane. Furthermore, sperm motility will decline as an increase in the number of broken membrane. Susilawati et al. (2014) fount that centrifugation process might also damage sperm membrane, thus causing a decrease on sperm motility. The centrifugation process provided free radicals namely reactive oxygen species (ROS) that damaged the sperm membrane. ROS are free radicals that play a crucial role in many sperm physiological processes such as capacitating, hyper activation and sperm-oocytes fusion (Aitken et al. 2012; Ball 2008; Bansal & Bilaspuri 2011).

X and Y Sperm Ratio of Filial Ongole Cattle Sexed Semen

Table 2. X and Y sperm ratio of Filial Ongole Cattle sexed semen with different methods

Sexing Methods	X and Y sperm ratio (%)	
	X sperm	Y sperm
Non Sexing	50,4 ± 1,17%	49,6 ± 1,17%
SGDP (Upper Fraction)	28,0 ± 2,26%	72,0 ± 2,26%
SGDP (Under Fraction)	77,5 ± 1,26%	22,5 ± 1,26%
Egg white sedimentation (Upper Fraction)	69,0 ± 15,35%	26,0 ± 4,37%
Egg white sedimentation (Under Fraction)	22,9 ± 1,44%	77,1 ± 1,44%

The result in Table 2. shows the significant effect (P<0,05) on ratio of X and Y sperm sexed semen between sexing methods. The sexed semen with egg white sedimentation method is better than percoll density gradient centrifugation on ratio of X and Y sperm. Under fraction have a high of X sperms, whereas the upper fraction have a high of Y sperms on percoll density gradient centrifugation method. The sexed semen with egg white sedimentation method produce a high of X sperms on upper fraction, whereas the under fraction have a high of Y sperms. From all of research results, sexing sperm technology using egg white sedimentation technique is better than percoll density gradient centrifugation on ratio of X and Y sperm.

CONCLUSION

The study concludes that the sexed with egg white sedimentation technique is better than percoll density gradient centrifugation on sperm motility and ratio of X and Y sperm.

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KEYWORD : Filial Ongole Cattle, Sexed Semen, Egg White Sedimentation, Density Gradient Centrifugation Percoll, Ratio of X and Y sperm

REFERENCES

Aitken R. J., Jones K. T. and Robertson S. A.. 2012. Reactive Oxygen Species and Review Sperm Function-In Stickness and In Health, J. Androl 22 (6): 1096-1106.

Ax R.L., M.R. Dally, B.A. Didion, R.W. Lenz, C.C. Love. D.D. Varner, B. Hafez and M.E. Bellin. 2008. Artifical Ainsemination. In: Hafez E.S.E and Hafez B., editors. Reproduction In Farm Animals. ed. Australia: Blackwell Publishing. p. 365-375.

Ball B.A. 2008. Oxidative Stress, Osmotic Stress and apoptosis: Impact on Sperm Function and preservation in the horse. Animal Reproduction Science 107: 257-267.

Bansal A.K. and Bilaspuri G. S. 2011. Impact of Oxidative Stress and Antioxidants on semen function: Review Article. SAGE- Hindawi Access to Research. Veterinary Medicine International: 1-7.

Garner D.L. and Hafez E.S.E. 2008. Spermatozoa and Seminal Plasma. In: Hafez E.S.E and Hafez B., editors. Reproduction In Farm Animals. ed. Australia: Blackwell Publishing. p. 96-109.

Hafez E.S.E. and Hafez B. 2008. X and Y Chromosome-Bearing Spermatozoa. In: Hafez E.S.E and Hafez B., editors. Reproduction In Farm Animals. ed. Australia: Blackwell Publishing. p. 390-394.

Susilawati, T. 2014. Sexing Spermatozoa (Hasil Penelitian Laboratorium dan Aplikasi pada Sapi dan Kambing). Malang: Universitas Brawijaya Press.

Susilawati T., Sri R., Setyabudi U., Herni S., and Eko N. 2014. Effect of Different Centrifugation on Simmental Bull Sperm Quality and Membrane Status after Sexing, Cooling and Freezing Processes. American-Eurasian Journal of Sustainable Agriculture. 8(7):28-34.

Susilawati T., Lieyo W., Nurul I. Aulia P.A.Y. 2015. Reproductive Performance of Ongole Crossbreed Cattle Using Artificial Insemination Sexed Semen With Different Methods. In: Rima A.E.W., Prisilia P.S., Miftahush S.H., Septi M., Aditya A., Riyan N.A., Pradiptya A.H., Satyaguna R., Zefanya A.G., Bagas P., editors. Integrated Approach in Developing Sustainable Tropical Animal Production. Proceeding The 6th International Seminar on Tropical Animal Production. Yogyakarta, 20-22 October. 2015. Yogyakarta (Indonesia): Faculty of Animal Science, Gadjah Mada University, Indonesia. p. 521-525.

Pineda, M.H. 2003. The Biology of Sex. In: McDonal's. Veterinary Endocrinology and Reproduction. Fifth Edition. USA: Blackwell Publishing. p. 201-238.

Purwoistri, R. F., Susilawati T., Rahayu S. 2013. Membran Spermatozoa Hasil Seksing Gradien Albumin Berpengencer Andromed dan Cauda Epididymal Plasma-2 Ditambahkan Kuning Telur. Jurnal Veteriner. 14 (3): 371-378.

O-16-3

Calf Birth Weight and Post Partum Estrus Bali Cow Fed Complete Feed From Palm Oil Plantation in Central Borneo Indonesia

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Introduction

The condition beef cattle that were kept by the farmers around palm oil plantations in Central Kalimantan, Indonesia are currently only rely on the grass for animal feed. The location of grazing field is far enough approximately 4-10 km for 4-5 hours per day. It is a distant and long time to feed the Bali cows. Therefore, the source of feed in palm oil can be used as the main feed to optimize cattle production.

Palm oil plantations have a very large source of feed for Bali cow, including the legume crop cover, by-product of palm oil plantations such as oil palm frond and by-product of palm oil mills such as palm kernel cake. As cattle feed, the potential feed plantation should be sufficiently visible in terms of production, nutrients, palatability and digestibility to produce a good Bali cow productivity. Jalaludin et al. (1991) stated that the cause of low reproduction and production was generally the difficulty of continuous quantity and quality of feed, especially during the dry season.

Legume cover crop planted between the the palm trees in general were Caloppo, Puero and Mucuna, crude protein content 16-23%, TDN 54,3-68,7% (Legel, 1990; Mudhita et al., 2016). Oil Palm Frond was a by-product of palm oil plantations that received less attention by farmer, but it had production of 7 kg per frond (aged>7 years) so it can be a source of fibrous feed for ruminants by processing it first by using chopper machine to soften the leaves (Hassan dan Ishida, 1992). The energy source of feed from palm oil plantations were palm kernel cake (Palm Kernel Cake), containing 13.9-15.4% crude protein, TDN 75% and ME 13.1 (MJ/kg) (Puastuti et al; 2014; Boateng et al. 2013).

Materials and Methods

This study was conducted on a palm tree plantation in West Kotawaringin Regency, in Central Borneo during August 2015 to February 2016. Twenty four (24) Bali cows at 5 month pregnancy were devided into two groups. 12 heads with average weight of 240.3 kg were given complete feed from palm oil plantation: *Pueraria javanica* 35%, palm oil frond 25% and palm kernel cake 40%, consisted of crude protein 13.89% and TDN 73.25% in the form of mash, compared to 12 heads with average weight of 235.9 kg that were given non palm oil plantation products (farmer feed): native grass 70%, elephant grass 30% consisted of crude protein 9% and TDN 51% (Table 1. and Table 2.). Feed was given 2,6% DM of body weight during pregnancy until post partum estrus.

The data collected were: feed consumption and digestibility nutrient, body condition score (start and finish), lenght of pregnancy, calf birth weight and post partum estrus.

Result and Discussion

Consumption

The average nutrients consumption of Bali cows that were fed with palm oil and and non palm oil plantations is shown in Table 3.

The consumption and percentage of dry matter intake and organic matter between the feed palm oil plantation with farmer feed were significantly different ($P<0.05$). Required nutrient for pregnant cow of 240.3 kg weight of farmer feed needed 6.25 kg of dry matter, 0.55 kg of crude protein, and 3.27 kg of TD. Meanwhile, the mash feed for cows with 235.9 kg body weight required 6.13 kg of DM, 0.54 kg of CP and 3.21 kg of TDN (Kearl, 1982). Dry matter consumption was occupied 71.2% from farmer feed and 92.7% from palm oil plantation feed. Low consumption of dry matter on farmer feed caused by the amount given were not sufficient because the ability of farmers to find native grass and nutrient content compare was lower compared palm oil plantation.

Dry matter consumption also affected the the consumption of crude protein (CP), TDN and crude fiber. It indicated that there was difference ($P <0.05$) between the feed from the palm oil plantations and farmer feed. Crude protein

consumption of farmer feed was less than 74.8% of the requirements, while palm oil plantation feed was 146.9%. Low consumption of crude protein of farmer feed was caused by low crude protein and high crude fiber (Table 1). Increased consumption of dry matter was in line with with TDN consumption, because the consumption of nutrients was affected by dry matter consumption and nutrient content of the feed. TDN of farmer feed consumption that should be 2.26 kg/head/day only reached 69% of the requirement (3.27kg/h/d), while feed palm oil plantation reached 130%. It shows that feed from palm oil plantations had a very sufficient nutrient quality, even exceed, for the needs for Bali cattle. Although palm oil frond had low nutrient, but it can be a source of fiber when it was mixed with legumes Puero and palm kernel cake that had high crude protein. It resulted in TDN consumption that was much higher from farmer feed.

Digestibility

The average of nutrients digestibility pregnant Bali cow fed from palm oil plantations in the form of mash and farmer feed shown in Table 4.

The results digestibility showed that the digestibility: dry matter, crude protein, crude fiber and NDF there were no difference between the feed palm oil plantation with farmer feed, while digestibility: organic matter, TDN and ADF was different and higher than farmer feed ($P<0.05$). Schneider and Flatt (1975) stated that high digestibility was reached when the value was 70% and lower at 50%. The low of dry matter digestibility of the feed from palm oil plantations was probably because there were still shell of the palm kernel cake and their sticks from the oil palm frond (OPF). As it is known, the shells of kernel and sticks OPF were difficult for cattle to digest. There was an increase in organic matter digestibility of 11.9%, 7.7% of crude protein, 54.9% of TDN and 10.9% of crude fiber in palm oil plantations feed than farmer feed. This was due to the ash content in farmer feed was high enough of 13.9% and palm kernel cake could be considered as concentrates. According to Tillman (1998), the concentrate was functioned as a stimulant for rumen activity, so it can improve the digestibility of forage.

Reproduction performance

Bali cow reproduction performance that was fed from palm oil plantations is shown in Table 5.

The average BCS of 5 months pregnant Bali cow with farmer feed was about 3.4 meaning that cattle conditions were fatter. Meanwhile, those which were given the mashed palm oil plantation feed was about 3.5 meaning in the same condition of cows were about the same with farmer feed. According Putro (2009), the optimum BCS value for cattle reproduction in early pregnancy was between 3.0 - 3.5 (in 1-5 scale). BCS final before calving had no difference of about 4.1 on the farmer feed and 4.2 on mashed palm oil plantation feed. This result indicates that high consumption of dry matter, crude protein, TDN caused high BCS also (fatter condition). Berry et al. (2007) stated that the ideal BCS for the cow when calving was 4 (BCS scale of 1 to 5). The pregnancy length of Bali cows that were fed with palm oil plantations and farmer feed had no difference. Feradis (2010) stated that cow length pregnancy varies between 276-295 days.

Calves birth weight were different between those which were feed with palm oil plantations and farmer feed ($P<0.05$). There was an increase of .3% in birth weight of those with palm oil plantations feed. High birth weight was caused by feeding nutrients from palm oil plantations were sufficient although it used only three feed stuffs (Table 1.). Therefore, its palatability increase, dry matter consumption was in accordance with requirement, even the crude protein and TDN consumption exceeded (130-140%), and high digestibility (> 60%).This means that the feed from palm oil plantations had enough nutrients, resulting the final BCS of Bali cow better, faster pregnancy length, and higher calf birth weight compares to farmer feed. These results were higher from Dahlanuddin et al. (2016) report stated that Bali calves birth weight was 16 kg with improved quality feed in Lombok.

Post Partum Estrus (PPE) of Bali cow that were fed with palm oil plantations was different from those with farmer feed ($P<0.05$), thus it accelerated PPE by 15.3 days with palm oil plantations feed. Toelihere (1985) stated that the PPE of cows was generally between 30-70 days post partum. PPE is influenced by lactation and involution (the depreciation of reproductive organs size). The faster the weaning period and returned the size of the reproductive organs to normal size, the faster was the PPE. Feed with high nutrients, especially protein and energy will accelerate involution of reproductive organs.

Conclusion

The results showed that the complete feed from palm oil plantation gave higher feed consumption, digestibility, high calves birth weight and shorter post partum estrus. Calves birth weight and post partum estrus in these group were 18.13 kg and 54.6 days better compared to others group which was 15.07 kg and 69.9 days. It can be concluded that complete feed from palm oil plantation can increase calves birth weight 20.3% and accelerated

post partum estrus of 15.3 days.

KEYWORD：Pueraria javanica, palm oil frond, palm kernel cake, birth weight, post partum estrus Bali cow

Table 1. Feedstuffs nutrien content (%DM)

Feedstuffs	DM	OM	CP	CF	EE	NFE	TDN	NDF	ADF
Palm Oil Plantation									
Pueraria javanica	23.43	91.49	18.3	43.1	1.71	29.31	57.51	53.35	29.48
Oil Palm Frond	23.38	95.98	1.96	47.14	0.64	47.35	47.33	74.56	51.43
Palm Kernel Cake	91.88	92.86	15.8	24.46	6.45	46.76	67.44	74.32	53.21
Non Palm Oil Plantation									
Native+Elephant grass	21.44	86.07	9.13	50.92	29.15	2.15	41.65	73.22	53.60

Table 2. Nutrient Content of Bali Cow Feed

Nutrient content	Non POP	Mashed POP
Dry Matter (%)	21.44	35.22
Organic Matter (%)	86.07	94.07
Crude Protein (%)	9.13	13.89
Total Digestible Nutrient (%)	50.92	73.52
Crude Fiber (%)	29.15	20.27
Ether extract (%)	2.15	9.85
Nitrogen free extract (%)	41.65	51.08
NDF (Neutral Detergent Fiber) (%)	73.22	67.02
ADF (Acid Detergent Fiber) (%)	53.60	44.54

Non POP: Non Palm Oil Plantation, POP: Palm Oil Plantation

Table 3. Bali Cow consumption fed from palm oil plantation

Consumption parameters	Non POP	Mashed POP
Dry Matter (kg)	4.45^{a}±0.44	5.69^{b} ±1.00
Percentage of body weight (%)	1.86^{a}±0.11	2.41^{b} ±0.06
Organic Matter (kg)	3.83^{a} ±0.38	5.34^{b} ±0.94
Crude Protein (kg)	0.41^{a} ±0.04	0.79^{b} ±0.14
Total Digestible Nutrient (kg)	2.26^{a} ±0.22	4.18^{b} ±0.73
Crude Fiber (kg)	1.30^{a} ±0.13	1.15^{b}±0.20
Neutral Detergent Fiber (NDF) (kg)	3.26^{a} ±0.32	3.81^{b} ±0.67
Acid Detergent Fiber (ADF) (kg)	2.38^{a} ±0.24	2.53^{a} ±0.44

The superscripts a and b indicate significant differences between the columns
Non POP: Non Palm Oil Plantation, POP: Palm Oil Plantation

Tabel 4. Bali Cow digestibility fed from palm oil plantation (%)

Digestibility	Non POP	Mashed POP
Dry matter	60.00±2.03	58.69±4.51
Organic Matter	60.63^{a} ±2.68	67.86^{b} ±3.49
Crude Protein	56.29 ±3.28	60.65 ±2.08
Total Digestible Nutrient (TDN)	49.81^{a} ±2.37	77.18^{b} ±4.19
Crude Fiber	53.27 ±4.75	59.05 ±8.68
Neutral Detergent Fiber (NDF)	62.15±2.63	57.93 ±6.12
Acid Detergent Fiber (ADF)	63.22^{a} ±2.83	49.81^{b} ±9.91

The superscripts a and b indicate significant differences between the columns
Non POP: Non Palm Oil Plantation, POP: Palm Oil Plantation

Table 5. Reproduction Performance of Bali Cow fed from palm oil plantation (%)

Reproduction parameter	Non POP	Mashed POP
Sample (head)	12	12
BCS start	3.40±0.52	3.50±0.53
BCS finish	4.10±0.32	4.20±0.42
Length of pregnancy (day)	283.20±6.16	282.50±5.46
Calf birth weight (kg)	15.07^{a}±0.68	18.13^{b}±1.59
Post Partus Estrus (day)	69.9^{a}±16.1	54.6^{b}±7.03

The superscripts a and b indicate significant differences between the columns
Non POP: Non Palm Oil Plantation, POP: Palm Oil Plantation, BCS: Body Condition Score, ADG: Average Daily Gain

REFERENCES

Berry D. P., J. M. Lee, K. A. MacDonald, and J. R. Roche. 2007. Body condition score and body weight effects on dystocia and stillbirths and consequent effects on postcalving performance. J. Dairy Sci. 90:4201–4211. American Dairy Science Association, 2007.

Boateng M, Okai DB, Donkoh A, Baah J. 2013. Effect of processing method on the quality of palm kernel cake: Chemical composition and nutrient utilization in enzyme supplemented diets. Afr J Agric Res. 8:5226-5231

Dahlanuddin, Zaenuri L A, Sutaryono Y A, Hermansyah, Puspadi K, McDonald C, Williams L J, Corfield J P and van Wensveen M. 2016. Scaling out integrated village management systems to improve Bali cattle productivity under small scale production systems in Lombok, Indonesia. *Livestock Research for Rural Development. Volume 28, Article* #79. Retrieved July 9, 2016, from http://www.lrrd.org/lrrd28/5/dahl28079.htm

Feradis. 2010. Animal Reproduction. Alfabeta Publisher. First Edition. Bandung.

Hasan, O.A. dan M. Ishida. 1992. Status of utilization of selested fibrous crop residues and animal performance with special emphasis on processing of palm oil frond (OPF) for ruminant feed in Malyasia. Trop. Agric. Series 24:135-143.

Jalaluddin, S., Z.A. Jelan, N. Abdullah, and Y.W.Ho. 1991. Recent development in plam oil by productbased ruminent feeding system. In: Y.W. Ho,H. K. Wong, N. Abdullah and Z.A. Tajuddin(Eds.). Recent Advances on

the Nutrition of Herbivores.Proceeding of the Third International Symposium on Nutrition of Herbivores. Malaysian Soc. Anim. Production.

Kearl, L.C. 1982. Nutrient Requirements of Ruminants in Developing Countries. International Feedstuff Institute, Utah State University. Logan Utah. USA.

Legel, S. 1990. Tropical Forage Legumes and Grasses. Institute of Tropical Agriculture of the Karl-Marx-University Leipzig. Deutscher Landwirtschaftsverlag. Berlin.

Mudhita I.K., N. Umami, S.P.S. Budhi, E. Baliarti, C.T. Noviandi, Kustono, I.G.S. Budisatria and J. Wattimena. 2016. Effect of Bali cattle urine on legume cover crop puero (*Pueraria javanica*) productivity on an East Borneo oil palm plantation. Pak. J. Nutrition 15 (5): 406-411

Puastuti W, D. Yulistiani, I.W.R. Susana. 2014. Evaluation of the nutritional value of palm kernel cake fermented with fungi as a source of ruminant protein. JITV Vol.19 No 2 Th. 2014: 143-151.

Putro, P.P. 1999. The phenomenon of Brahman cross cattle reproduction: problems and workarounds. Papers Procurement Evaluation of Cross Brahman cattle. Breeding Directorate. Directorate General of Livestock. Agriculture Department. Jakarta.

Schneider, B.H. and W.P. Flatt. 1975. The Evaluation of Feeds Through Digestibility Experiment. The University of Georgia Press, Athens.

Toelihere, M.R., 1985. Reproduction in Animal Physiology. 4th Edition. Publisher: Angkasa Bandung.

O-16-4

Milk Production of Sows Superovulated with PMSG and hCG Through Superovulation Before Mating

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Abstract

This research has been conducted to study the effect of superovulation prior to mating toward gilts reproduction performance. Sixty gilts were divided into two treatments *1)* gilts without superovulation and *2)* gilts with superovulation. Once the gilts shows a standing heat symptoms, the boar inserted into the pig pen to mat the gilts. During the study, the pregnant gilts kept together in postal pens, then two weeks before farrowing each pregnant gilts then placed in 2.5 x 3.5 m2 individual cages equipped with feeding and drinking devices. A Completely Randomized Design (CRD) was used in the first phase of study, consisting of two treatments with 30 replicates each, while analysis of data based on the mathematical model procedures, as follows: Yij = $\mu + \alpha$ i + ε ij.

All data were then analysed using variance (Steel and Torrie, 1989).The results showed that the superovulation treatments were highly significant affected (P <0.01) respectively against the daily sow ration consumption (DPRC) 4.87 ± 0.77 and 5.48 ± 0.45 kg, the sows milk production (SMP) per suckling 0.32 kg ± 0.10 and 0.39 ± 0.05 kg, the daily sows milk production (SMP) per day 6.23 ± 1.89 and 7.74 ± 1.00 kg, the sows milk production (SMP) per lactation 305.54 ± 92.40 and 379.44 ± 11.08 kg

It is concluded that the superovulation treatment in the parent before mating can improve milk production of sow which is described by the improvement of consumption of rations and the sow milk production.

OBJECTIVE

Pig reproduction performance is highly dependent on the success of the reproductive process.The ability of the sows to produce milk would be an advantage to the growth of the piglets.Piglet growth is determined by the production of milk from the mother to the care of children during pre-*weaning period (Kimet al.2000; valroset al.2003).*

Improvement of milk production that can be done with superovulated using hormones such as PMSG and hCG has been proven in sheep (Manalu*et al.*1998), cattle (Sudjadmogo*et al.*2001), goat (Adriani*et al.,*2005) and swine (Mege*et al.*2007).Through increased production of milk, the growth and development of piglets can be improved. This study aims to assess the effect of superovulation in sows before mating to the milk production of the sows.

MATERIALS AND METHODS

As many as 60 *Landrace*, *Yorkshire*, and *Duroc* sows weighing between 100-107 kg was used in this study.

Analysis using completely randomized design (CRD), consisting of two treatments with 30 replications was designed. Data analysis followed the procedures of mathematical models as follows: Y_{ij} $\mu + \alpha$ i + ε_{ij}. All data were analyzed using analysis of variance (Steel and Torrie, 1989).

Research procedure

30 sows was injected by PMSG and hCG with dose 400/200 (superovulation 600) IU per cow and another 30 sows were only injected with physiologic saline 0.95%.Before the injection of PMSG and hCG, all animal was injected with one ml PGF_2 α twice with an interval of 14 days for heat synchronizing. After having birth, all sows were measured daily sow ration consumption, the sows milk production per suckling, the daily sows milk production, and the sows milk production per lactation

RESULTS AND DISCUSSION

Effect of Treatment to sows milk production can be seen on Table 1

Table 1. Sows Reproduction Performance With (SO) and Without (TSO) Superovulation

Description: Different superscript on the same row and column showed highly significant different (P <0.01) and significantly different (P<0.05); TSO = without superovulation, SO = superovulation

Sows Milk Production

Sows milk productions are measured based on the frequency of lactating sows, milk production per suckling, daily milk production, and milk production per lactation.

Frequency of Lactating Sows Average of lactating sows frequency is 19.41 ± 0.58 times per day, with a range of 13.57-24.29 times/day.Based on the value of, it can be obtained that the duration of lactation of piglets was every 74.81 minutes (1:15 hours).Observations obtained are still lower than the results of Xu and Cranwell (2003) that shows as much as 20 times.Data frequency lactating sows are presented in Table 1. The results of analysis of variance showed that the effect of different superovulation treatment were significantly difference (P<0.01).The average frequency of lactating sows per day with and without superovulation is 18.93 ± 0.23 times and 19.89 ± 0.40 times respectively.

Superovulation treatment in sows increases the frequency of sows to lactate piglets.Sows with high milk production will more often to lactate piglets.This shows that the piglets from superovulation parent consistently and better maintain the frequency of lactation until pre weaning than piglets from sows without superovulation. This is due piglets born without superovulation parent body weight (1.34 ± 0.14 kg) lower than superovulation (1.46 ± 0.19 kg), so it needs more milk and more aggressive than those without superovulated.

Figure 1. Frequency of Lactating Sows Without and with Superovulation

Figure 1 explains that the frequency of lactation will follow the amount of milk production from its mother. The frequency of lactation is very high at the beginning of lactation up to week 2 with the average 22 times for superovulated sows and 21 times for sows that are not superovulated.The frequency of lactation began to decline after the 3rd week to the lowest frequency at week 7 with the average 15 times.

Sows Milk Production Per Suckling The average for milk production (PASI) per suckling is 0.35 ± 0.08 kg.Milk production of sows per suckling is very important for piglets, especially in early lactation where piglets are totally dependent on its mother's milk before the piglets learn to eat other foods such as rations.When the piglets cannot utilize milk in early lactation the growth and development will be disrupted.Analysis of variance showed that the effect of treatment were significantly different (P <0.05) against PASI pigs per feeding.The mean PASI per suckling without and with superovulation is 0.32 ± 0.10 kg and 0.39 ± 0.05 kg respectively.

Milk production of superovulated sows increased because of the work of endogenous hormones of pregnancy which increase the concentration of progesterone and estradiol, so as to enhance the growth and development of the mammary glands are depicted with an increasing number of cell secretory glands udder formed and increased activity of the synthesis of milk (Manalu*et al.*1999; Mege *et al.*2007). The role of progesterone and prolactin are indispensable for the development of alveoli.Milk production aircraft produced by the mammary gland depending on the number of cells in the gland, so the more a producer of milk, the more milk production that will be produced (Delaval 2008). Figure 7 presents the sows milk production per suckling from the parent without and with superovulation.

Figure 2. Sows milk production per suckling

Figure 7 shows that the pattern of milk production of lactating sows reached the peak at week 3 and then decreases linearly until the lowest production on the 7th week. Figure 7 also explained that the production of milk per lactation of the superovulated sows higher than the production of milk of non-superovulated sows.

Daily Sows Milk Production The average of Daily Milk production (PASI) was 6.99 ± 1.68 kg/day.This is higher than PASI obtained from the research of Silalahi (2011) that shows as many as 5.45 ± 1.64 kg/day. According to Mepham (1987) milk production of sows depending on the number of children who suckle although not necessarily guarantee optimum needs of piglets (Parakkasi 1983). The analysis of variance showed that there is highly significant effect (P <0.01) in the PASI pigs per day.The average PASI without and with superovulation respectively is 6.23 ± 1.89 kg/day (KK = 30.24%), and 1.00 ± 7.74 kg/day (KK = 12.97%).Milk production of sows per day can be enhanced through superovulation treatment because of the increase in the number of cells that form the secretory gland or udder due to increased activity of milk synthesis.Increasing in growth and development of the mammary gland is influenced by hormones of pregnancy, especially estradiol and progesterone and placental lactogen (Manalu*et al.*1998; Manalu*et al.*1999; Manalu*et al.*2000; Sudjatmogo*et al.*2001; Adriani 2005; Hurley*et al.*2001). Increasing in growth and development of the mammary gland during pregnancy due to increased secretion of estrogen and progesterone sows superovulated (Mege *et al* , 2007).

Observations of daily PASI in each week of the measurement are shown in Figure 8. The results of this study are

consistent with the statement of Kim*et al.*(2000) that the mammary gland of sows during lactation reached the peak from 5 to 21 days of lactation.

Figure 3. Average of Sows Milk Production/Day/Animals

The level of development of the udder gland in early lactation will determine the peak of livestock lactation (Forsyth, 1986) and start to decrease from the fourth week to the seventh week.Sows milk production per day on a weekly measurements show that superovulated sows better in terms of maintaining production as compared than sows without superovulation.

Sows Milk Production Per Lactation The average of sows milk productions per lactation was 342.49±82.12 kg.In detail the effect of treatment of swine PASI per lactation can be seen in Table 3.Results of analysis of variance showed that the treatment was highly significant (P <0.01) in the sows PASI per lactation.The average sows PASI without and with superovulation was 305.54±92.40 kg/lactation, and 379.44±49.20 kg/lactation respectively. Sows milk production per lactation are capable of being upgraded through superovulation treatment, because increasing in sows PASI per lactation would increase animals lactation per day and per lactation.

CONCLUSION

Reproduction performance of sows through multiple ovulation by PMSG and hCG before mating can improve milk production of sows.

KEYWORD：superovulation, milk production, sow

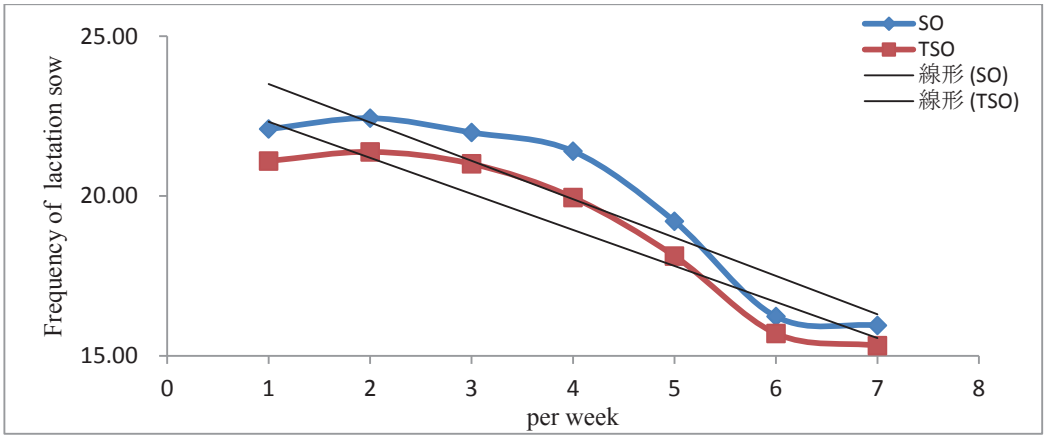

Figure 1. Frequency of Lactating Sows Without and with Superovulation

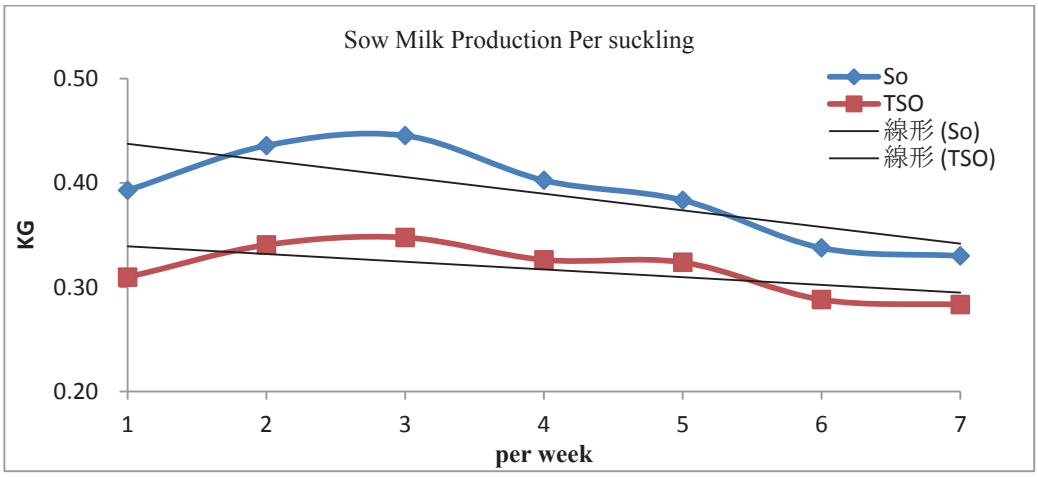

Figure 2. Sows milk production per suckling

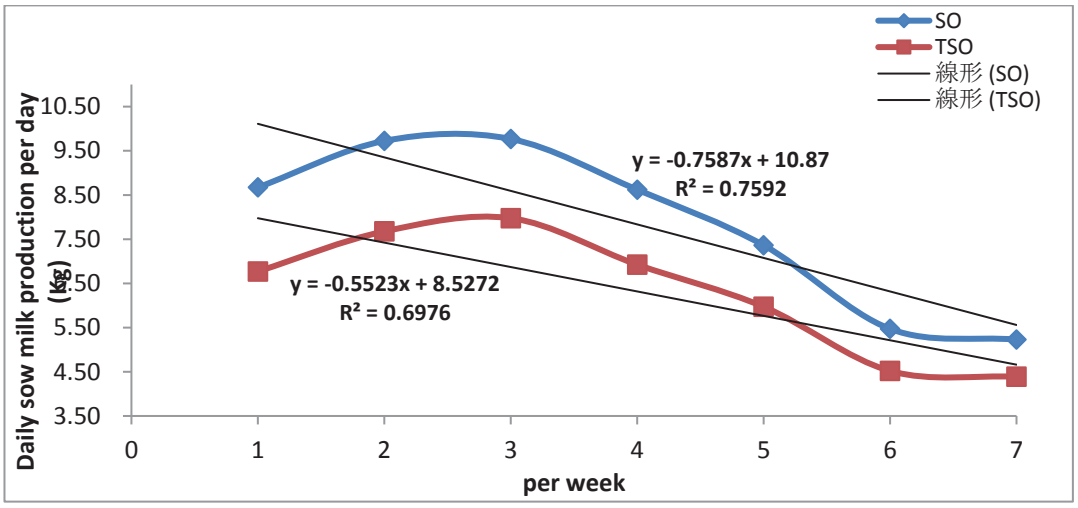

Figure 3. Average of Sows Milk Production/Day/Animals

Table 1. Sows Reproduction Performance With (SO) and Without (TSO) Superovulation

Parameter	Treatment		Mean
	TSO	SO	
Sows milk production (PASI)			
1. Frequency of lactating sows	18.93 ±0. 23 A	19. 89 ±0:40 B	19:41 ±12:58
2. Sows Milk Production Per Suckling (kg)	0. 32 ±0. 10 a	0. 39 ±0:05 b	0:35 ±12:08
3 Daily Sows Milk Production (Kg)	6. 23 ±1. 89 A	7. 74 ±1:00 B	6.99 ±1.68
4. Sows Milk Production Per Lactation (Kg)	305. 54 ±92. 40 A	379. 44 ±11:08 B	342.49 ±82.12

Description: Different superscript on the same row and column showed highly significant different (P <0.01) and significantly different (P<0.05); TSO = without superovulation, SO = superovulation

REFERENCES

Adriani IK, Sutama, Sudono A, Sutardi dan Manalu W. 2005. Pengaruh superovulasi sebelum perkawinan dan suplementasi seng terhadap produksi susu kambing peranakan etawa. *J. Anim. Production* 6:86-94

Delaval . 2008. Milking Technology. Di dalam : Delaval editor. The Lactating Dairy Cow. USA Delaval Publishing [Terhubung berkala]. http://www.milkproduction.com/ library [10 Januari 2010].

Forsyth IA. 1986. Varition among species the endokrine control of mammary growth and function. The role of prolactin, growth hormone and plasental laktogen. *J Dairy Sci* 46 : 1293-1298.

Hurley WL. 2001. Mammary gland growth in the lactating sow. *Livestock Prod Sci* 70:149-157.

Kim SW, Hurley WL, Han IK, Easter RA. 2000. Growth of nursing pigs related to thecharacteristics of nursed mammary glands. *J Anim Sci* 78:1313- 1318.

Manalu W, Sumaryadi MY, Sudjatmogo dan Satyaningtijas AS.1998. Pemanfaatan kelimpahan folikel melalui teknik superovolasi untuk meningkatkan sekresi endogen hormon kebuntingan dan hormon mammogenik dalam upaya peningkatan efisiensi dan produksi domba. Prosiding Seminar Nasional Peternakan dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan, Badan Penelitian dan Pengembangan Pertanian Departemen Pertanian. Hal. 55-66.

Manalu W dan Sumaryadi MY. 1998. Correlations of litter size and maternal serum progesterone concentration during pregnancy with mammary gland growth and development indices at parturition in Javanese thin-tail sheep. *Asian-Austr. J Anim Sci* 11:300-306.

Manalu W dan Sumaryadi MY. 1999. Correlations between lamb birth weight and the concentrations of hormone and metabolites in the maternal serum during pregnancy. *J Agric Sci* 133:227-234.

Manalu W. 1999. Correlations of serum progesterone concentration with uterine and fetal weights at weeks 7 and 15 of pregnancy in Javanese thin-tail ewes. *Asian-Aust J Anim Sci* 12:854-861.

Manalu W, Sumaryadi MY, Sudjatmogo, Satyaningtijas AS. 1999. Mammary gland differential growth during pregnancy in soperovulated Javanese thin-tail ewes. *Small Rumin Res* 33:279-284

Manalu W, Sumaryadi MY, Sudjatmogo, Satyaningtijas AS. 2000. The effects of superovulation of Javanese thin-tail ewes prior to mating on lamb birth weight and preweaning growth. *Asian-Aus. J Anim Sci* 13:292-299.

Mege RA, Manalu W, Nasution SH, Kusumorini N, 2007. Pertumbuhan dan Perkembangan Uterus dan Plasenta Babi dengan Superovulasi. *J Hayati* Vol 14 hal.1-6.

Mepham, T. B. 1987. Physiology of Lactation. Open University Press. Melton Keynes, Philadelphia

Parakkasi, A.1983. Ilmu Gizi dan Makanan Ternak Monogastrik.PenerbitAngkasa, Bandung.

Steel RGD, Torrie JH. 1993. *Prinsip dan Prosedur Statistik*. Alih bahasa Sumantri B. PT Gramedia. Jakarta.

Sudjatmogo B, Utomo, Subiharta, Manalu W, Ramelan. 2001. Tampilan produksi susu akibat peningkatan perumbuhan ambing sapi perch Friesian Holstein yang disuntik PMSG pads program perkawinannya. *J Trop Anim Dev* 26:8-13.

Silalahi P. 2011. Pengaruh penambahan tepung tanaman bangun-bangun (Coleus amboinicus Lour) dalam ransum terhadap penampilan reproduksi induk dan anak babi menyusui (tesis). Bogor: Fakultas Peternakan, Institut Pertanian Bogor

Valros *et al.* 2003. Metabolic state of the sow, nursing behaviour and milk production livest. *Prod Sci* 79:155-167.

Xu RJ. Cranwell PD. 2003. *The Neonatal Pig*. Gastrointestinal physiology and Nutrition. Nottingham. University Press.

O-16-5

Effect of Sericin Supplementation on Motility and Plasma Membrane Integrity of Frozen-Thawed Dairy Cattle Bull Semen

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ABSTRACT

Semen cryopreservation decrease posted thawed sperm quality and affect to fertility. To improve the quality, antioxidant is considered as one of important factor should be supplemented in an extender. The present study was designed to improve semen quality by determine the effect of sericin supplementation (antioxidants) on sperm motility and plasma membrane integrity of frozen-thawed semen in dairy cattle. Fifteen semen samples from three bulls were collected by electroejeculation technique. The ejaculates were frozen in egg yolk-Tris-glycerol extender supplemented with different concentrations of sericin (0, 0.025, 0.05 and 0.1%). The semen quality including motility and plasma membrane integrity was assessed after freezing by phase contrast microscope and hypo-osmotic swelling test (HOST), respectively. Post-thawed semen evaluations showed that extenders supplemented with 0.025 and 0.05% increased sperm motility ($P<0.01$) meanwhile sperm motility was decreased in 0.1% but did not differ to control. The plasma membrane integrity was higher in group supplemented with 0.025% sericin compared with 0.05% ($P<0.01$). In conclusion, extender supplemented with 0.025% sericin improves sperm motility and plasma membrane integrity of frozen-thawed semen in dairy cattle. (**Keywords**: Frozen Semen; Semen Motility; Plasma Membrane Integrity; Dairy Cattle)

INTRODUCTION

Nowadays, artificial insemination with frozen-thawed semen is breeding standard due to the reduction of costs associated with bull's maintenance, the protection of infectious diseases and the increasing of superior genetic spread.Frozen semen has low fertility because cryopreservation damage sperm plasma membrane resulted in the decreasing of sperm viability (Watson, 2000). The important factor to decrease the quality of frozen semen is free radical and engender to lipid peroxidation ensue. These processes decrease the motility and fertility of sperm. Therefore, the antioxidants have been supplemented in the semen extender to protect the lipid peroxidation.

Sericin derived from cocoon of silk worms (*Bombyx mori*). It is a natural macromolecular and water-soluble globular protein. Sericin has 18 kinds of amino acid such as hydroxyl, carboxyl and amino group (Wai et al., 2015). Kato et al. (1998) was report that sericin have role antioxidant and inhibits lipid peroxidation. The addition of sericin supplementation in extender of buffalo bull semen could improve semen quality which protected sperm from oxidative stress (Kumar et al., 2015). This result was similarly to another report which conducted in Thai native bull by Dorji et al. (2015). However, there is no study about sericin supplementation on semen quality of frozen-thawed in dairy bull. Therefore, the present study was to investigate the effect of sericin supplementation on motility and plasma membrane integrity of frozen-thawed in dairy bull.

MATERIALS AND METHODS

Semen collection

Three bulls were collected by electroejeculation technique. Semen of these bull was collected one per week for five time. The ejaculates were frozen in egg yolk-Tris-glycerol extender supplemented with different concentrations of sericin (0, 0.025, 0.05 and 0.1%).

Assessment of sperm motility

Post-thawed semen samples were placed into slide and were closed by cover slip for sperm motility assessing under phase contrast microscope (10X).

Assessment of plasma membrane integrity

Plasma membrane integrity was evaluated using hypo-osmotic swelling test (HOST) (Jeyendran et al., 1984). Samples were determined by phase contrast microscope (40X). The semen sample were counted at least 300 sperm and determined sperm tail coiling. Sperm tail coiling referred to an intact sperm plasma membrane.

Statistical analysis

Experimental data were analyzed using a program SAS 9.0 statistical software package. Duncan's New Multiple Range Test (Steel and Torrie, 1980) was used to test the difference in the mean of group experiment.

RESULTS

The results of sperm motility revealed sericin supplemented in extenders with 0.025 and 0.05% increased sperm motility ($P<0.01$) meanwhile sperm motility was decreased in 0.1% but did not differ to control (Table 1). Furthermore, plasma membrane integrity was higher in group supplemented with 0.025% sericin compared with 0.05% ($P<0.01$; Table 1).

DISCUSSION

It is well known that sericin has an important role as an antioxidant for decreasing ROS reaction. ROS are common products of normal cellular metabolism but an excessive production of ROS resulted in an oxidative stress and lipid peroxidation (Mazur et al., 2000). The lipid peroxidation decreased motility and fertility of sperm. Kato et al. (1998) reported sericin could suppress lipid peroxidation and tyrosinase activity. Sercin supplementation with 0.025% resulted in greater sperm motility. Furthermore, sericin increased the percentage of sperm intact plasma membrane. In conclusion, sericin supplementation improves sperm motility and plasma membrane integrity of frozen-thawed semen in dairy cattle.

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KEYWORD : Frozen Semen, Semen Motility, Plasma Membrane Integrity, Dairy Cattle

Teble 1 Effect of Sericin concentration on motility and Membrane integrity

Sericin concentration (%)	0	0.025	0.05	0.1
Motility	49.33 ± 8.14^{B}	57.00 ± 9.64^{A}	56.00 ± 9.64^{A}	51.00 ± 6.24^{B}
Plasma Membrane integrity	39.21 ± 3.20^{C}	43.19 ± 4.30^{A}	41.44 ± 3.86^{B}	37.99 ± 3.62^{C}

Different letter in the same row indicate statistical differences ($P<0.01$).

REFERENCES

Dorji, P., T. Vongpralub, V. Pattarajinda, V. Chankitisakul and P. Chuawongboon. 2015. Effect of sericin supplement in Tris-egg Yolk Citrate extender on quality of cryopreserved semen from Thai native bulls. BJNRD, 2(1): 11-18.

Jayendran, R. S., H. H. van der Ven, M. Perez-Pelaes, B. G. Crabo and L. J. D. Zaneveld. 1984. Development of an assay to assess the functionalintegrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil. 70: 219-288.

Kato, N., S. Sato, A. Yamanaka, H. Yamada, N. Fuwa and M. Nomura. 1998. Silk protein sericin inhibits lipid peroxidation and tyrosinase activity. Biosci. Biotechnol. Biochem. 62: 145-147.

Kumar, P., D. Kumar, P. Sikka and P. Singh. 2015. Sericin supplementation improves semen freez ability of buffalo bulls by minimizing oxidative stress during cryopreservation. Animal Reproduction Science. 152: 26-31.

Mazur, P., I. Katkov, N. Katkova and J. Cristar. 2000. The enhancement of the ability of mouse sperm to survive freezing and thawing by the use of high concentrations of glycerol and the presence of an Escherichia coli membrane preparation (oxyrase) to lower the oxygenconcentration. Cryobiology, 40: 187-209.

Steel, R. G. D. and J. H. Torrie. 1980. Principle and procedures of statistics: A Biomaterial Approach (2ndEd). McGraw Hill Book Co., New York, U.S.A.

Wei, T., M. Z. Li and R. J. Xie. 2005. Preparation and structure of porous silk sericin materials. Macromol. Mater. Eng. 290: 188–194.

O-16-7

Effects of straws size on the quality of cryopreserved Thai native chicken semen

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Introduction

Semen sample in cryobanks are of great value and require an economic effort for a long time (Barbas and Mascarenhas, 2009). For this reason, at least one assessment after cryopreservation is necessary to verify the accomplishment of quality standards and subsequently decide if a given sample is suitable enough to be store and used in artificial insemination (AI). Nevertheless, freezing and thawing induce detrimental effects on the ultra-structure, biochemistry, and functional integrity of sperm (Watson, 2000), resulting in a reduction of motility, membrane integrity and fertility ability (Purdy, 2006). Many factors, such as extender, cryoprotectant, dilution rate, freezing rate and packaging method affect the quality of frozen - thawed semen from difference species (Watson, 2000).

The history of preserving poultry semen in straws was reviewed by Donoghue and Wishart (2000). Some clear benefits existed for freezing extended semen in straws (Seigneurin and Blesbois, 1995) compared with glass ampoules (Lake and Ravie, 1984) and plastic cryovials (Wishart, 1995) comparable or better sperm survival in storage, dose of sperm to achieve optimal fertility and reduced storage space. Plastic straws for packaging liquid semen were first introduced in Denmark in 1940. Later, techniques for freezing semen in straws were developed and refined by Cassou and Jondet (Pickett and Berndtson, 1974). During the 1960s, Cassou (1972) introduced a medium size, polyvinyl chloride straw with a volume of 0.5 - mL with replaced a larger straw with a volume of 1.2 mL. In, 1968, Cassou suggest the 0.25 - mL straw that remains in the use today. Many studies have been conduced to assess the influence of different packaging methods on sperm survival. For bull, 0.25 - mL straw was superior to 0.5 - mL (Stevenson, 2009). However, the results in dogs (Nöthling and Shuttleworth, 2005) and rams (Paulenz et al., 2004) showed that 0.5 - mL straw gave higher post-thawed sperm quality than the 0.25- mL straw. To our knowledge, there was no report on comparison of straw size on post-thaw semen quality of the chicken.

The present study was, therefore aim to compare the effects of 0.25 - and 0.5 - mL straws on motility and viability after thawing chicken semen.

Materials and methods

Animal

Twelve Thai native cocks (Pradu hang dam; one year old) were kept in individual cages. Cockerels were fed (commercial poultry ration) 130g/head/day and water was provided *ad libitum*. The animals were reared under natural environmental condition.

Semen collection and qualification

The semen samples from 12 individuals Thai native cocks was collected twice a week, by the dorso-abdominal massage method (Burrows and Quinn, 1937). Semen from an individual cock was collected in a 1.5 mL microtube containing 0.1 mL Schramm diluents (compose of 0.7 g magnesium acetate, 28.5 g sodium glutamate, 5 g glucose, 2.5 g inosital and 5 g potassium acetate dissolved in double-distilled water 1,000 mL). Following semen collection, the sperm were analyzer for motility. Motility was expressed as the percentage of motile sperm and survivability with the use of contrast light microscopy, using a scale of 1 to 5. Ejaculates having good motility ($\geq$ 85%) were used in this study. The quality semen samples were pooled and used in this study.

Freezing and thawing protocol

Indeterminate of the treatment, the pooled ejaculates in every experiment were diluted using Schramm diluents (Schramm, 1982), cooled down to 5 ºC in 60 min. Then, diluted with DMF (N, N - Dimethylformamide; Sigma - Chemical Co., St. Louis, USA, D-4551) to the final concentration of 6% (v/v) in diluted semen. The semen were

loaded into either 0.25 - mL (CRYO - VET ref. AM163SEP) or 0.5 - mL (IMV ref. 005569) plastic straws. Semen straws were placed in stylofoam box containing liquid nitrogen at -35ºC for 10 min and located at -135 ºC for 5 min and then plunged into liquid nitrogen (Vongpralub et al., 2011). After storage, the straws were thawed individually in an iced water bath at 5 ºC for 5 min and then evaluated for various sperm functions.

Analysis of the post-thaw sperm motility and viability

Analysis of post-thaw sperm motion parameters were determined using a computer assisted sperm analysis (CASA) (HTM-IVOS Model 10 Spermatozoa Analyzer; Hamilton Thorne Biosciences, Beverly, MA, USA)

Sperm membrane integrity was assessed with dual fluorescent probes, SYBR-14 and propidium iodide (PI) (Live/dead® sperm viability kit L7011 Invitrogen USA), according to the method described by Partyka et al., (2010). Briefly, each sample was diluted to a concentration of 50×10^6 spz/mL. Portions (250 µL) of the diluted samples were dropped into a cytometric tube and 5 µL of SYBR-14 working solution was added. The working solution was obtained by diluting a solution of SYBR-14 in distilled water at a ratio of 1:49. Samples were mixed and incubated at room temperature for 10 min. The cells were counterstained with 5 µL PI for 5 min and then fixed with 30 mL 20% formaldehyde. The sperm was then evaluated under a fluorescent microscope IX71 (Olympus, Tokyo, Japan). The PI - negative and SYBR-14 - positive population showing green fluorescence was considered to be live, with sperm plasma membrane intact (PMI).

Statistical analysis

The experiment was conducted as randomized complete block design (RCBD) using SAS statistical software and differences in number of particular categories of spermatozoa in frozen-thawed semen were analyzed with ANOVA and Duncan's multiple range tests. All percentage data were arcsine transformed before statistical analysis. The results are presented as mean ± SE of measurements on sample from 12 replicate determinations ($P \leq 0.05$).

Results and discussion

The results from experiment are shown in Table 1. The percentages of total motility (50.80 ± 4.47 and 49.10 ± 4.86 respectively), progressive motile (28.60 ± 4.43 and 26.60 ± 4.67 respectively) and viability (44.62 ± 4.05 and 48.05 ± 3.80 respectively), the post-thawed significant semen quality were not difference ($P \geq 0.05$).

Comparing the relative of 0.25 - and 0.5 - mL straws reveals at least three apparent differences: 1) the 0.25-mL straw required less extender to fill and less space to store, potentially reducing production, storage, and shipping costs (Kroetsch, 1992; Johnson et al., 1995); 2) the larger 0.5-mL straw was easier to handle, easier to read, and may (De Jarnette, 2003) or may not suffer less breakage during storage (Kroetsch, 1992); and 3) the 0.25-mL straws responded to temperature changes faster than the 0.5-mL straws (Berndtson et al., 1976). Whether this latter characteristic is positive or negative has been the subject of dispute, and the research regarding the chicken semen with frozen-thawed semen packaged in 0.25- or 0.5-mL straws over those years has often had conflicting results, conclusions, and interpretations. The superior quality results achieved for 0.25 - mL compared to 0.5 - mL straws have to be carefully evaluated in relation to the possible application of a more rational semen production, fertility rates and simplified semen handling at AI.

In conclusions, it is recommended that Thai native chicken semen can be frozen in 0.25 - and 0.5 - mL straws under conditions of simple vapor freezing method.

Acknowledgments

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KEYWORD : package, frozen semen, chicken

Table 1 Mean values (± SE) for characteristics of frozen chicken semen packed into 0.25 or 0.5 mL straws (n=12)*.

Item	0.25 – mL	0.5 - mL
Viability (%)	44.62±4.05	48.05±3.80
Motility (MOT) (%)	50.80±4.47	49.10±4.86
Progressive motile (PMOT) (%)	28.60±4.43	26.60±4.67

* mean ± standard error of the mean.

EFERENCES

Barbas, J. P. and R. D. Mascarenhas. 2009. Cryopreservation of domestic animal sperm cells. Cell Tissue Bank.10:49–62.

Berndtson, W. E., B. W. Pickett, and C. D. Rugg. 1976. Procedures for field handling of bovine semen in plastic straws. Pages 51–60 in Proc. 6th NAAB Tech. Conf. Artif. Insemin. Reprod., Columbia. MO.

Burrows, W. H., and J. P. Quinn. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Sci.16:19–24.

Cassou, R. 1972. A technique for semen preservation and its application to breed improvement programmes. AI Co-op Centre, 61, L'Aigle, France. Personal publication.

De Jarnette, J. M. 2003. Pros and cons of the half- and quarter-cc straws. Selections. Winter 2003. http://www.selectsires.com/ selections/2003_q1_page10-11.aspx Accessed Dec 12, 2015.

Donoghue, A. M., and G. J. Wishart. 2000. Storage of poultry semen. Anim. Reprod. Sci. 62:213–232.

Johnson, M. S., P. L. Senger, C. H. Allen, D. D. Hancock, B. M. Alexander, and R. G. Sasser. 1995. Fertility of bull semen packaged in .25- and .5-milliliter French straws. J. Anim. Sci. 73:1914– 1919.

Kroetsch, T. G. 1992. Experiences with mini-straws. Pages 64–67 in Proc. 14th Natl. Assoc. Anim. Breeders Tech. Conf. Artif. Insemin. Reprod., Columbia, MO.

Lake, P. E., Ravie, O., 1984. An exploration of cryoprotective compounds for fowl spermatozoa. Br. Poult. Sci. 25: 145–150.

Nöthling, J. O. and R. Shuttleworth. 2005. The effect of straw size, freezing rate and thawing rate upon post-thaw quality of dog semen. Theriogenology 63: 1469-1480.

Partyka, A., W. Nizanski and E. Lukaszewicz. 2010. Evaluation of fresh and frozen-thawed fowl semen by flowcytometry. Theriogenology 74:1019–1027.

Paulenz, H., L. Söderquist and T. Ădnoy. 2004. Fertility results after different thawing procedures for ram semen frozen in minitubes and mini straws. Theriogenology 61:1719-1727.

Pickett, B. W. and W. E. Berndtson. 1974. Preservation of bovine spermatozoa by freezing in straws: A review. J. Dairy Sci. 57:1287–1301.

Purdy, P. H. 2006. A review on goat sperm cryopreservation. Small Rumin. Res. 63: 215-225.

Schramm, G. P. 1982. Ein neues Medium zur Verdu ¨ nnung und Flu ¨ ssigkonservierung von Hahnensperma. Monatsh. Veterina ¨ ermed. 37:900–902.

Seigneurin, F. and Blesbois, E., 1995. Effects of the freezing rate on viability and fertility of frozen-thawed fowl spermatozoa. Theriogenology 43: 1351–1358.

Stevenson, J. S., J. J. Higgins and Y. Jung. 2009. Pregnancy outcome after insemination of frozen-thawed bovine semen packaged in two straw sizes: A meta-analysis. J. Dairy Sci. 92: 4432-4438.

Vongpralub, T., A. Utha, J. Pongpeng, P. Sonseeda and W. Yindee. 2011. A comparison of simplevapour method and programmable freezer on motility and fertility of frozen Thai native chicken semen. Pages 649–652 in Proceedings of the 3rdInternational Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2011), Nakhon Ratchasima, Thailand.

Watson, P. F. 2000. The causes of reduced fertility with cryopreserved semen. Anim. Reprod. Sci. 60-61: 481-492.

Wishart, G.J., 1995. Cryopreservation of avian spermatozoa. In: Day, J.G., McLellan, M.R. Eds., Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology Humana Press vol. 38, pp.167–177.

O-17-2 The Survey of nutritive value of By-products from Oil palm that use for raise Beef Cattle

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INTRODUCTION

The most important of the ruminant species in Thailand are beef cattle which include native Thai cattle, crossbred cattle and a small number of purebred beef type cattle (OAE, 2014). This land has a shortage of forage in the wet season. Cattle are also supplemented with cut-and-carry naturalized grasses from roadsides, paddy bunds and community lands. Productivity of livestock in such traditional systems is limited by the variable availability of forages and the low quality of grasses and crop residues obtained in this manner (Phaikaew *et al*. 1997). Most ruminants in Southern Thailand are grazed in the rice growing areas by smallholder farmers owning only 2-2.5 ha of land per household (Sophanodora 1997). The palm oil industry plays an important role in Thailand's economy. Palm oil occupies 70% of the Thai vegetable oil market (Chavalparit *et al*., 2006). Palm oil industry produces amount of by-products, including crude palm oil (PO), oil palm frond, palm press fiber, palm kernel cake (PKC) and palm oil decanter cake (DC). These by-products represent an alternative, as they are readily available and represent sustainable feed resources for ruminants and other farm animals. Their inclusion in diets can be an effective measure in overcoming the lack of grazing pasture for small ruminants. Palm kernel cake and DC can be used as feed for dairy goats (Silva et al., 2005) lambs (Ribeiro et al., 2011) and cattle (Seephueak et al., 2011). The objective of this study was made to determine the nutritive values of the by-products from oil palm used for beef cattle in South of Thailand.

MATERIALS AND METHODS

The sample of 169 farmers was randomly selected from five provinces in South of Thailand (Yala, Pattani, Narathiwat, Songkhla and Satun). The research tool was a structural questionnaire and collected by interview. The statistic employ were frequency, distribution and percentage. In addition, collecting and sampling of by-products from oil-palm that farmer use to fed cattle (oil palm leaf, oil palm front, Decanter cake and Empty fruit), Total Mixed Ration Oil Palm (TMR-OP) (oil palm leaf and oil palm front 2 kg + concentrate 1 kg+ palm kernel cake 1 kg + Decanter cake 1 kg as fed basis) and Silage oil palm (Silage-OP) (oil palm leaf and front) that farmer raised cattle. The 3 samples of each forages were evaluated the dry matter (DM), crude protein and ash (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were evaluated using the methods of Goering and Van Soest (1970).

Statistical analysis

The data were also collected by interview and statistically analyzed using frequency, distribution and percentage.

RESULTS AND DISCUSSION

The result showed that most of farmers were male, Muslim and 51-60 years (37.87%) of age with a primary education (45.56%). The most of farmer raised Thai Native breed cattle (37.87%). All of them which raised their cattle1-5 heads per family (88.76%). The farmers raised their cattle tether in natural pasture (72.19). They have not a pasture for raised the cattle (63.31%).They used by-products from oil palm for fed cattle about 21.81% (oil palm leaf, palm kernel cake and Decanter cake). The by-products from oil-palm that farmer use to fed such as oil palm leaf and oil palm front (DM=44.43, CP=3.09, EE= 1.73, ash =1.97, NDF= 56.53 and ADF=42.43%) and Decanter cake (DM= 20.33, CP= 10.72, EE=7.01, ash=12.51%). Sudin (1988) reported the different levels of POS in concentrate (0, 15, 30 and 65%) did not affect total DMI in Sahiwal-Friesian growing heifers because POS used in the ration had low EE content (12.1%). Moreover, The farmer usually used by- product from oil palm to feed Total mixed ration (TMR) and Silage for cattle. The TMR oil palm compose of oil palm leaf and oil palm front 2 kg + concentrate 1 kg+ palm kernel cake 1 kg + Decanter cake 1 kg as fed basis for feed cattle. Furthermore, they used silage oil palm from oil palm leaf and oil palm front raised our cattle. The chemical composition was shown in Table 1.

CONCLUSION

It was concluded in this experiment that the farmer in South of Thailand were male, Muslim and 51-60 years of age with a primary education. Their raised Thai-Native Cattle. They used by-products from oil-palm to feed cattle. The chemical composition decanter cake and oil palm front can be produce to TMR or Silage for feed cattle.

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KEYWORD：Oil palm fronds, beef cattle

Table1. The chemical composition of by- products from oil palm and feed for cattle

Chemical composition	Oil palm front	Decanter cake	Empty fruit	TMR-OP[1]	Silage-OP[2]
DM	44.43	20.33	31.32	42.16	61.82
CP	3.09	10.72	1.43	14.86	5.37
EE	1.73	7.01	2.86	3.85	2.34
Ash	1.97	12.50	5.35	4.55	5.61
NDF	56.53	26.07	34.75	51.75	65.68
ADF	42.43	14.59	16.40	26.45	30.77

[1] oil palm leaf and oil palm front 2 kg + concentrate 1 kg+ palm kernel cake 1 kg + Decanter cake 1 kg as fed basis
[2]oil palm leaf and front silage

REFERENCES

AOAC. 1990. Official Methods of Analysis, 14th ed., Washington,D. C.: Association of Official Analytical Chemists.

Phaikaew, Chaisang, Nakamanee, Ganda, and Klum-em, Kiatisak (1997). FSP Activities in Thailand. In: Stür, W.W. (ed). *Feed Resources for Smallholder Livestock Production in Southeast Asia*. Proceedings of regional meeting in Vientiane, Lao PDR. CIAT Working Document No. 156. pp. 49-50.

Sophanodora, P. (1997). Crop-livestock integration in Southern Thailand: prospects and constraints. In: Stür, W.W. (ed). *Feed Resources for Smallholder Livestock Production in Southeast Asia*. Proceedings of regional meeting in Vientiane, Lao PDR. CIAT Working Document No. 156. pp. 77-82.

Chavalparit, O. Rulkens, W. H., Mol, A. P. J. and Khaodhair, S. 2006. Options for environmental sustainability of the crude palm oil industry in Thailand though enhancementof industrial ecosystems. Environmental, Development and Sustainability. 8, 271-287.

Silva HGO, Pires AJV, Silva FF, Veloso CM, Carvalho GGP, Cezario AS, et al. (2005) Apparent digestibility of diets containing cocoa meal and palm kernel cake in lactating goats. Pesquisa Agropecuária Brasileira40: 405–411.

Ribeiro RDX, Oliveira RL, Macome FM, Bagaldo AR, Silva MCA, Ribeiro CVDM, et al. (2011) Meat Quality of Lambs Fed on Palm Kernel Meal, a By-product of Biodiesel Production. Asian-Australasian Journal of Animal Sciences24: 1399–1406.

Seephueak, W., Ngampongsai ,W. and Chanjula, P. 2011. Effects of palm oil sludge in concentrate on nutrient utilization and rumen ecology of thai native cattle fed with hay. Songklanakarin Journal of Science and Technology 33: 271–280.

Sudin, Y. B. 1988. Performance of Sahiwal-Friesian growing heifers on different levels of dried palm oil sludge in their concentrate ration. Malaysian Agricultural Journal.54, 165-171.

O-17-4 FORAGE GROWING AND HAY MAKING OF CLITORIA TERNATEA FOR DRY SEASON FEED SUPPLEMENT IN EAST NUSA TENGGARA, INDONESIA

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INTRODUCTION

Cattle production problems in East Nusa Tenggara include: high calf mortality (30-40%) in Bali Cattle breed and long calving interval in Sumba Ongole cattle thus low calf crop per year, high body weight loss during the dry season (up to 60% of the wet season gain), all caused by the lack of forage availability especially during the dry season (May to December) which results in overall low beef cattle productivity in the province. One of the important problems in Bali Cattle in Timor is high calf mortality caused by high number of calves born during the dry season (May to December). The Bali cattle is well known for its high fertility trait even under poor body condition score (BCS), though with low milk production, and thus very often fails to feed the new born calf during the dry season causing high mortality. In contrast Sumba Ongole Cows (SO cows) responded to the poor feed condition during the dry season by lengthening calving interval, thus produced a calf in 2-3 years time.

Native grasses under the dry tropical condition of the region grows quickly and matured in a relatively short time, and thus quickly to get down to low quality (3-5% of crude protein), therefore causes low cattle productivity if totaly dependent on the native grasslands only (Nulik and Bamualim, 1998; Bamualim and Wirdahayati, 2002).

The main food crop grown in the short wet season (December to April), is maize, integrated with local beans (cow pea, pigeon pea, peanut, and mung bean), pumpkin and tubers (cassava, tarro, and *Dioscorea species), which are rarely applied with fertilizer, thus soil degradation in fertility is a common fenomena seen every year. Therefore the introduction of herbaceous legumes integrating into the food crop planting systems will be expected to improve soil fertility as well as an alternative to provide high quality forages for the animals.*

The current works in the province in integrating herbaceous legumes into livestock and food crops farming (with maize and or rainfed rice), conducted forage conservation (in silage or hay forms), combine with the cultivation and use of the deep rooted forage tree legume such as Leucaena leucocephala need more detail studies (Nulik *et al*., 2013; Kana Hau and Nulik, 2015).

MATERIAL AND METHODS

The legumes, planted (in 2014/2015) included *Clitoria ternatea* cv Milgarra, *Dolichos lablab* cv Highworth, and *Centrosema pascuorum* cv Bundey and Cavalcade. The adaptation of the legumes to the regional climate was described by Nulik *et al*., (2013). During the experiments and assessments year, with less rainfall compared to the normal year (2015 and 2016), production of biomass was then expanded year round with the help of additional irrigation from the bore water. However, as only *Clitoria ternatea* that produced reasonable biomass from several sites planted, the production of hay was only of the species to be used for the supplement assessments, while the other two species were only for the comparison purposes on their performances during the time (i.e. in flower and seed formation characteristics).

Soils used for growing the legumes were mainly of black sandy clays (light vertisol) (in Kupang, West Timor and in Wanga, East Sumba) and red alfisol soil at one of the biomass production site (in Milipinga, East Sumba). The legume was planted either in pure stand, in relay stands (with corn and sorghum), or in the alleys of *Leucaena leucocephala* hedge rows during the wet season, as well as after the harvest of corn or rainfed rice (rotation) in the dry season by irrigation from bore water.

In the experiment Bali Calves in the village of Oefafi in the sub-district of central Kupang in Kupang District were used. At least 20 calves were allocated to each supplement treatment, thus 20 calves for control (no supplement), 20 calves received hay of *Clitoria ternatea* only, 20 calves were given *Clitoria ternatea* hay + dried cassava, and 20 calves fed with concentrate feed as formulated according to Copland *et al*., (2011). Calves were allowed to have access to the supplement feed when yarded at night by provide a creep feeder at each individual participating farmer sites. The legume hay and dried cassava chips were cut into small sizes before putting into the creep feeder (2% DM of animal weight) every afternoon before the animals return to the night yard pens.

Sumba Ongole cows were used for a simple supplement study, in which 5 cows received supplement as hay of

Clitoria ternatea (1.6 kg/hd/d) when pen yarded at night while 10 cows received no supplement (farmer practices) as the control animals. Supplement was given in the form of *Clitoria ternatea* hay, harvested during the planting season to the early dry season (December 2014 to May 2015) with no additional irrigation.
Data were mainly collected in a monthly interval, included body weight of the calves and records of mortality if occured for the Bali calves, while SO cows were recorded for their monthly weight and Body Condition Score (BCS), and reproduction performances (such as hit and pregnancy). Weighing of SO cattle was conducted by providing a simple crush pen at the experimental site on farm (Melolo village, East Sumba District).

RESULTS AND DISCUSSION

Though previous results have provided solution to the important problems of high calf mortality in Bali Cattle in west Timor (Copland *et al*., 2011), however adoption was negligible as the formulation of the concentrate would be a problem for farmers, as some ingredients (fish meal) need to be purchased, which also may not be available in the village, therefore growing herbaceous legumes and producing cassava tuber may be a more practical way to cope with the problem. The results of the initial works have shown promising solutions for the low income farmers in the villages in west Timor as well as to overcome the problem of long calving interval in Sumba Ongole cattle in Sumba Island by supplementing the cows to improve their reproduction capacity.

The legumes in pictures and performances

The growth of *Clitoria ternatea* was related to radiation, identified as "neutral day" plant that can set flowers at any time, and not depending on the length of the day or night, while CP and LP were significantly related to the temperature and daylength and could be identified as long day plants, though with inconsistence (Hosang *et al*., 2016 in this proceedings) by producing few flowers during the short days, but significantly have smaller leaves during the peak of the dry season (August to October).

Harvesting, drying for hay and balingpictures

Biomass production

Biomass production was much related to amount of water able to be given (in the dry season) and the fertility of the soils, i.e. for CT, while CP and LP were also related to temperature and radiation, and production in vertisol soil was higher than on red alfisol soil in Sumba (Table 1). In average CT produced 3,5 tons of DM from 1 ha of land size, where production was significantly lower during the dry season (1-2 tons DM per ha) (May to December) compared with that of during the wet season (2.28-3.2 tons per ha/harvest) (February to April 2015). Under favorable condition in East Nusa Tenggara the legume may produce up to 5 ton of DM/ha/harvest (at 3 months interval) in the wet season (Nulik *et al*., 2013).

Table 1. Dry matter (Biomass production) of *C.ternatea* for supplement trials

Sites (village/Hamlet)/Soil	**Wet season harvest (Feb - Apr) (ton)**	**Dry season harvest (May - Dec) (ton)**	**Total Biomass (kg)**
Kupang District / Vertisol			
(plot size, ha)			
(plot size, sqm)			
Lili	760 kg (0.25 ha) or 3.04 ton/ha	340 kg/0.25 ha or 1.36 ton per ha	1100 kg
Uel	570 kg (0.25 ha) or 2.28 ton/ha	320 kg/0.25 ha or 1.28 ton per ha	890 kg
Manusak 1-	850 kg/0.60ha or 1.42 ton/ha (2 harvests)		850 kg
Manusak 2-			

300 kg/0.30ha or 1.0 ton/ha (1 harvest)
300 kg
Naibonat-
600 kg/0.25 ha or 2.4 ton/ha (2 harvests)
1.200 kg
Oefafi-
400 kg/0.25 ha (1 harvest)
400 kg
Total Kupang DM

4740 kg
East Sumba District

Wanga / vertisol636.4 kg (0.2 ha) or 3.18 tons/ha
360.6 kg (0.2 ha) or 1.80 tons/ha
997 kg
4.98 tons/ha
Milipinga / alfisol230 kg (0.2 ha) or 1.15 tons/ha
No harvest (dry)
230 kg (0.2 ha) or 1.15 tons/ha
Total East Sumba DM

1,227 kg
Hay supplement trials

Calves mortality in Bali Cattle was not significantly affected by the treatments, as calves death was only recorded one each in the control and supplemented groups, which was not as the result of the feed treatments, but rather due to wet condition in January 2016. This indicated that the native grassland used for the grazing of the animals (from June to December, and January the early wet season) may still have sufficient feed supply (mainly of native grasses) to at least sustain the survival of cows and calves grazing in there. However looking at the DWG of the animals, treatments significantly have effects with DWG as much as 0.02 to 0.09 at the supplemented groups. It was observed that calf does not like to eat most of the small stems of *Clitoria ternatea* hay, thus it is suggested that in a better legume supplement trial in the future, calf may need to be given a course ground of the legume to improve the intake.

In SO cows the supplemented group significantly has stabel DWG and BCS, while the unsupplemented group significantly had a reduction at the end of the dry season. In this experiment, *C.ternatea* hay was quite palatable to the animals in that none of the legume hay was left in each feed served at the night feeding. All the supplemented cows were pregnant at the observation after the experiment, while only 30% of the unsupplemented animals were pregnant. However, considering that only few animals were used in the supplemented treatment, owing to the restriction on the availability of hay with the strike of dry condition, a similar experiment is planned to be conducted this year (2016) by producing more biomass for hay to be able to supplement more cows for confirming the results. However, the initial experiment has demonstrted the potential to improve the reproduction performance of SO cows to go through the dry season grazing.

Feeding and BCS assessment/weighing pictures

CONCLUSSION AND IMPLICATIONS

The initial activities have demonstrated a solution to overcome problems in cattle production farming in East Nusa Tenggara, especially in west Timor and East Sumba to reduce calf mortality and to improve BCS of Sumba Ongole cattle aiming at the improvement of reproduction capacity and thus shorten the calving interval to produce a calf from each cow each year.

Clitoria ternatea, the selected herbaceous legumes has shown its exellent potential to produce forage for the improvement of feeding management by its capacity to produce forage, and also seed, year round, provided some additional irrigation can be afforded. Year round seed production capacity of the legume will ensure sustainable seed availability for growing and use of the legume in the province to provide an alternative of high quality hay

especially for dry season feed supply.

KEYWORD：Clitoria ternatea, feed supplement, agronomy, Sumba Ongole cows, Bali Cattle calves, Herbaceous legumes

The legumes in pictures and performances

Picture 1. *Clitoria ternatea* produced in the early of wet season (left), end of wet season with some additional irrigation (midle), and in the dry season with 100% irrigation (right).

Harvesting, drying for hay and baling (picture 2)

Picture 2. Harvest and sun drying of *C.ternatea* (left), baling (middle) and prepared for storage and used (right) as supplement produced at wanga site of East Sumba.

Feeding and BCS assessment/weighing pictures

Picture 3. Sumba Ongole cows in the night yard prepare for feeding trial (left) preliminary feeding (middle), and put into simple crush for BSC assessment and weighing (right).

REFERENCES

Bamualim, A.M., Wirdahayati Ramli Barkri (2002). Peternakan Lahan Kerin di Nusa Tenggara (Dryland Animal Husbandry in Nusa Tenggara). BPTP Nusa enggara Timur (The East Nusa Tenggara Assessment Institute for Agriculture Technology). 95 pages.

Copland, R., G. Jelantik, M. Mullik, H.L.L. Belli, J. Sogen, and A. Nalley (2011). Evaluating strategies to improve calf survival in West Timor villages. LPS/2006/005, Final Report. ACIAR, GPO Box 1571, Canberra ACT 2601, Australia.

Hosang Evert, Nulik Jacob, Kana Hau Debora, Liunokas Yanto, Aby Yendri, Uran Yakobus, Kendrick Cox (2016). The influence of sowing date and trellising on the flowering of some promising herbaceous legumes for eastern Indonesia. In This proceedings (17th AAAP).

Kana Hau, D., and J. Nulik (2015). Body weight gain response of Sumba Ongole Cattle to the improvement of feed quality in East Sumba District, East Nusa Tenggara, Indonesia. Proceedings of Th 6th ISTAP International Seminar on Tropical Animal Production, October 20-22-2015 Yogyakarta, Indonesia. Pp. 143-14.

Nulik, J., Bamualim, M. (1998). Pakan Ruminansia Besar di Nusa Tenggara (Fodderr for Large Ruminants in Nusa Tanggara). Balai Pengkajian Teknologi Pertanian Naibonat Bekerja Sama dengan (In Collaboration with) Eastern Islands Veterinary Services Project. 127 pages.

Nulik, J., N. Dalgliesh, K. Cox, and S. Gabb (2013). Integrating Herbaceous Legumes into Crop and Livestock Systems in Eastern Indonesia. ACIAR Monograph Series, No. 154.

O-17-6

Fermentation characteristics of tropical grass using in vitro gas production technique

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Abstract: The aim of this study was to investigate the effect of various tropical grasses with different ratios of roughage to concentrate (R:C) on gas production kinetics and digestibility of dry matter and organic matter using an *in vitro* gas production technique. Two male, rumen fistulated dairy steers were used as rumen fluid donors. The treatments were arranged according to a 5x4 Factorial arrangement in a Completely randomized design with five roughage sources namely Ruzi (*Brachiaria ruziziensis*), Guinea (*Panicum maximum*), Napier pakchong1 (*Pennisetum purpureum x Pennisetum americanum*), dwarf Napier (*Pennisetum purpureum cv.* Mott) and Sweet grass (*Pennisetum purpursum, Schum*) and four R:C ratios (80:20, 60:40, 40:60 and 20:80). Under this investigation, the results revealed that increasing concentrate ratio resulted in increasing gas production, DM and OM digestibility. Sweet grass has the highest CP and NFC content (15.2 and 12.1 %, respectively), revealed the highest DM and OM digestibility up to 80 % roughage. Based on this study, it could be concluded that R:C ratio had an effect on rumen gas production and digestibility.. Sweet grass could be used as a good quality roughage source in ruminant feeding for enhancing rumen ecology, fermentation and digestion. However, *in vivo* trials should be subsequently conducted to investigate more on the effect of feeding Sweet grass in diets on rumen ecology and productivity such as meat and milk of ruminants.

Introduction

Roughages are the main feed source and importantly for ruminants. High quality roughages can reduce the use of concentrate diet for lactating dairy cows (Wanapat, 1999). High quality roughages will allow rumen microbes to increase the digestion of roughage, proving more nutrients to the host animal, and hence, decrease the concentrate supplementation (Wanapat et al., 2006). In tropical climate areas, as well as during the summer in subtropical regions, the productivity of cattle fed on forages is limited due to the low quality of the forage species that grow in this area, among other factors. However, some tropical grasses, such as elephant or napier grass (*Pennisetum purpureum*) and napier pakchong 1 (*Pennisetum purpureum x Pennisetum americanum*) produces large amounts of yield per area (Almeida et al., 2000). Those grasses are the tall varieties of elephant grass. Moreover, the short or dwarf variety such as dwarf napier grass had a higher overall nutritive quality compared to the taller varieties mainly because the former had a higher leaf-to-stem ratio (1.4 in dwarf and less than 0.8 in tall varieties, (Halim et al., 2013). Sweet grass (*Pennisetum purpursum, Schum*) is a Mott (dwarf) napier grass and is a perennial bunch grass which is potentially high nutritive value. Sweet grass is short variety similar to dwarf napier grass (*Pennisetum purpureum cv.* Mott). However, the knowledge of the intrinsic values of this forage and exactly how they affect the digestion, the metabolism, and the efficiency of feed utilization for productive processes by the animal still need to be determined. This grass might be persistent, highly productive and produces a substantial amount of high quality roughage. Nevertheless, there is no research information relating to its nutritive value and for ruminant feeding. Therefore, the objective of this experiment was to evaluate different of tropical grasses with different of roughage to concentrate ratio using an *in-vitro* gas production technique.

Materials and Methods

Dietary substrate treatments, animals and experimental design

Five roughage sources were Ruzi, Guinea, Napier pakchong1, dwarf Napier and Sweet grass. These were collected sample at 45 ± 3 days of growth. All fresh grasses were chopped manually to a length of about 4-6 cm. Subsamples of each grass were collected to be analyzed for dry matter (DM). The grasses and concentrate were dried in a hot air-oven at 60℃ for 48 hour and ground through sieve 1 mm apertures before using in the experiment. The feed ingredients of concentrate were shown in Table1.

The method used for *in vitro* fermentation was based on the technique. (Menke and Steingass, 1988). Cumulative gas production data were fitted to the model of Orskov and McDonald (1979). At 24 and 48 hour post inoculation a set of samples were tested for *in vitro* digestibility. *In vitro* degradability was determined *in vitro* dry matter

degradability (IVDMD and *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963). All obtained data were subjected to the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998) according to a 5 × 4 factorial arrangement in a completely randomized design (CRD). The statistical model included roughage sources, R:C ratio, R:C ratio × roughage sources interactions. For all parameters, differences among treatments means were contrasted by Tukey's Multiple Comparison Test (Crichton, 1999).

Results and Discussions

At 45 day of growth, the moisture and crude protein content were highest in Sweet grass and lowest in ruzi grass (Table2). ADF and NDF content were highest in Guinea grass. Moreover, NFC was highest in Sweet grass. Different in nutrients level due to amount of leaf and stem. Generally, higher leaf grass varieties had higher nutrients. According to Halim et al. (2013) reported that shorter varieties of napier grass had higher CP due to higher leaf to stem ratio (LSR) because the leafier swards make the whole-plant nutritive quality better than the tall varieties that were stemmier which LSR of shorter varieties was 1.15-1.63 and tall varieties was 0.68-0.92 .

Effect on gas production kinetics

Gas production level was highest in high concentrate proportion diet. Moreover, it was highest in Sweet grass diet as showed in Table3. The higher fermentable carbohydrates and available nitrogen of high concentrate ratio is a better nutrient availability for rumen microorganisms. Higher gas values obtained for the experimental rations were indicating a better nutrient availability to rumen microorganisms (Ahmed and Abdel, 2007). The higher gas production of rations containing higher proportion of concentrate might be due to the increased production of propionate as CO_2 is produced when propionate is made by ruminal bacteria via the succinate-propionate pathway. Moreover, the decreased gas production was having more roughage content was due to the suppressing effect of high cell wall and lignin present in these feeds resulting in decreased attachment of ruminal microbes to feed particles (Paya et al., 2007)(see also Figure 1).

Effect on DM and OM digestibility

DM and OM digestibility (Table4) were significantly different among roughage sources ($p<0.01$). At 60 and 80 % of Sweet grass were higher DM and OM digestibility when compared with other grass. These results were related with nutrient component of roughage, especially low content of NDF and ADF of this experimental feeds resulted in increased microbial populations to digestion of animal feeds. According to Ramin and Huhtanen (2013) reported that feedstuffs with lower NDF, ADF and NFC related to higher feeds digestion and total gas production effect by high microbial activity.

Moreover, DM and OM digestibility were highly significant different among R:C ratio ($p<0.001$). On the other hand, no differences were found in R:C ratio and roughage sources interactions. The digestibility of measured organic matter is closely correlated with that predicted from gas production and the crude protein and ash contents of feeds. According to Nagalakshmi et al. (2005) reported that increase in roughage component of the ration decreased IVOMD. Increasing of nutrient digestibility from increasing of concentrate ratio due to increase in readily available energy and protein contents which have improved microbial growth and fermentation.

Conclusions and recommendations

Based on this finding, it could be concluded that Sweet grass has high nutritive value and resulted in highest DM and OM digestibility. At 60 and 80 %DM of Sweet grass in the diets, it showed higher DM, OM digestibility and gas production than other roughage. Further research using Sweet grass in high roughage diets to improve rumen fermentation and feed efficiency in ruminants are recommended.

KEYWORD : tropical grasses, digestibility, fermentation, ruminants

Table 1 Feed ingredients of the concentrate

Feed ingredients	% of dry matter
Cassava chip	60.1
Rice bran	1.8
Soybean meal	19.7
Palm kernel meal	4.5
Coconut meal	7.2
Urea	1.5
Molasses	3.7
Salt	0.5
Sulfur	0.5
Mineral mixture	0.5
Total	100.0

Table 2 Chemical composition of experimental feeds

Items	DM	CP	ADF	NDF	NFC	Fat	Ash
	---%---	----------% of DM----------					
Ruzi grass	25.3	8.9	36.2	67.8	7.2	2.2	14.1
Guinea grass	20.1	9.2	39.7	67.2	8.0	2.0	12.6
Napier pakchong 1 grass	18.2	11.1	38.3	64.4	9.8	2.0	12.7
drawf Napier grass	14.8	13.4	36.4	61.4	10.4	2.1	10.7
Sweet grass	13.4	15.2	34.9	59.5	12.1	2.2	10.0
Concentrate	91.1	17.9	8.9	17.7	55.5	3.2	5.7

CP, Crude Protein; NDF, Neutral detergent Fiber; ADF, Acid Detergent Fiber

Table 3 Gas production kinetics and gas production of the experiment

Treatment	Roughage	R:C ratio	Gas production kinetics [c]				Gas [d]
			a	B	c	a+b	
T1	Ruzi grass	80:20	-1.0	79.0	0.06	77.9	77.7
T2	Ruzi grass	60:40	-1.7	79.9	0.07	78.2	78.1
T3	Ruzi grass	40:60	-2.4	81.7	0.09	79.3	79.3
T4	Ruzi grass	20:80	-0.1	79.6	0.08	82.0	81.9
T5	Guinea grass	80:20	-2.6	74.6	0.08	72.0	71.9
T6	Guinea grass	60:40	-1.9	75.3	0.08	73.3	73.3
T7	Guinea grass	40:60	-1.0	76.5	0.09	75.5	75.5
T8	Guinea grass	20:80	-1.4	80.2	0.09	78.8	78.8
T9	Napier pakchong1 grass	80:20	-0.2	75.3	0.06	75.1	74.7
T10	Napier pakchong1 grass	60:40	-0.8	77.0	0.06	76.2	76.0
T11	Napier pakchong1 grass	40:60	-1.9	78.6	0.07	76.7	76.6
T12	Napier pakchong1 grass	20:80	-2.9	83.6	0.09	80.7	80.7
T13	dwarf Napier grass	80:20	-0.4	76.2	0.06	75.8	75.6
T14	dwarf Napier grass	60:40	-0.8	79.2	0.07	78.4	78.3
T15	dwarf Napier grass	40:60	-1.3	83.0	0.08	81.7	81.6
T16	dwarf Napier grass	20:80	-3.8	84.5	0.09	80.7	80.7
T17	Sweet grass	80:20	-1.8	80.2	0.07	78.4	78.3
T18	Sweet grass	60:40	-2.7	81.7	0.08	79.0	79.0
T19	Sweet grass	40:60	-1.3	82.2	0.08	80.9	80.9
T20	Sweet grass	20:80	-1.8	85.3	0.09	83.5	83.5
SEM			0.16	0.51	0.001	0.46	0.47
Interaction							
	Roughage		ns	***	***	***	***
	R:C ratio		ns	***	***	***	***
	Roughage x R:C ratio		**	Ns	***	ns	ns

[a], the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); a+b, the gas potential extent of gas production. d Cumulative gas production at 96 h (mL/0.2 g DM substrate). * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 4 Digestibility of *in vitro* incubation

Treatment	Roughage	R:C ratio	*In vitro* digestibility (%)			
			DM		OM	
			24 h	48 h	24 h	48 h
T1	Ruzi grass	80:20	48.3	62.5	53.4	67.9
T2	Ruzi grass	60:40	53.8	68.6	59.3	74.4
T3	Ruzi grass	40:60	57.3	72.9	60.7	76.6
T4	Ruzi grass	20:80	60.8	77.0	63.6	80.1
T5	Guinea grass	80:20	45.0	62.1	50.5	67.9
T6	Guinea grass	60:40	49.5	67.1	51.4	69.3
T7	Guinea grass	40:60	54.0	72.0	58.3	76.6
T8	Guinea grass	20:80	58.2	77.0	62.2	81.3
T9	Napier pakchong1 grass	80:20	43.9	61.5	49.6	67.5
T10	Napier pakchong1 grass	60:40	48.6	66.6	52.4	70.7
T11	Napier pakchong1 grass	40:60	53.1	71.7	58.0	76.9
T12	Napier pakchong1 grass	20:80	57.7	76.8	60.7	80.1
T13	dwarf Napier grass	80:20	49.2	63.2	55.7	70.0
T14	dwarf Napier grass	60:40	53.4	67.9	58.3	73.1
T15	dwarf Napier grass	40:60	56.7	72.5	59.7	75.8
T16	dwarf Napier grass	20:80	61.3	77.3	64.0	80.3
T17	Sweet grass	80:20	51.3	64.5	57.1	70.6
T18	Sweet grass	60:40	55.1	68.9	59.9	74.0
T19	Sweet grass	40:60	58.7	73.2	63.7	78.5
T20	Sweet grass	20:80	62.6	77.6	65.6	80.9
SEM			1.29	1.34	1.14	1.15
Interaction						
	Roughage		**	ns	**	ns
	R:C ratio		***	***	***	***
	Roughage x R:C ratio		ns	ns	ns	ns

* p<0.05; ** p<0.01; *** p<0.001.

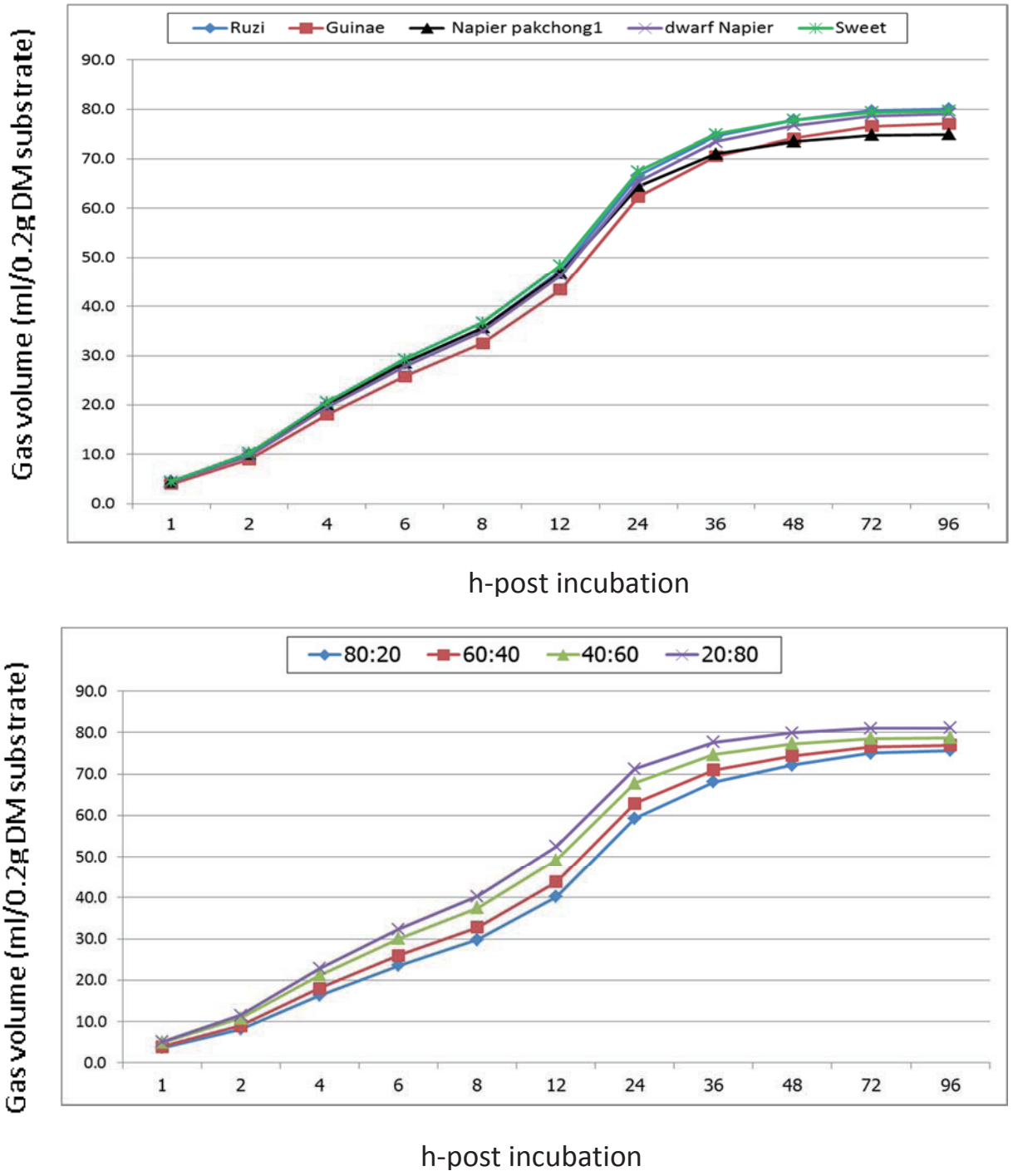

Figure 1 Cumulative gas production at different time of incubation from roughage sources and R:C ratios

REFERENCES

Almeida E.X, G.E., Maraschin, O.E.L. Harthmann, H.M.N. Ribeiro-Filho, and E.A. Setelich. Oferta de forragem de capim elefante anão cv. Mott e o rendimento animal. Revista Brasileira de Zootecnia.2000;29:1288–1295. doi: 10.1590/S1516-35982000000500005.

Ahmed G.M. and N.M.A.F.E. Abdel. 2007. Chemical composition and in vitro gas production characteristics of six fodder trees, leaves and seeds. Research Journal of Agriculture and Biological Sciences, 3: 983-986.

Crichton, N. 1999. Information point: Tukey Multiple Comparison test. Blackwell Science Ltd, J. Clinical Nursing, 8:299-304.

Halim RA; Shampazuraini S; Idris AB. 2013. Yield and nutritive quality of nine Napier grass varieties in Malaysia. Malaysian Journal of Animal Science 16:37–44.

Menke, K. H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev. 28:7-55.

Nagalakshmi, D., D.N. Reddy, and M.R. Prasad. 2005. Evaluation of complete ration with different levels of deseeded sunflower heads and concentrate by in vitro and in situ techniques. Animal Nutrition and Feed Technology, 5: 21-30.

Orskov, E. R. and I. McDonal. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499- 503.

Paya, H., A. Taghizadeh, H. Janmohammadi, and G.A. Moghadam. 2007. Nutrient digestibility and gas production of some tropical feeds used in ruminant rations estimated by the in vivo and in vitro gas production techniques. American Journal of Animal and Veterinary Sciences,2: 108-113.

Ramin, M. and P. Huhtanen. 2013. Effects of dietary and animal factors on methane production: A meta-analysis. J. Dairy Sci. (in press).

SAS. 1998. User's Guide: Statistic, Version 6, 12th Edition. SAS Inst. Inc., Cary, NC.

Tilley, J.M. and R.A.Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18, 104–111.

Wanapat, M. 1999. Feeding of ruminants in the tropics based on local feed resources. Khon Kaen Publ. Comp. Ltd., Khon Kaen, Thailand. pp. 236.

Wanapat M, C, Promkot, S, Wanapat. 2006. Effect of cassoy-urea pellet as a protein source in concentrate on ruminal fementation and digestibility in cattle. Asian-Aust J Anim Sci.19:1004–1009.

O-17-7

Increasing Fattening Performance of Bali cattle by Improving Post Weaning Growth Through Supplementation with Sesbania grandiflora

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Introduction

One of the constraints to improving Bali cattle productivity in Indonesia is the slow growth rate due to low quality of available feeds. Wirdahayati (1994) reported that under traditional management system, male Bali cattle aged 21.5 to 31.5 months grew at the rate of 0.2 kg/d, and the growth rate declined to 0.14 kg/d at the age of over 30 months. Under an improved village management system (Panjaitan, 2012) the male Bali cattle grew at a slightly higher rate of 0.24 kg/day from weaning to maturity.

Growth rate of male Bali cattle can be increased to 0.76 kg/d when fed 40% grass and 60% concentrate with a diet crude protein content of 20.7% (Mastika, 2003). Under this feed lot condition, Bali cattle can reach a slaughter weight of 300 kg within 2 years compared to 3-4 years under the traditional management system. However, feeding high levels of concentrate is not applicable to small holder cattle farmers as the price of concentrate is very high and often not available locally. Therefore, an alternative protein (and energy) source that can potentially replace concentrate is tree legumes such as *Sesbania grandiflora* should be promoted.

Sesbania (*Sesbania grandiflora*) is a tree legume successfully established in the southern part of Lombok island since the 1970s and has now became an important part of diets for fattening cattle in the region (Dahlanuddin et al. 2005). Typically, sesbania is fed at 12% of the diet as a supplement to elephant grass resulting in an average daily gain (ADG) of 0.4 kg/day (Dahlanuddin et al., 2014). This ADG is much higher than the 0.14 to 0.24 kg ADG under traditional systems reported by Wirdahayati (1994) and Panjaitan (2012).

Availability of sesbania is limited due to the very small land area. The average land ownership of cattle farmers in the region is 0.6 ha per household that they can use to plant an average of 406 seabania trees (Dahlanuddin et al, 2014). The use of sesbania should therefore be optimized to improve efficiency of Bali cattle fattening.

Based on our observation, under the traditional management system farmers do not normally pay additional attention to male Bali cattle from weaning until they enter the fattening period. Weaners are mostly sold to other farmers to be raised until they are big enough to enter fattening period. During this period, farmers do not offer high quality feeds so they are slow to reach fattening weight, resulting in a long time to reach slaughter weight. This paper compares the performance of Bali bulls being fattened (aged 16-24 months) when post-weaning diets are improved with sesbania from weaning to 16 months old.

Materials and methods

Site, animals, management and treatments

This experiment was conducted at a demonstration farm of Nyerot (8° 41'13"S, 116° 13'01"E) central Lombok eastern Indonesia. The experiment was conducted in two stages. Stage 1 was conducted from 11 March 2014 to 12 February 2015 and stage 2 was conducted from 13 February 2015 to 14 September 2015.

All calves used in this study were purchased from the local livestock market and drenched with an anthelmintic (albendazole) on arrival. All animals were individually housed in concrete floor pens measuring 1.0 x 1.3 m. Each pen was completed with feed trough to enable measurements of feed offered and feed refused. Water was provided *ad libitum* using bucket brought separately to each pen three times a day.

In the first stage, 24 male weaned Bali calves, approximately 6 months old weighing 75.5 ± 1.4 kg (mean ± SE)

were randomly allocated into four treatments with 6 replicates. The four treatment diets were mixed grasses (mostly elephant grass) *ad libitum* (Grass) as the control, control with a supplement of sesbania offered at 5 g DM/kg live weight (LW) per day (GSes5), control with a supplement of sesbania offered at 10 g DM/kg live weight (LW) per day (GSes10) and control with a supplement of sesbania offered at 15 g DM/kg live weight (LW) per day (GSes15).

In Stage 2), all young bulls from Stage 1 were fed the same diet of grass ad *libitum* with supplements of sesbania and rice bran each offered at 5 g DM/kg LW. This diet is typically fed by the local farmers during fattening period. All animals remained in the same individual pens.

Measurements

In both stages, liveweight was measured every two weeks. Total feed intake was measured for seven consecutive days in the middle of each stage by recording the amount of feed and supplement offered and the amount of feed and supplement refused each day. Dry matter and organic matter digestibility weremeasured following the intake measurement by determining total feed intake and total fecal output of individual animals over seven consecutive days. The digestibility was based on approximately 90% feed intake. During the fecal collection period, animals were monitored closely and feces were collected from the concrete floor from each pen regularly over 24 hour period. Samples of feed, supplement and feces offered were collected and dried to constant weight at 65°C.

Analyses

The DM, OM contents of feeds, refusals and feces, crude protein (CP) content of feeds and supplements, rumen ammonia concentrations were determined according to Association of Official Agriculture Chemists (2005). Data were analysed using One Way ANOVA. All data analyses were conducted using Statistical Analysis Software (SAS, 1999).

Results and discussion

Nutrient compositions of the diets

Table 1 shows that nutrient compositions of the feed ingredients are as expected to provide enough essential nutrients especially crude protein (CP). The CP content of sesbania was more than 20% and thiscontributed significantly to the CP content of the diet. All feed ingredients have very high organic matter content contents (83-92% of dry matter).

Feed intake, rumen ammonia and blood urea nitrogen

The feed intake, rumen ammonia concentration and blood urea nitrogen during stage 1 and stage 2 of this experiment are presented in Table 2.

Rumen ammonia concentration reflected the high availability of soluble nitrogen (from sesbania) in the diet. The rumen ammonia concentrations were within the suggested normal range of 50-250 mg NH-N/liter (Preston and Leng, 1987). Rumen ammonia declined from 220 mg/liter in the bulls fed 15g DM sesbania/kg live weight during stage 1 to 157-173 mg/liter during fattening period. This is due to higher availability of soluble carbohydrate from supplemented rice bran (Orskov and Grubb,1978).

Plasma urea nitrogen increased significantly with increasing levels of sesbania (Panjaitan et al, 2010). The blood urea level recorded in this experiment were at the lower end of the values reported form Bali cattle in other studies(Dradjat et al, 2008) .

Live weight gain

Live weight of young bulls fed grass only was 0.28 kg/day, comparable to that of bulls fed grass in other experiments (Dahlanuddin et al, 2012, Panjaitan 2012). Supplementation with increasing levels of sesbania significantly increased live weight gain up to 0.46 kg/day (Figure 1).

During stage 2 (fattening period), when all bulls were fed the same diet, the live weight gain of bullspreviously fed

grass and those supplemented with 5, 10 and 15 g DM sesbania/kg live weight were 0.52, 0.47, 0.47 and 0.53 kg/d. There was no effect of previous diets on the ADG during fattening.

The overall live weight gain (Figure 2) was significantly higher for the bulls supplemented with 15 g Sesbania DM/ kg live weight from weaning to 18 months old, compared to those fed lower CP diets.

When fed the same diet of grass+ 5 g DM sesbania + 5 g DM rice bran (18-21 months old) the bulls previously fed grass only grew much faster (almost 0.6 kg/day) than when they were fed grass only (0.28 kg/d), which demonstrates compensatory growth. Those previously fed grass+5 g DM sesbania/kg LW, grass+10 g DM sesbania/kg LW grew at the same rate as as they did 6-18 months old. The bulls previously fed grass+15 g DM sesbania/kg LW continued to grow at the same rate and reached 300 kg live weight faster.

Overall, the ADG of bulls fed grass+15DM Sesbania from weaning to 18 months old (0.47 kg/day) was higher ($P<0.05$) than those fed lower CP diets (0.37, 0.42 and 0.41 kg/day respectively). Consequently, the bulls fed grass+15DM Sesbania from weaning to 18 months old finished (at 24.5 moths old) at 336 kg , 53.5 kg higher ($P<0.05$) than those fed grass only. However the final weight of those fed grass only did not differ ($P>0.05$) with the final weight of the bulls supplemented with 5 or 10 g DM sesbania/kg LW (300 and 3002 kg respectively). The highest ADG of Bali bulls in this experiment was comparable to the ADG of Bali bulls fed similar high protein diets (Dahlanuddin et al., 2014). However, they were lower than the ADG reported by Mastika (2003) because the quality of the diets fed in the current experiment were not as high as the diets fed in the experiment reported by Mastika (2003).

Conclusion

This growth path study demonstrated that male Bali cattle fed high quality diet from weaning and during fattening can reach 300 kg (a preferable slaughter weight of Bali bull) at less than 24 months old. Feeding lower quality diet from weaning to 18 months old will result in bulls taking longer time to reach 300 kg even if they are fed a high quality diet during fattening.

KEYWORD : Bali cattle, Diet quality, Fattening, Growth, Sesbania grandiflora

Table 1. Dry matter (DM), organic matter (OM), crude protein (CP), fat, crude fibre (CF) and ash content of feeds used in stage 1 and 2 of the experiment (% DM)

Feeds		DM	OM	CP	Fat	CF	Ash
Grass	stage 1	16.78	85.55	9.80	*	30.3	14.45
	stage 2	21.48	83.44	9.19	2.01	34.77	16.56
Sesbania	stage 1	23.00	90.73	21.31	*	25.36	9.27
	stage 2	24.15	91.48	22.71	3.65	28.92	8.52
Rice bran	stage 2	90.00	89.44	13.29	16.65	10.83	10.56

Table 2. Total feed intake, rumen ammonia and blood urea N concentrations during stage 1 and stage 2 of this experiment

Variables		Grass (G)	G+5gDM Sesbania	G+10gDM Sesbania	G+15gDM Sesbania
Total intake (g DM/Kg LW/day)					
	Stage 1	23.0±0.2	22.0±0.5	21.9±0.6	22.8±0.14
	Stage 2	22.3±0.39	19.9±0.65	18.8±0/58	19.6±1.16
Rumen NH_3-N (mg N/L)					
	Stage 1	84.1±8.9^{a}	172.0±32.1^{b}	162.1±15.4^{b}	220.1±21.6^{b}
	Stage 2	157±15.3	173±11.4	161±14.0	161±26.8
Plasma urea nitrogen (mg/dl)					
	Stage 1	18.9±1.3^{a}	28.3±0.5^{b}	30.5±2.0^{bc}	34.5±0.4^{c}
	Stage 2	24±2.74	24±1.94	24±2.66	28±6.59

Figure 1. ADG of young bulls fed grass only or grass supplemented with increasing level of sesbania from weaning to 18 months old (left) and when they were fed the same diet during fattening (right)

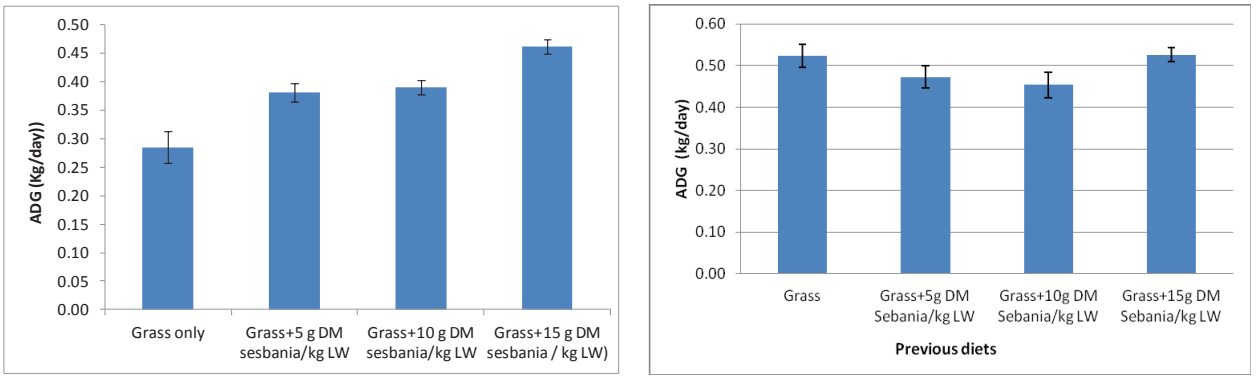
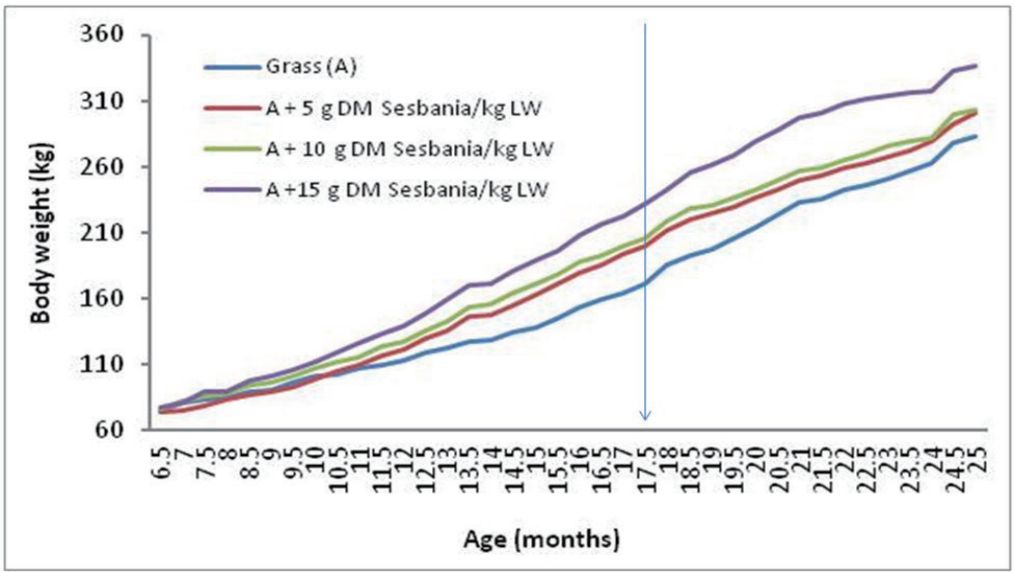


Figure 2. Growth path of male Bali cattle fed grass only, grass+5 g DM sesbania/kg LW, grass+10 g DM sesbania/kg LW, grass+15 g DM sesbania/kg LW from 6-17 months old then fed the same diet of grass+ 5 g DM sesbania + 5 g DM rice bran from 18-25 months old).



REFERENCES

Association of Official Agriculture Chemists (2005) 'Official methods of analysis.' Available at http://www.oma.aoac.org [Verified 2 October 2013]

Dahlanuddin Hasniati Shelton HM (2005) Sesbania grandiflora: a successful tree legume in Lombok, Indonesia. Tropical Grasslands 39, 217.

Dahlanuddin, B T Yuliana, T Panjaitan, M J Halliday, E van de Fliert and H M Shelton (2014) Survey of Bali bull fattening practices in central Lombok, eastern Indonesia, based on feeding of Sesbania grandiflora. Animal Production Science, 2014, 54, 1273–1277.

Dahlanuddin, TB Yulianto, A Priyanti, DP Poppi and SP Quigley (2012). Weaning and supplementation increase liveweight gain of Bali (*Bos javanicus*) cattle of small-holder farmers in Central Lombok, Indonesia. Animal Production 14(3):173-179.

Dradjat A S, Dahlanuddin, M Ali, Imran, Lestari and Maskur (2008). Pemberian Pakan, Pemeliharaan dan Gambaran Darah Pada Sapi Bali (*Bos sondaicus*) Infertil. Research Report University of Mataram.

Mastika, M (2003) Feeding Strategies to Improve the Production Performance and meat quality of Bali cattle (*Bos sondaicus*). ACIAR Proceedings no 110. p 10-13.

Orskov, E. R. and Grubb, D. A. (1978). Validation of new systems for protein evaluation in ruminants by testing the effect of urea supplementation on intake and digestibility of straw with or without sodium hydroxide treatment. J. Agric. Sci. 91:483-486.

Panjaitan T, S P Quigley, S R McLennan, T Swain and D P Poppi (2010) Intake, retention time in the rumen and microbial protein production of Bos indicus steers consuming grasses varying in crude protein content. *Animal Production Science*, 50, 444–448.

Panjaitan, T (2012) Performance of male Bali cattle in village system of Lombok. In: Koonawootrittriron S; Suwanasopee T; Jattawa D; Boonyanuwat K; Skunmun P, eds. Improving smallholder and industrial livestock production for enhancing food security, environment and human welfare. Proceedings of the 15th AAAP Animal Science Congress, 26 — 30 November 2012, Bangkok, Thailand. p. 956 — 959.

Preston, T. R. and Leng, R. A. (1987). Matching Ruminant Production Systems with Available Resources in the Tropics and Subtropics. Penambul Books. Armidale.

SAS (1999) 'SAS/STAT user's guide. Version 8.'(SAS Institute: Cary, NC)

Wirdahayati, R.B. 1994. Reproductive characteristics and productivity of Bali and Ongole cattle in Nusa Tenggara, Indonesia. PhD Thesis, University of Queensland, Brisbane Australia.

O-17-8 Study of Digestibility Value of Oil Palm By Product Ration and Its Effect on The Performance of Bali Cattle

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Objective: The objectives of the study was to evaluate digestibility value of palm oil by product based of ration and its effect on the performance of Bali cattle in Indonesia.

Methodology: The in vitro and in sacco experiments initiated by nutrition determination of Treatment and Control Feed ration. Treatment feed ration were composed by the mixtures of palm oil by products e.g. palm frond, ammoniated oil palm leaf, palm oil mill effluent, mineral mix and molasses. Control feed ration were composed by mixture of Guinea grass hay and local concentrate. All ingredient on both ration (treatment and control) were milled and mixed to obtain homogenous mixtures by using batch mixer. Nutrition determination were conducted by using AOAC (2000) method for proximate analysis and Van soest (1992) for fiber fraction analysis. Data of ingredient and nutrition composition of treatment and control feed ration are describe on Table 1.

The digestibility value were determined by using two stage in vitro method (Tilley and Terry, 1963) and in sacco method were conducted as described by Orskov and McDonald (1963). About 1 g of milled treatment and control ration sample filled in each 10 in vitro bottles which used for in vitro determination and fresh rumen liquid obtained from fistulated Bali cow were strained through 4 layer of surgical gauze diluted with artificial saliva (1:2) was used as inoculum.
Triplicate nylon bags (bag size, 80mmx150mm; pore size 45 μ m) containing 5 g of milled dry sample were weighed and then incubated in the rumen of fistulated Bali cow 38 months old and weighing 310 kg. The bags were then withdrawn after 2, 4, 8, 12, 24, 48, 72 and 96 hours. The zero hour was obtained by soaking the bags in a water bath maintained at 39^{o}C for 1 hour. After the incubation period, the bags were withdrawn then hand washed under running tap water until the water coming out of the bags was clear. The washed bags and contents were then dried for 48 hours at 60^{o}C in oven to determined apparent dry matter disappearance.
Data were pooled from 3 consecutive experiments followed by the feeding experiment of the ration on Bali steer performance. Six Bali steer 26 month old were grouped into treatment and control group. Each group consists of 3 steers with average body weight 184 $\pm$ 3.2 kg. Initial weight and body condition (BCS) score of each steer were determined and each group were given total 18 kg feed ration 3 times per day (08.00, 13.00 and 17.00 h respectively) for 90 days. T test independent sample analysis were applied for the data (Steel and Torrie, 1980)

Result and Conclusion: Digestibility value of palm oil by products feed ration and its effect on performance of Bali cattle are shown in table 2. Treatment feed rations of palm oil by products has higher dry matter digestibility than control. This result are related to the report by Bamikole and Ikhatua (2009) regarding to the digestibility value of palm oil by products. The treatment feed rations also contain ammoniated palm oil leaves which provide availability of non-protein nitrogen for the rumen fermentation. Higher degradation of dry matter of palm oil by product obtained by the treatment of nitrogen supplementation (Bengaly et al., 2010) while Islam et al. (2000) reported that different level of palm oil frond has significant result on dry matter digestibility. Total VFA value of rumen fermentation represent the fermentability value of samples, in this experiment, total VFA value of palm oil by products feed rations has higher value than control. This result may related to more ingredient composition of treatment feed ration than control. However, total VFA of treatment and control feed ration were lower than the range of optimum requirement (>100 mM/L) for the rumen fermentation as stated by Perdock and Leng (1989).

in sacco dry matter disappearance of both feed rations has similar pattern. Dry matter disappearance has increased over 12h incubation time and decrease between 24h to 48h. Final dry matter disappearance of treatment and control feed ration were 79.71 $\pm$ 1.34 % and 72.85 $\pm$ 1.66 % respectively. Chart 1 show the pattern of in sacco dry matter disappearance of treatment and control feed ration. High dry matter disappearance rate were initiated over 24h incubation time, this may reflect the feed ration need longer time in the rumen for the

fermentation.

Dry matter digestibility on feeding trial has higher value than in vitro dry matter digestibility for both feed ration and no significance result obtained for the all parameters. Average daily gain of treatment feed ration almost similar to the control treatment. Similar result on BCS score between group of treatment feed ration and control feed ration of Bali steer. This may represent the high possibility of palm oil utilization as feed for ruminant.

KEYWORD：Bali steer, Digestibility Value, In Vitro, In Sacco, Palm Oil Mill Effluent

Table 1. Ingredient and Nutrition composition of treatment and control feed ration

Treatment Ration	%		Control Ration	%
Palm frond	40		Guinea grass hay	80
ammoniated palm oil leaf	34		Local commercial concentrate	20
Palm oil mill effluent	15			
Mineral mix	1			
Molasses	10			
Nutrient Composition (%)	Treatment	SD	Control	SD
Dry matter	67.15	2.14	89.21	3.78
Crude Protein	12.43	0.53	14.02	0.22
Crude Fat	9.54	0.66	4.65	0.92
Crude Fiber	28.26	1.43	26.14	2.13
Ash	3.54	0.36	6.82	0.98
Neutral Detergent Fiber	56.43	3.11	62.21	2.56
Acid Detergent Fiber	48.21	1.65	28.94	2.54
lignin	15.22	0.58	7.46	1.17

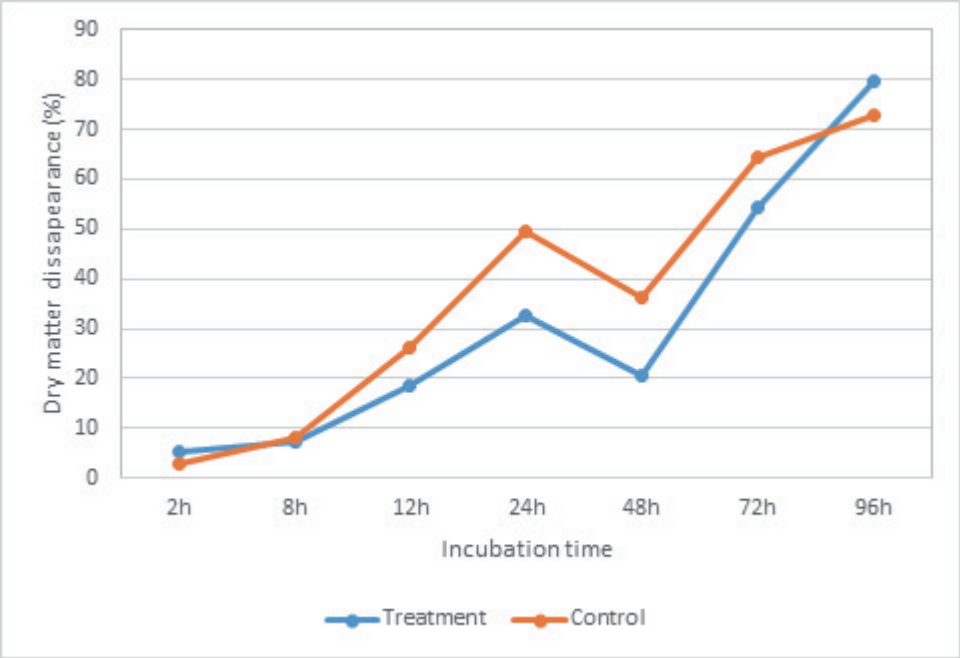
SD: Standard deviation

Table 2. Digestibility value and feeding experiments of palm oil by products feed rations

Parameters	Treatment	SD	Control	SD
In vitro experiment				
Dry matter digestibility (%)*	58.22	1.27	52.45	1.85
Total VFA (mM/L)*	87.26	2.21	91.56	2.05
Feeding experiments				
Dry matter digestibility (%)	69.32	2.35	72.43	2.87
Average daily gain (kg day^{-1})	0.62	0.03	0.69	0.02
Initial Body Condition Score	4.5		4.5	
Final Body Condition Score	5.5		5.5	

*significantly different ($p<0.05$)

Chart 1. In sacco dry matter disappearance of treatment and control feed ration



REFERENCES

AOAC. 2000. Official methods of Analysis, Vol.1, 19th Edition. Association of Official Analytical Chemists, Arlington, Virginia, USA. pp. 69-90.

Bamikole, M. A. Ikhatua, U. J. 2009. Variety diversity effect on the chemical composition and dry matter degradation characteristics of residue and by-products of oil palm fruits. Anim. Sci. J., 80 (3): 239-249.

Bengaly, K. Liang, J. B. Jelan, Z. A. Ho, Y. W. Ong, H. K. 2010. Utilization of steam-processed oil palm (*Elaeis guineensis*) frond by ruminants in Malaysia: investigations for nitrogen supplementation. Afr. J. Agric. Res., 5 (16): 2131-2136.

Islam, M. Dahlan, I. Rajion, M. A. Jelan, Z. A. 2000. Rumen pH and ammonia nitrogen of cattle fed different levels of oil palm (*Elaeis guineensis*) frond based diet and dry matter degradation of fractions of oil palm frond. Asian-Aust. J. Anim. Sci., 13 (7): 941-947.

Ørskov ER Mc Donald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agri Sci (Camb) 92: 499-504.

Perdock, H. B. R. A. Leng. 1989. Rumen ammonia requirements for efficient digestion and intake of straw in cattle. In: The Roles of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). pp. 291-293 (Penambul Books, Armidale, Australia).

Steel RGD Torrie JH. 1980. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw Hill, New York.

Tilley, J.M.A. R.A.Terry. 1963. A two stage technique for the in vitro digestion of forage crops, J.Br.Grassland Soc.18, pp 104-111.

Van Soest PJ Robertson JB Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci; 74: 3583-97.

O-17-9

Optimizing Rice Straw Utilization for Bali Cattle Fattening as Adaptation Strategy to Climate Change for Smallholder Farmers in Lombok Indonesia

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Introduction

The fluctuated feed resources availability becoming main cause of liveweight fluctuation of beef cattle in West Nusa Tenggara province. This was also worsened by the climate change that caused uncertainty of dry and wet season and also prolonged draught. To overcome this it was necessary to find the alternative feed source that available in abundance, easy to collect and cheap. This alternative feed is rice straw which is abundance in number, highly available but less use by farmer. Gumilar (2010) stated that each hectare land produced between 12 to 15 tonnes of rice straw in one harvest, but 70-80% of this rice straw was merely burned straight after harvest. On the other hand, this rice straw have potential to be used as cattle feed since it contains high organic matter and could be digested by cattle.

Improvement of nutrient quality and digestibility of rice straw could be achieved through certain physical treatment and fermentation. Winarno (2010) stated substrates that undergo fermentation usually contain higher nutrition value compared to its origin caused by microorganisms activities that able to break the complex components so it was easier to digested and also provide more nutrients. There are many fungus isolated from soil such as *Trichoderma viride, Pleuratus sp, Phanerachaete chrysosporium,* and *Fusarium sp.* These microorganisms are able to degraded cellulose and lignin that originally from nature (Malekzadeh *et al*., 1993). Previous research by Sutaryono and Ali (2007) showed that the used of local microorganisms on rice straw fermentation could reduced the NDF, ADF, cellulose and lignin content of rice straw.

It is expected that the impact of the rice straw use as a base of complete feed for cattle will increase the scale of breeding and fattening activities by smallholders and at the same time also as an adaptation strategy to overcome climate change so the cattle population keep increasing.

Materials and Methods

The ground rice straw was fermented with *Trichoderma viride* a particularly fungus which were cultured in petridish. Rice straw in 100 kgs was sprayed with 400 mls of starter solution (the mixture of 2.5 kg urea, 2.5 kg molasses, 2.5 litres of prepared *T. viride* which was diluted in 20 litres aquadest). The rice straw then were put in the bunker and sealed with hard plastic. Furthermore the fermented rice straw was mixed with concentrate to make complete feed with varies composition as follows: Formula 1 (80% rice straw + 20% concentrate); Formula 2 (60% rice straw + 40% concentrate); Formula 3 (40% rice straw + 60% conscntrate); dan Formula 4 (20% rice straw + 80% concentrate). Meanwhile the concentrate was composed of rice bran (63.5%), ground corn (30%), fish meal (5%) and urea (1.5%). This rice straw base complete feeds were then fed to young Bali bull for 4 months. Several parameters were observed such as feed quality, average daily gain, meat physical and chemical characteristics.

Results and discussion

The fermentation of rice straw with *Trichoderma viride* with addition of urea and molasses reduced the neutral detergent fibre (NDF) and acids detergent fibre (ADF) content of rice straw as shown in Figure 1.

Higher NDF and ADF content of feed showed that the feed quality is not good. With lower fiber content of fermented rice straw indicated that the fermentation process could increase the rice straw nutrients. The increase of digestibility of rice straw after fermentation was also reported by Han *et al*., (1978). Feed consumption tended to increase inline with the adaptation of cattle on the ration of the rice straw base complete feed provided to the cattle. The complete feed which consist of 80% rice straw and 20% concentrate (Formula 1) and 60% rice straw % and 40% concentrate (Formula 2) showed the higher consumption compared to those two other formulas with higher concentrate content (Formula 3 and 4). This result suggests the good palatability of rice straw base complete feed. Hence, the rice straw base complete feed could be developed as quality feed resource for cattle.

Although complete feed Formula 3 and 4 contain higher concentrates, the palatability of complete feed Formula 1 and 2 were better. So eventhough the quality of Formula 1 and 2 was lower compared to those of Formula 3 and

4, the nutrient content of Formula 1 and 2 still in the significant level to increase the Bali cattle productivity and provide reliable average daily gain (Figure 2).
Although the growth rate of Bali cattle fed with complete feed Formula 1, 2 and 3 all showed significant good result, the best gain was showed by complete feed Formula 1 with average daily gain of 0.63 kg/day. This result similar to average daily gain of cattle reported by Thalib (2008) with average daily gain varies between 0.3 - 0.75 kg/day.

Average carcass showed the positif correlation between liveweight and carcass weight. Average carcass weight was 46.30% of liveweight, meanwhile the meat percentage of carcass was 70.80% and leg plumpness was 85.29%. All values were the normal value of percentage could be achieved from livestock carcass. Hence, the use of rice straw base complete feed could provide good growth for cattle and maintain cattle condition although without using grasses as cattle feed.
Although there was decrease of meat pH from fresh meat to 4 hours after slaughtering (pH ultimate), the pH value of meat in this research was in the normal range of meat pH value (Table 3). Postmortem decrease of meat pH was affected by postmortem glikolysis rate and meat postmortem glikogen reserve, in which the normal value of meat pH ultimate was 5.40-5.80 (Silva *et al*., 1999). The pH value is the factor influence the physical characteristic of meat such as colour, water holding capacity, tenderness and cooking loss.

Meat tenderness was achieved by measuring the the total *breaking value* of meat, the lower the breaking value the more tender the meat (Tambunan, 2010). Factors affected the meat tenderness have relationship with the meat composition itself, such as meat cross linkage, meat fibres, marbling of the meat and meat rigor mortis that happened after cattle slaughtered. The meat collagen and age of cattle also influenced the meat tenderness caused by cross linkage of meat fibre individually increased accordingly to the cattle age (Swatland, 1984). Based on Warner Bratzer category, the top side of Bali cattle meat fed with rice straw based complete feed fall into tender category (4.51 kg/cm^2) while the tenderness of rump falls in slightly tender category (6.41 kg/cm^2; Suryati dan Arif, 2005).
Cooking loss value of top side meat was 13.68% was lower than normal value which was varied between 15.00-40.00%. Meat with lower cooking loss indicates higher quality compare to meat with higher cooking loss value, because with lower cooking loss the meat will have lesser nutrition loss during cooking process. The cooking loss value in this research was lower compared to cooking loss value reported by Bolink *et al* (1999) for youg Limousine beef with cooking loss value of 31.2 + 0.6%. The difference in cooking loss value may be caused by differences in duration and temperature of cooking of the research. This inline with statement made by Suparno (2005) that cooking loss was influenced by temperature and duration of cooking. The higher the temperature of cooking the higher the loss of meat juice until it achieved the constant level.
The proximate analysis value of rump and top side was similar and all values were at the normal ranges (Table 4). The water content and crude protein content was close to the value those reported by Arka (1990) which found the water content of 73.63% and crude protein content of 20.91% in meat of young Bali cattle at 2-3 years old. Hence, base on this result it is clear that the quality and chemical characteristic of meat produced by feeding cattle with rice base complete feed were in good quality.

Conclusions

Based on the results found in this research it was concluded that:
Rice straw ferementation with *Trichoderma viride* isolate plus urea and molasses improved the quality of rice straw as cattle feed which was shown by decrease in NDF and ADF content.Rice starw base complete feed Formula 1 (80% fermented rice straw + 20% concentrates) provide the highest average daily gain of cattle at 0.63 kg/ dayPhysical and chemical characteristics of meat from cattle fed with rice straw based complete feed were comparable to meat of cattle raised traditionally by smallholder farmers.Rice straw base complete feed could be used as basic feed for Bali cattle raising and as a climate change adaptation strategy for smallholder farmer

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KEYWORD : Bali cattle, Rice straw fermentation, Complete feed, Meat quality

Table 1. Proximate analysis of rice straw base complete feed.

Content	Rice straw base complete feed			
	Formula1	Formula2	Formula3	Formula 4
Dry matter (%)	100.00	100.00	100.00	100.00
Ash (%)	23.62	19.01	14.87	12.18
Crude fibre (%)	22.52	17.97	14.16	11.66
Crude protein (%)	10.76	12.24	14.16	14.67

Table 3. Physical characteristics of Bali cattle meat

	pH (fresh)	pH ultimate (after 4 hrs)	Water Holding Capacity (%)	Tenderness (kg/cm^2)	Cooking Loss (%)
Rump	5.63 ± 0.10	5.26 ± 0.19	235.79 ± 4.69	6.14 ± 0.53	21.39 ± 2.41
Top side	5.35 ± 0.09	5.30 ± 0.05	238.13 ± 12.18	4.51 ± 0.25	13.68 ± 0.75

Table 4. Proximate analisys and Fatty acids content of Bali cattle meat

	Water content (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Fatty acids (mix; w/w)
Rump	77.38 ± 0.20	1.10 ± 0.06	22.29 ± 0.53	15.0 ± 0.14	40.0
Top side	76.87 ± 0.22	1.04 ± 0.07	22.90 ± 0.05	14.0 ± 0.01	

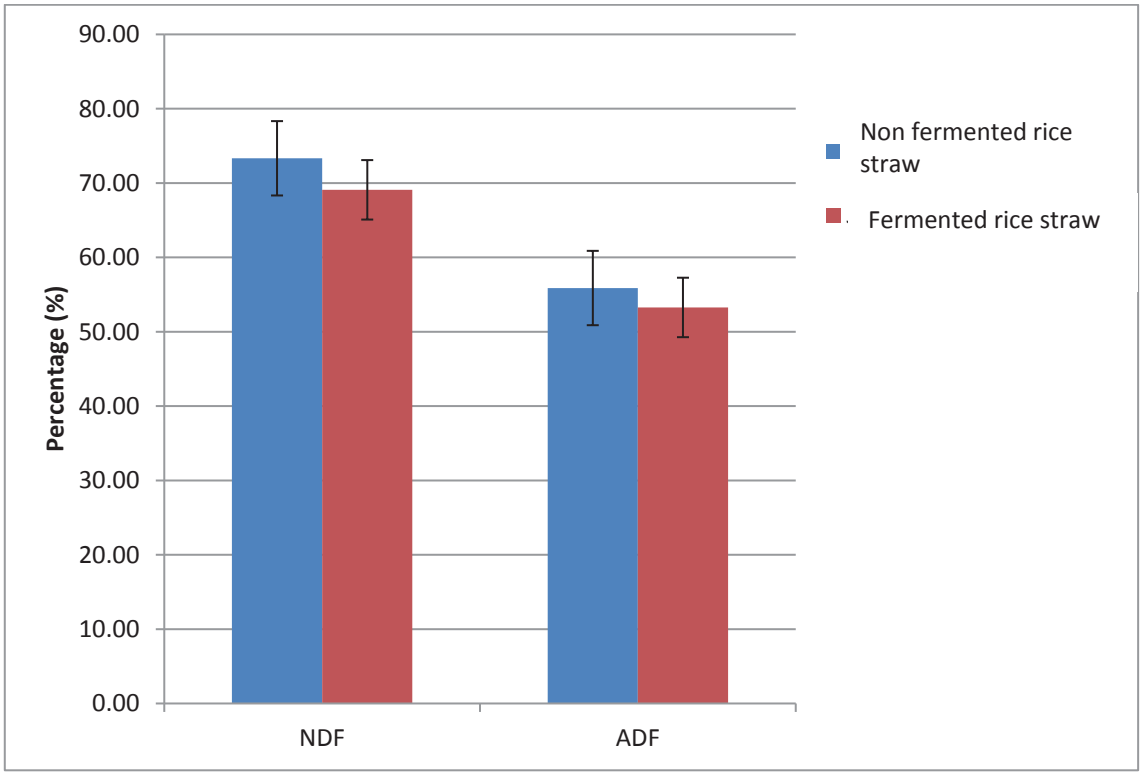

Figure 1. NDF and ADF of non fermented and fermented rice straw

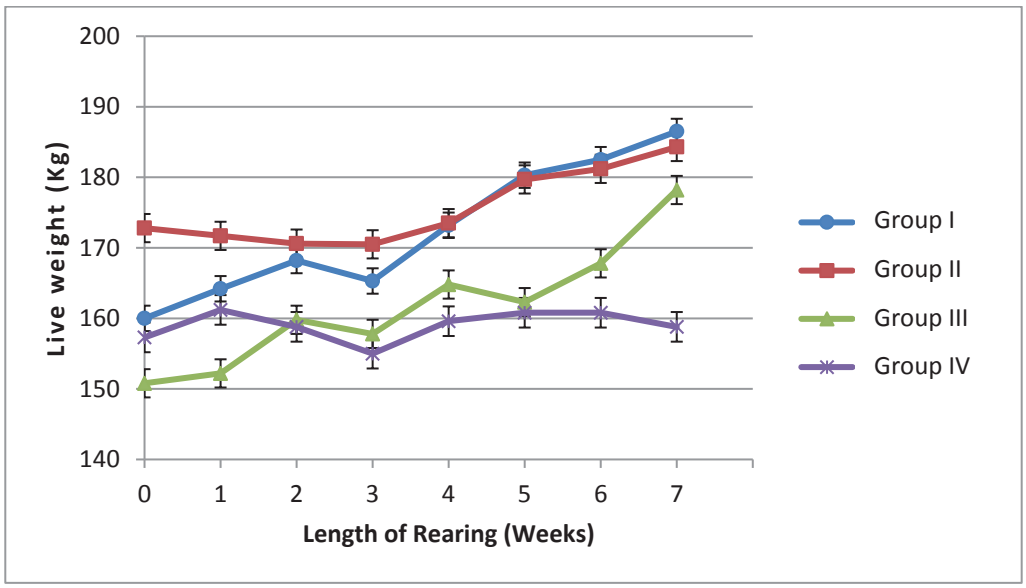

Figure 2. The liveweight development of Bali cattle fed with rice straw base complete feed with different composition of rice straw and concentrate. Group I fed with 80% rice straw+20% concentrate, Group II fed with 60% rice straw+40% concentrate, Group III fed with 40% rice straw+60% concentrate, and Group IV fed with 20% rice straw+80% concentrate.

REFERENCES

Arka, I.B. 1990. *Kualitas Daging Sapi Bali.Bali 20-22 September.Prosiding Sapi Bali; Bali. p A – 108*.

Bolink, A.H. Hoving. , W.J.A Hanekamp, P Walstra (1999). Effect of sire breed and husbandry system on carcass, meat and eating quality of Piemontese and Limousin crossbred bulls and heifers. Livestock Production Science, vol 57, Issue 3, p.273-278

Gumilar, S. 2010. *Penanganan dan pengolahan limbah jerami padi. http://kebuntani.com/2010/10/penanganan-dan-limbah-jerami-padi*.

Han, Y.W., Smith, S.K. and Cheeke, P.R., 1978. Response of weanling meadow voles to a diet containing fermented straw. Journal of Animal Science 47: 648-653.

Malekzadeh F., Azim M., Shahamat M., Colwel R.R. 1993. Isolation and identification of three cellulomonas spp. from forest soils. World Jurnal of Microbiology and Biotechnology. Rapid Communications of Oxford Ltd., 9, 14-18.

Silva, J.A., L. Patarata, and C. Martins, "Influence of ultimate pH on bovine meat tenderness during ageing," Meat Science, vol. 52, no. 4, pp. 453–459, 1999. View at Publisher · View at Google Scholar · View at Scopus.

Soeparno. 2005. *Ilmu dan Teknologi Daging.Cetakan Ke-4. Gadjah Mada University Press, Yogyakarta*.

Suryati, T. dan I.I. Arif. 2005. *Pengujian daya putus Warner Bratzler, susut masak dan organoleptik sebagai penduga tingkat keempukan daging sapi yang disukai konsumen. Laporan Penelitian. Fakultas Peternakan. Institut Pertanian Bogor. Bogor*

Swatland, H.J. 1984. Structure and Development of Meat Animals. *Second Edition*. Prentice Hall Inc. Englewood Cliffs. New Jersey.

Sutaryono Y.A., dan Ali, M. *Isolasi Mikroba Pemecah Jerami Padi untuk Meningkatkan Nilai Nutrisi Jerapi Padi sebagai Pakan. PPKD, Bappeda NTB, Mataram*.

Tambunan, R. D. 2010. *Keempukan Daging dan Faktor-faktor yang Mempengaruhinya. www.lampung.litbang.deptan.go.id [18 September 2012*].

Winarno, F.G. 1980. Microbial Conversion of Lignocellulose Into Feed. Straw and Other Fibrous by-Product as Feed. Elsevier. Amsterdam-Oxford- New York.

O-18-1

The Effects of Using Corn with Various Phytic Acid in Diets on Performance, Phosphorus and Calcium Serum in Nursery Pigs

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INTRODUCTION

Animal feed is an important factor of animal production because the animal feed costs 60-70% of the total expense. Corn is still a majority of energy feedstuff to be used in feed formulation in which 4.0 million tons of corn in each year are used in Thailand feed mill (Poommarin, 2010). Corn contains metabolizable energy 3,420 kcal/kg, crude protein 8.3%, calcium 0.03%, total phosphorus 0.28 (NRC, 1998), moreover, it is abundant of provitamin A and xanthophyll source (Khajarern, 2003). However, the antinutritional factor is also found in corn such as phytic acid (PA). The PA content in corn was reported to account for about 75-80% of total phos phorus (Raboy et al, 2000; Tongoona, 2005) and the available phosphorus of corn is only 0.2%. Corn breed has been often developed into corn low phytic in agricultural countries. Therefore, the objectives of the research are to develop corn breed into corn low phytic and explore its utilization on growth performance and level of phosphorus and calcium serum of nursery pigs.

MATERIAL AND METHODS

Animals and treatments: A total of 36 (Landrace x Large white x Duroc jersey) nursery pigs (18 male and 18 female) with an initial BW of 9.10 kg were used. Pigs were randomly allotted to 1 of 3 treatment diets (**Table 1**). There were 4 replications (pens) in each treatment with four pigs (two male and two female) in each pen. The treatment diets 1) were commercial corn as basal diet (BD), 2) low-phytic acid hybrid corn (LPC) and 3) high-phytic acid corn (HPC). The PA content of diets were 478.17, 454.68 and 491.07 mg/100 g, respectively. Diets were formulated as follow nutrient requirement of NRC (1998) for nursery pigs (10-20 kg). Diets were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF) Calcium (Ca) and total phosphorus (tP) (AOAC., 2005). Analysis of gross energy (GE) was using isoperibol bomb calorimetry (CAL2K). The PA was analyzed in the protocol as described by Haug and Lantzsch (1983)

Sampling and measurements: Pigs were allotted to consume feed and water *ad libitum* from feeder and an automatic waterer. Pigs and diets were weighted on beginning and final of experiment to calculate the body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), gain per feed (G/F). Blood sampling were collected on day-28 of experimental period.

Statistical analysis: Data collected were analyzed by ANOVA as a completely randomized design. Difference between treatment means were determined by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). A level of significance was set at $P \leq 0.05$ for all statistical tests.

RESULTS AND DISCUSSION

According from **Table 2**, after finished the collecting data of 28 days, the data were pooled and calculated for growth performance parameters, we found that there were no differences ($P > 0.05$) in ADG, pig fed LPC and BD had ADG 0.53 and 0.51 kg/pig/day and there was not different ($P>0.05$) from pig fed HPC (0.46 kg/pig/day). The ADFI was not significantly different ($P>0.05$) in all group of pigs. The G/F was not different ($P>0.05$) among diets. FCG of pig fed LPC was cheaper than pig fed BD and HPC as 0.21 and 0.91 baht/kg.gain. Results shown that growth performance were not different between all treatment diet groups. It might be the amount of PA in all treatment diets was hardly different (454.68 - 491.07 mg/100 g). However, Veum et al. (2001) reported pig fed diet with LPC (low-phytate hybrid corn from *lpa 1-1* allele) had more body gain 17% ($P <0.01$) and more efficient ($P <0.01$) in feed conversion (gain/feed) 11% compared with pigs fed semi-purified diet or NC diet, where as the NC diet has 0.14 % of PA composition and LPC diet has 0.06% PA, the PA of NC diet was higher than LPC diet as 2.33 times. Spencer et al. (2000) reported that low-phytic acid corn increased the availability of P and other nutrients. Corn with this trait would be predicted to have a greater concentration of energy because of changes in the chemical composition of the kernel.

Conclusion

From this research, the effect of using low phytic acid corn in diet was not improved growth performance, serum level of phosphorus and calcium. However, the feed cost per gain of pig fed low phytic acid corn in diet (LPC) was cheapest.

KEYWORD : hybrid corn, phytic acid, nursery pig, productive performance, serum

Table 1. Diet composition (as-fed basis)

Ingredient (%)	Treatment diet		
	BD	LPC	HPC
Corn-Low phytic	-	-	15.0
Corn-High phytic		15.0	-
Corn-Commercial	15.0	-	-
Whey	10.0	10.0	10.0
Broken rice	36.0	36.0	36.0
Soybean meal, 44% CP	18.0	18.0	18.0
Fish meal, 60% CP	3.0	3.0	3.0
Full fat soybean	15.0	15.0	15.0
L-lysine.HCl	0.20	0.20	0.20
DL-methionine	0.10	0.10	0.10
Salt	0.30	0.30	0.30
Limestone	0.50	0.50	0.50
Palm oil	0.20	0.20	0.20
Monocalcium Phosphate, P21%	1.20	1.20	1.20
Vitamins-Minerals Premix*	0.50	0.50	0.50
Total (kg)	100	100	100
Calculated composition			
Dry matter (%)	88.48	88.53	88.58
Gross energy (Kcal/kg)	3,268.6	3,268.6	3,268.6
Crude protein (%)	20.6	20.6	20.6
Crude fiber (%)	3.0	3.0	3.0
Calcium (%)	0.70	0.70	0.70
Total phosphorus (%)	0.60	0.60	0.60
Phytic acid in diet, mg/100 g**	478.17	454.68	491.07

*The vitamin mineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg;pyridoxine, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg, Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

** Chemical analyzed (DM basis)

BD; Basal diet was contained commercial corn (PA of diet = 478.17 mg/100 g diet)
LPC; Low phytic acid corn diet was contained low-phytic acid hybrid corn (PA of diet = 478.17 mg/100 g diet)
HPC; High phytic acid corn diet was contained high-phytic acid corn (PA of diet = 478.17 mg/100 g diet)

Table 2. Growth performance, phosphorus and calcium serum level of nursery pig fed treatment diets

Parameter	Treatment diet			SEM	*P*-value
	BD	LPC	HPC		
Pig number (pig)	12	12	12	-	-
Observation period (day)	28	28	28	-	-
Body weight gain (BWG; kg/pig)	14.27	14.82	12.80	1.45	0.1855
Average daily gain; (ADG; kg/pig/day)	0.51	0.53	0.46	0.01	0.1807
Average daily feed intake (ADFI; kg/pig/day)	0.82	0.84	0.75	0.01	0.4714
Gain per feed (G/F; kg/kg)	0.62	0.63	0.61	0.01	0.8088
Phosphorus serum, mg/dL	8.00	7.60	7.07	0.24	0.1437
Calcium serum, mg/dL	10.40	10.63	10.33	0.18	0.6726
Feed cost per gain (FCG; Baht/kg. gain)	25.81	25.60	26.51	-	-

SEM = Standard error of mean
BD; Basal diet was contained commercial corn (PA of diet = 478.17 mg/100 g diet)
LPC; Low phytic acid corn diet was contained low-phytic acid hybrid corn (PA of diet = 478.17 mg/100 g diet)
HPC; High phytic acid corn diet was contained high-phytic acid corn (PA of diet = 478.17 mg/100 g diet)

REFERENCES

AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Analytical Chemists; Washington, DC, USA.

Haug W, Lantzsch H.-J,. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric 34: 1423-1426

Khajarern, S. 2003. Feed Stuffs. In Non-Ruminant Nutrition. Department of Animal Science, Faculty of Agriculture, Khon Kaen University.

NRC. 1998. National Research Council. Nutrient Requirements for Swine, 10th ed.National Academy Press, Washington, DC

Poommarin, P. 2010. Feed ingredients. In Animal Feed. Faculty of Animal Sciences and Agricultural Technology, Silpakorn University.

Raboy V, Gerbasi PF, Young KA, Stoneberg SD, Pickett SG, Bauman AT, Murthy PPN, Sheridan WF, Ertl DS,. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol 124: 355-368

Spencer, J. D., G. L. Allee, and T. E. Sauber. 2000. Growing-finishing performance and carcass characteristics of pigs fed normal and genetically modified low-phytate corn. J. Anim. Sci. 78:1529–1536.

Steel R G and Torrie J H. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co. New York, NY, USA.

Tongoona P,. 2005. Development of tropical maize varieties with low phytic acid to improve the nutri¬tional status of poor communities in sub-Saharan Africa. [on-line]. Available: http://www.african¬crops.net/ abstracts2/ maize/tongoona.htm

Veum, T. L., D. R. Ledoux, V. Raboy, and D. S. Ertl. 2001. Low-phytic acid corn improves nutrient utilization for growing pigs. J. Anim. Sci. 79:2873–2880

O-18-2 Dietary fish oil regulates glucose and lipid metabolism genes via porcine peroxisome proliferator-activated receptor γ

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Objective

Peroxisome proliferator-activated receptor γ (PPAR γ) is a transcription factor that promotes adipocyte differentiation and insulin sensitivity. To activate PPARγ, a ligand is needed for binding its ligand-binding domain. It has been demonstrated that DHA treatment increases PPARγ-responsive gene expression in a cell model (Yu et al., 2008a). Plasma adiponectin is elevated when mice are fed fish oil (Neschen et al., 2006). Adiponectin is involved in insulin sensitivity and its gene is PPARγ-responsive. These results imply that the enhancement of insulin sensitivity by PUFA is mediated by activation of PPAR γ and PUFA may be endogenous ligands for PPARγ. However, most research demonstrating PPARγ ligand activity uses in vitro cell models. No direct evidence is available to indicate that PUFA is able to activate PPARγ in vivo. In previous studies, we demonstrated that porcine PPAR γ and PPAR δ regulate lipid metabolism (Yu et al., 2006; Yu et al., 2008a). The PUFA, DHA or its metabolites are able to activate porcine PPARγ (Yu et al., 2008b). Therefore, we generated muscle-specific expression of porcine PPAR γ in transgenic mice. These mice were fed diets containing potential PPARγ ligands to study the function of porcine PPARγ.

Methodology

The current experiment was designed to determine the potential for PUFA, particularly eicosapentaenoic acid and docosahexaenoic acid, to activate the function of porcine PPAR γ in vivo. Transgenic mice, expressing porcine PPARγ in skeletal muscle (Figure 1A) were generated by microinjection of the transgene into pronuclei of fertilized FVB/NJ mouse embryos. Age-(7-8 week old) and gender-matched wild-type (FVB/NJ) and PPAR γ transgenic mice were fed with high-saturated fat (13% beef tallow) or high-unsaturated fat (13% fish oil) diets for 4 months. Body weight, blood biochemical parameters, histochemistry and gene expression were analyzed at the end of the experiment. The treatment effects were analyzed using an ANOVA procedure to determine the main effects of PPARγ and diets. Statistical analysis of results was performed by a 2x4 factorial design (mouse genetic backgrounds and diet treatments) and Duncan's new multiple range test was used to evaluate differences among means (SAS Inst. Inc., Cary, NC). A significant difference indicates that the P value is not greater than 0.05.

Result

Successful integration and expression of the transgene was confirmed by the Southern blot and western blot (Figure 1B and 1C). After feeding experiments, dietary treatments had a significant effect on feed intake, body weight and fat pad weights (Figure 2). The mouse genetic backgrounds had no effect on feed intake, body weight and fat pad weights. Compared with standard diet-fed wild-type mice, PPARγ ligand-fed (rosiglitazone) mice had a significantly lower feed intake (Figure 2A), but dietary rosiglitazone had no significant effect on body weight (Figure 2B) and fat pad weight (Figure 2C). PPARγ transgenic mice ate less than wild-type mice when fed with the standard diet. Fish oil feeding significantly decreased feed intake and body and fat pad weight in wild-type mice. In PPAR γ transgenic mice, fish oil feeding did not significantly decrease feed intake, but significantly decreased body and fat pad weight. In wild-type mice, dietary beef tallow decreased feed intake but had no effect on body weight. The beef tallow fed mice had greater fat pad weight than the fish oil fed mice. Both diet treatments and genetic backgrounds had significant effects on plasma triacylglycerol, plasma free fatty acid and plasma glucose. Fish oil feeding reduced plasma triacylglycerol in both the wild-type and transgenic mice (Figure 3A). Compared with wild-type mice, PPAR γ transgenic mice had an overall low plasma free fatty acid concentration across all diets (Figure 3B). On each diet, PPAR γ transgenic mice had lower plasma glucose concentration than wild-type mice (Figure 3C). Feeding wild-type mice a PPARγ-ligand, rosiglitazone markedly reduced plasma glucose concentration and the effect was enhanced in PPAR γ transgenic mice (Figure 3C). Dietary fish oil treatments significantly increased of the plasma adiponectin concentration. The transgene did not affect the adiponectin secretion. Dietary rosiglitazone increased the plasma adiponectin concentration in both genotypes (Figure 3D).

Fish oil feeding had a moderate stimulatory effect on adiponectin secretion in wild-type mice and an even greater effect in PPAR γ transgenic mice (Figure 3D). Adipocyte size was not different between wild-type and transgenic mice when fed either the standard or the rosiglitazone diets (Figure 4). Fish oil feeding markedly decreased adipocyte size in wild-type mice and adipocyte size was further decreased in the PPAR γ transgenic mice fed fish oil. Compared to the standard diet, adipocyte size was increased in wild-type mice fed beef tallow. However, in PPAR γ transgenic mice, adipocyte size was the same when mice were fed either the standard or beef tallow diets. The mRNA level of the PPARγ-regulated adipogenic marker gene, lipoprotein lipase (LPL), was low in wild-type mice even in the presence of the PPARγ ligand, rosiglitazone, fish oil or beef tallow (Figure 5A). PPARγ transgenic mice had a high level of LPL mRNA when fed rosiglitazone. These results demonstrated that porcine PPAR γ is functional in transgenic mice and that an exogenous ligand greatly enhanced LPL expression in both genotypes. Fish oil significantly enhanced the expression of PPARγ target gene, LPL in both genotypes. Dietary beef tallow did not enhance expression of LPL in either genotype. The mRNA level for the fatty acid uptake gene, fatty acid translocase (FAT) was increased in wild-type mice when fed fish oil (Figure 5B). In PPAR γ transgenic mice, FAT mRNA levels were increased by dietary rosiglitazone and increased even more by fish oil. The mRNA level of sterol regulatory element-binding protein-1c (SREBP-1c), a nuclear transcription factor involved in lipogenesis, was unaltered in wild-type mice when fed any of the diets (Figure 5C). In PPARγ transgenic mice, the SREBP-1c mRNA levels were elevated by rosiglitazone feeding. The mRNA levels for the SREBP-1c target gene, fatty acid synthase (FAS), were not different in wild type mice fed any of the diets (Figure 5D). Feeding PPAR γ transgenic mice rosiglitazone or beef tallow increased FAS mRNA levels. In contrast, the mRNA level for FAS was decreased when fish oil was provided. The mRNA for the glucose uptake gene, glucose transporter-4 (GLUT-4) was increased in wild-type mice and even more so in PPAR γ transgenic mice fed rosiglitazone compared to the standard diet (Figure 5E). High fat feeding did not change the GLUT-4 mRNA levels in wild-type mice. In PPAR γ transgenic mice dietary fish oil, but not beef tallow caused the GLUT-4 mRNA levels to increase. The pattern for expression of mRNA for another glucose metabolism-related and PPARγ-regulated gene, adiponectin (ADN) was similar to that for GLUT-4 in both wild-type and PPAR γ transgenic mice (Figure 5F). The interaction of the two major factors on the expression of LPL, FAT, and GLUT4 mRNA was significant, indicating that the regulation of these genes by PPAR transgene depended on the dietary treatments.

Conclusion

We demonstrated that adipogenic genes and glucose metabolism genes were elevated in PPAR γ transgenic mice when fed fish oil. This transgenic mouse model provided direct evidence to demonstrate PUFA, especially EPA and DHA, regulate glucose homeostasis through interaction with PPAR γ .

KEYWORD：porcine, peroxisome proliferator-activated receptor γ , fish oil, gene, metabolism

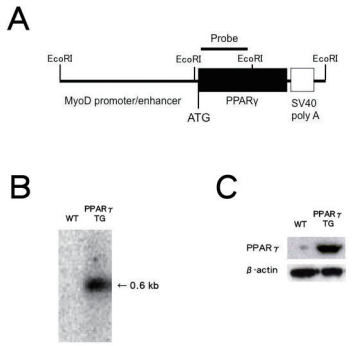

Fig1

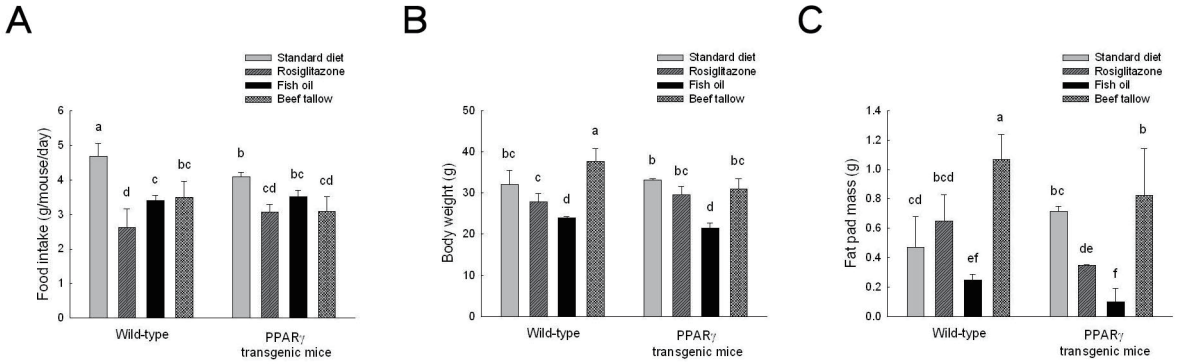

Fig2

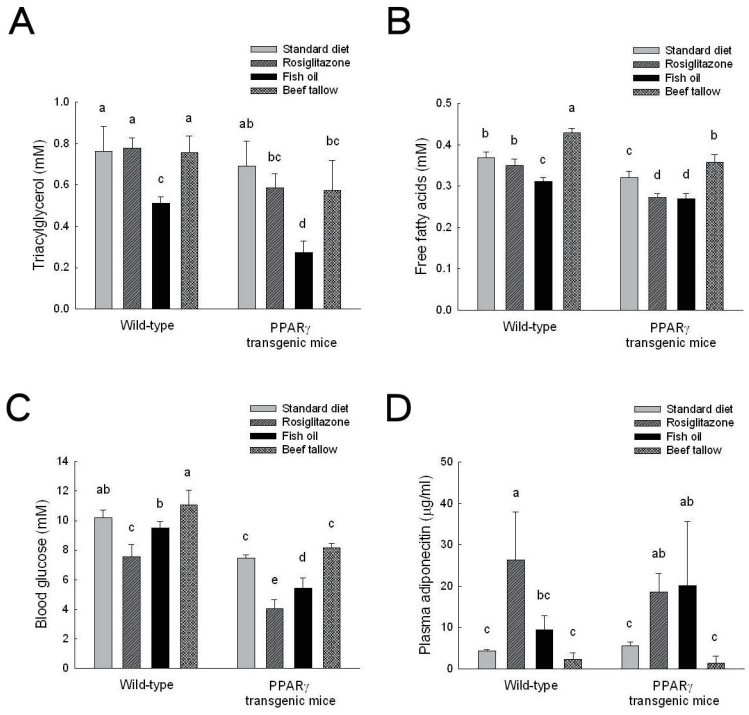

Fig3

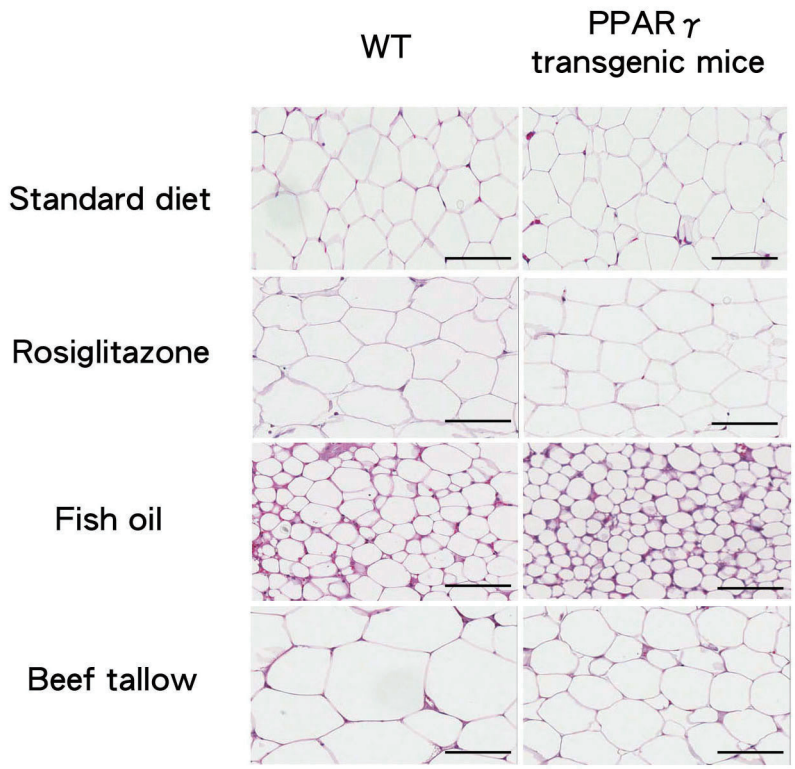
WT
PPARγ transgenic mice
Standard diet
Rosiglitazone
Fish oil
Beef tallow

Fig4

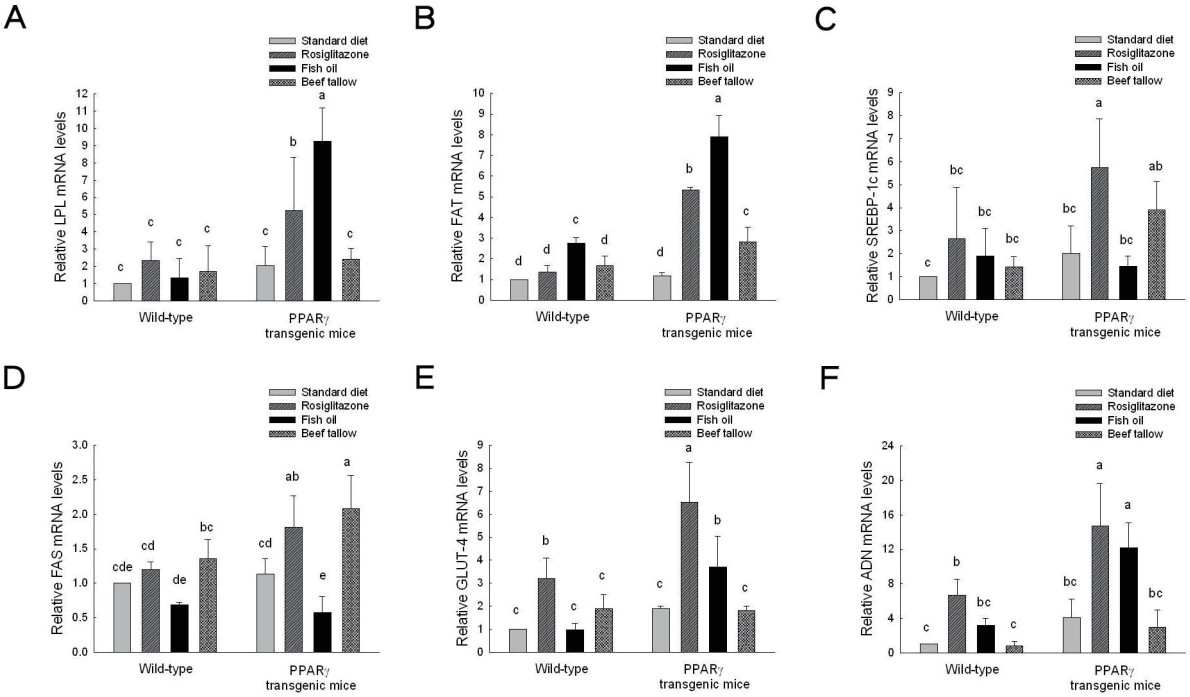
A
B
C
D
E
F
Standard diet
Rosiglitazone
Fish oil
Beef tallow
Relative LPL mRNA levels
Relative FAT mRNA levels
Relative SREBP-1c mRNA levels
Relative FAS mRNA levels
Relative GLUT-4 mRNA levels
Relative ADN mRNA levels
Wild-type
PPARγ transgenic mice

Fig5

REFERENCES

Neschen, S., K. Morino, J. C. Rossbacher, R. L. Pongratz, G. W. Cline, S. Sono, M. Gillum, and G. I. Shulman. 2006. Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor- γ -dependent mechanism in mice. Diabetes 55:924-928.

Yu, Y. H., B. H. Liu, H. J. Mersmann, and S. T. Ding. 2006. Porcine peroxisome proliferator-activated receptor γ induces transdifferentiation of myocytes into adipocytes. J. Anim. Sci. 84:2655-2665.

Yu, Y. H., S. C. Wu, W. T. K. Cheng, H. J. Mersmann, and S. T. Ding. 2008a. Ectopic expression of porcine peroxisome proliferator-activated receptor δ regulates adipogenesis in mouse myoblasts. J. Anim. Sci. 86:64-72.

Yu, Y. H., E. C. Lin, S. C. Wu, W. T. K. Cheng, H. J. Mersmann, P. H. Wang, and S. T. Ding. 2008b. Docosahexaenoic acid regulates adipogenesis genes in myoblasts via porcine peroxisome proliferator-activated receptor γ . J. Anim. Sci. 86:3385-3392.

O-18-3

Effect of feeding of noni fruit powder (Morinda citrifolia) based herbal supplement on production and meat quality in hybrid duck

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INTRODUCTION

The bioactive substance of medicinal plants have been widely studied as substitution of antibiotic for improvement of productivity, quality and immunity in poultry. Feeding diets containing bioactive substance may result in inhibition of the growth and colonization of enterophatogenic microbes in the digestive tract, thus contributing to improve balance of gut microflora (Harris *et al*., 2001).). Improvement of balance digestive microflora could enhance performances and health of poultry. Changes structure and morphology of villi might be increase performances production of poultry which play a substantial role in the digestion and absorption of nutrients in the gastrointestinal tract. An increases in the size and height of intestinal villi contributes to the nutrients absorption which leads to improvement on the productivity (Jamroz and Kamel, 2002). The health effects may be partly due to the development and responses of the host toward immune system against pathogenic and non-pathogenic antigens (Jang *et al.,* 2007).

The bioactive substance of medicinal plants have been widely studied to improve food quality such as fatty acid composition in the products. Adriani et al. (2014) reported that use of noni fruit juice by 0.3% and palm sugar (*Arenga pinnata*) around 0.2-0.4% through drinking water was able to decrease total cholesterol, LDL and triglyceride of blood serum of broiler chickens meat. Antioxidant activity on noni plant as feed additive was expected able to prevent free radical and reduce lipid oxidation on bird to produce better quality poultry products in terms of fatty acid profile. Noni fruit (*Morinda citrifolia*) is a medicinal plant containing a number of bioactive substances with good effects for health, likely phenolic compounds, organic acids and alkanoids (Chan-Blanco et al., 2006). This study was aimed to determine effect of noni powder on performance production, intestinal characteristics, lipids serum profile and fatty acid composition of hybrid duck meat.

MATERIAL AND METHODS

Environment of the study: The research was conducted in Animal Nutrition and Feed Science Department, Faculty of Animal Husbandry, Brawijaya University, Malang, Indonesia. Noni (*Morinda citrifolia* L.) fruit was purchased from the local market in Malang, East Java, Indonesia. Noni fruit powder was prepared by selecting ripe yellowish fruit, cutting into small slice, dried in the 50 oC oven for 48 h and then ground into powder.

Experimental design: A total of one hundred and fifty of 2-weeks-old hybrid ducks unsexing from local hatchery were used in a Complete Randomized Design (CRD), randomly assigned into five treatment groups of T0, T1, T2, T3 and T4 with thirty birds per treatment group replicated six times of five birds per replicate. The birds in the first group (T0) were given only basal diet without supplementation, while as other groups were supplemented with 1% noni fruit powder (T1), 2% noni fruit powder (T2), 3% noni fruit powder (T3) and basal diet supplemented with 0,3 g/kg feed tetracycline antibiotic (T4). Each experimental unit was 70x80x40 cm in size and it was used for 5 duck up to they reach 56 days of age. Feed and water were provided *ad libitum* throughout the experimental period. The basal experimental diet was formulated according to the standards of National Research Council (1994). The composition of basal diet used shown in Table 1 was formulated as an antibiotic-free diet.

Sample preparation and analysis: Duck were weighed at 14 d, 28 d, and 56 d of age. Feed offered and refused per replicate were daily recorded. Body weight gain, feed intake and feed conversion ratio (FCR) were calculated. No mortality was observed in any of the treatments during the whole experimental period. At 56 d of age, another 24 ducks in each treatment were slaughtered for carcass analysis. After feed deprivation overnight, two birds were randomly selected from each pen, individually weighed, carcasses were manually plucked and eviscerated. Abdominal fat (including mesenteric fat), breast meat (including the muscles *penctoralis major* and *penctoralis minor*) and leg meat (including thigh and drumstick) were manually excised and weighed. All weights, including eviscerated carcass, breast meat, leg meat and abdominal fat were expressed as a percentage relative to live weight before slaughter. The eviscerated carcass were weighed and frozen at -30^{o}C until further fatty acid composition assay.

Intestinal microflora: Approximately 1 g intestinal digesta samples were taken gently from 15 cm end of small intestine of 56 d old duck per experimental unit. The digesta samples were then placed in a sterile test tube, kept in container box containing ice tubes, then brought to the laboratory. In the laboratory, the samples were then immediately diluted with distilled water at a ratio of 1:1. The diluted samples were then plated onto selective agar, of which Mac-Conkey agar and de Man Rogosa Sharpe agar were used for growing *Escherichia coli* and lactic acid bacteria, respectively. All plates were incubated at 37oC for 24 h, before calculating the number of colony (Benson, 2002). Intestinal characteristics: Parameters recorded included villus height (from trip of villus to the crypt opening), villi surface area (μm^2/villi) calculated as [(villi basal width + villi apical width)/apical width] x villi height, and crypt depth (from the base of the crypt to the level of crypt opening) (Iji *et al.*, 2001). The ileum, defined as region Mackel's diverticulum to a point 10 cm proximal to the ileo-caecal junction, was dissected and the contents were collected. The collected samples were put into bottles immersed with 10% formalin solution. Then the samples were cut perpendicular to the longitudinal axis and embedded in paraffin wax. Transversal sections were cut (2-3µm), stained by Hematoxylin-Eosin and analyzed under a light microscope (Sugito *et al*., 2007).

Lipid profile of blood serum: Blood sample collection was taken from wing vena by 3 ml syringe and then was placed in the sample tube. Blood sample was placed in container box containing ice cubes and transported to the laboratory. Analysis of lipid profile of blood serum consisted of triglyceride level, total cholesterol, Low Density Lipoprotein (LDL), and High Density Lipoprotein (HDL) using CHOD-PAP method based on DSI (2005). Fatty acid identification using the chromatography gas. Testing procedure of meat fatty acid composition was according to AACC (1983).

Statistical analysis: All data were statistically analyzed by a one-way ANOVA as a completely randomized design. Any significant differences were further analyzed by Duncan's multiple-range test. All statements of significance were based on probability of 0.05.

RESULTS AND DISCUSSION

Production Performance: The effects of noni fruit powder on duck production, carcass characteristics, intestinal characteristics and microflora were summarized in Table 2. The supplementation of noni fruit powder did not significantly (P>0.05) effect live weight, FCR, eviscerated carcass, breast meat and leg meat, but abdomen fat was significantly decreased (P>0.05).

Intestinal microflora: The supplementation of noni fruit powder increased significantly (P<0.05) population of small intestinal lactic acid bacteria and *Escherichia coli* as compared with positive control (P4). However, comparative result toward negative control (P0) indicated that lactic acid bacteria was significantly decreased (P<0.05) as levels of noni extract increase, except for P2. The result for *Escherichia coli* count indicated that positive control group was significantly (P<0.05) able to kill more *Escherichia coli* than those of noni powder added groups. The antimicrobial compounds of noni fruit which was able to decrease *Escherichia coli* count in the intestine included phenolic compounds, organic acid and alkanoids. Singh (2012) reported that *Morinda citrifolia* has bioactive compounds which function as antimicrobial enable to suppress *Escherichia coli* count in small intestine.

Intestinal characteristic: The supplementation noni fruit powder effected significantly (P<0.05) on villus height, villus surface area and crypt depth of hybrid duck. Sunder *et al*. (2014) reported that broiler fed noni fruit juice, *Lactobacillus acidophilus* and noni fruit juice and *Lactobacillus acidophilus* mixture supplemented diets significantly improved the villi height and crypt depth. This improved intestinal characteristics of villi by the noni fruit powder justified the data on better feed efficiency and overall growth performance as reported by Singh *et al.* (2008). In this study, supplementation of 1% noni fruit powder showed the increase villi height (753.24 µm), villi surface area (72,00 mm) and crypt depth (170,27 µm) if compared negative control. The increase may be indicated that the development of intestinal characteristics is a function of ratio between pathogenic and non-pathogenic population.

Lipid profile of blood serum: Effects of noni fruit powder to lipid serum profile and composition of fatty acid of duck meat is presented in Table 3. Noni fruit meal used as feed additive had no significant effect (P<0.05) on triglyceride, total cholesterol, HDL and LDL. The supplementation of noni fruit meal up to 3% in diet was not able to decrease triglyceride level and total cholesterol of duck's blood serum. This shows that active compound in noni fruit meal is unable to reduce lipid oxidation and prevent cholesterol formation. This result is not in accordance with Adriani et al. (2014) who reported that there was a decrease of triglyceride and total cholesterol of broiler

chicken's blood serum by administrating noni fruit juice through the drinking water.

Composition of meat fatty acid: Supplementation of noni fruit powder in diet did not affect (P>0.05) overall fatty acid composition of duck meat, saturated fatty acid, and unsaturated fatty acid, except stearat (C18-0) which showed significant effect (P<0.05). This result shows that administration of noni fruit meal up to 2% in diet was unable to change composition of meat fatty acid. Active compound in the noni fruit meal was unable to prevent lipid oxidation, so that duck meat fatty acid composition was not different from the control. Unsaturated fatty acid is a compound that is susceptible to auto-oxidation. Lipid oxidation is the main cause of bird products damage. Antioxidant compound is a component that may retard and prevent lipid oxidation by free radical. Alloui et al. (2014) said that antioxidant in several herbal plants was able to protect lipid in diet from oxidation, so that the products from animal became more stable to the oxidation. Purba et al. (2010) said that composition of fatty acid of boiled duck meat supplemented by antioxidant showed higher total composition of unsaturated fatty acid than the total of saturated fatty acid.

CONCLUTION

In the present study, hybrid duck production did not increase with noni fruit supplementation in diet, but did decrease abdomen fat. The supplementation noni fruit powder can decrease of pathogenic bacteria and improve small intestinal morphology. The addition noni fruit powder up to 3% in diet has not been able to change lipid profile of blood serum and composition of fatty acid of hybrid duck.

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KEYWORD : Noni fruit, meat quality, intestinal characteristic, hybrid duck, phytobiotic

Table 1. Composition and nutrition content of basal diet used.

Feedstuffs	-- (%) --
Yellow corn	56.52
Soybean meal	11.68
Polished rice	20
Fish meal	10
Coconut oil	1.5
Premix**	0.3
Diet Nutrition Content, DM (%) *	
Metabolizable Energy (Kcal/kg)	3150
Crude protein (%)	18.28
Crude fat (%)	5.93
Crude fiber (%)	4.08

(*) Analysis result of Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, University of Brawijaya, Malang (2015)
(**) Premix per kg consists of: Vit A 12.000 IU; Vit D3 2.000 IU; Vit E 8 IU; Vit K3 2 mg; Vit B1 2 mg; Vit B2 5 mg; Vit B6 05 mg; Vit B12 0.012 mg; Vit C 25 mg; Ca-D-pantothenate 6 mg; Niacin 40 mg; Cholin Chloride 10 mg; Methionine 30 mg; Lysine 30 mg; Manganese 120 mg; Iron 20 mg; Iodine 0,2 mg; Zinc 100 mg; Cobalt 0.2 mg.

Table 2: Effect of level of noni fruit powder on duck performance, carcass characteristics intestinal characteristics and microflora

Variables	Treatments				
	T0	T1	T2	T3	T4
Performance production					
Live weight (g/bird)	1317±176	1302±116	1356±133	1261±110	1297±134
FCR	4.75	4.91	4.49	4.73	5.29
Carcass characteristics (% of live weight)					
Eviscerated carcass	68.49±1.09	67.06±2.08	67.93±3.00	67.41±2.40	68.58±2.45
Breast meat	11.26±1.88	10.64±2.13	11.35±1.98	10.38±2.15	12.13±2.47
Leg meat	12.12±1.44	12.25±2.08	11.07±1.27	11.44±2.32	11.37±2.20
Abdominal fat	1.74±0.61 ^{c}	1.40±0.88 ^{b}	1.40±0.79^{b}	1.07±0.87 ^{a}	1.00±0.59^{a}
Intestinal characteristics					
Villi height (µm)	571.74±145.8^{a}	753.24±161.1^{b}	514.32±37.3^{a}	588.10±33.7^{a}	568.74±78.8^{a}
Villi surface area (mm^{2})	52.40±27.74^{a}	72.00±23.17^{b}	29.72±12.23^{a}	33.08±11.17^{a}	36.54±20.40^{a}
Crypt depth (µm)	137.52±13.72^{a}	170.27±32.63^{b}	160.71±16.8^{b}	166.06±25.9^{b}	125.25±33.1^{a}
Intestinal microflora (log cfu/ml)					
Escherichia coli	5.444±0.106^{d}	4.444±0.106^{b}	4.772±0.042^{c}	4.882±0.029^{c}	3.710±0.153^{a}
Lactic acid bacteria	6.818±0.114^{c}	6.628±0.105^{bc}	6.832±0.092^{c}	6.524±0,123^{b}	5.654±0.201^{a}

T0: basal feed serving as negative control, T1: basal feed + 1 % noni fruit powder, T2: basal feed + 2 % noni fruit powder, T3: basal feed + 3 % noni fruit powder, T4: basal feed supplemented with tetracycline antibiotic (300mg/kg feed) as positive control.

^{ab}Means with different in treatment are significantly different (P<0.05).

Table 3: Effect of level of noni fruit powder on lipid serum profile and composition of fatty acid of hybrid duck meat

Variables	Treatments	
	Control	Noni fruit powder (2 %)
Lipid profile of blood serum (mg/dL)		
Cholesterol	203.33±43.82	186.33±65.06
Triglyseride	155.33±43.82	183.00±11.45
HDL	111.40±43.82	92.77±28.76
LDL	60.87±43.82	56.97±23.69
Composition of fatty acid (mg/g)		
Butyrate (C4)	-	0.01±0.00
Caproate (C6)	-	0.01±0.00
Caprilate (C8)	-	0.01±0.00
Caprate (10)	-	0.01±0.00
Laurate (C12)	0.04±0.00	0.02±0.00
Myristate (C14)	0.09±0.01	0.11±0.02
Palmitate (C16-0)	3.85±0.41	4.60±0.57
Stearate (C18-0)	1.32±0.12[a]	1.89±0.17[b]
Total saturated fatty acid	5.30±0.49	6.65±0.66
Oleate (C18-1)	7.04±0.83	7.95±0.94
Linoletae (C18-2)	2.32±0.28	2.85±0.33
Linoleate (C18-3	0.09±0.01	0.10±0.02
Total unsaturated fatty acid	9.45±1.15	10.90±1.76

[ab]Means with different in treatment are significantly different (P<0.05) - (undetected)

REFERENCES

[AACC] American Association of Cereal Chemist. 1983. Approved methods of the American Association of Cereal Chemist. 8th ed. USA: American Association of Cereal Chemist.

Adriani L, Widjastuti T, Sujana E, Mushawwir A, Yulianti A. 2014. Effect of Noni (*Morinda clitrifolia* L) juice and Palm sugar (*Arenga pinnata*) supplementation in drinking water on lipid profile broiler chicken. Pak J Nutr. 14:531-534.

Alloui MN, Agabou A, Alloui N. 2014. Application of herb and phytogenic feed additive in poultry production- a review. Glob J Anim Sci Res. 2:234-243.

Benson, H. J. 2002. Microbiological Applications 8th edn. Bacterial Population Counts. Fig. 21-1. McGraw Hill, New York.

Chan-Blanco, Y., F. Vaillant, A. M. Perez, M. Reynes, J. M. Brillouet and P. Brat.2006. The noni fruit: A review of Agriculture Research, Nutritional and Teurapeutic Properties. J. Food Compos. Anal. 19: 645-654.

[DSI] Diagnostic System International. 2005. Cholesterol FS.

Harris, J. C., S. Cottrell, S. Plummer and D. Lioyd. 2001. Antimicrobial properties of *Allium sativum* (garlic). Appl. Microb. Biotech. 57: 282-286.

Iji, P. A., R. J. Hughes, M. Choct and D. R. Tivey. 2001. Intestinal structure and function of broiler chickens on wheat- based diets supplemented with a microbial enzyme. Asian-Aust. J. Anim. Sci. 14: 54-60.

Jamroz, D. and C. Kamel. 2002. Plant extracts enhance broiler performance. J. Anim. Sci. 80: 41-46.

Jang, I. S., Y. H. Ko, S. Y. Kahn and C. Y. Lee 2007. Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chikens. Anim. Feed Sci. Technol. 134: 304-315.

National Research Council. 1994. Nutrien Requrement of Poultry. 9th Revision edn. National Academi Press, Washington DC.

Purba M, Laconi EB, Keraten P, Wijaya CH, PS Hardjosworo. 2010. Kualitas sensori dan komposisi asam lemak daging itik lokal jantan dengan suplementasi santoquin, vitamin E dan C dalam ransum. JITV. 15:47-55

Singh, D. R., J. Sunder and R. C. Srivastava 2008. Peptide and mineral profile of *Morinda citrifolia* fruits and leaves. Int. J. Noni Res. 2: 72-78.

Singh, D. R. 2012. *Morinda citrifolia* L: A review of the scientific validation for its nutritional and therapeutic properties. J. Diabetes and Endocrinology 3: 77-91.

Sugito, W. Manalu, D. A. Astuti, E. Handharyati, dan Chairul. 2007. Morfometrick usus dan performa ayam broiler yang diberi cekaman panas dan ekstrak n-Heksan kulit batang taloh (*Salix tetrasperma* Roxb). Media Peternakan 30: 198-206.

Sunder, J., S. Tamilvana, P. Natesan, A. Kundu and M. S. Kundu. 2014. Effect of *Morinda citrifolia* fruit juice and *Lactobacillus acidophilus* on broiler duodenal morphology. Adv. Anim. Vet. Sci. 2: 28-30.

O-18-4

Effects of Supplemental β-Mannanase to Provide Substrates for Binding to Aflatoxin in Feed on Growth and Health of Pigs

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OBJECTIVE

β-mannanase has been shown to reduce digesta viscosity in the gut of pigs by hydrolyzing mannan-polysaccharides to mannan-oligosaccharides which can potentially be used to detoxify aflatoxins in the feed.

METHODOLOGY

Two studies were conducted to investigate the use of β-mannanase to provide mannan-oligosaccharides to detoxify aflatoxin in feed for pigs to help their growth and health. First study was in vitro digestion of guar gum as a source of mannans with β-mannanase (10%). Digesta were freeze dried, ground, and used for in vitro binding test to aflatoxin in comparison to a commercially available mycotoxin binder as positive control. Second study used 135 pigs (6 wk old) in 3 treatments: PC (no aflatoxin), NC (aflatoxin, 150 μg/kg), and MAN (NC + 0.2% β-mannanase). There were 15 pens/treatment and 3 pigs/pen. Pigs were fed experimental diets for 42 days measuring growth performance, morbidity, and blood cell counts. Data were analyzed using Proc MIXED of SAS.

RESULT AND CONCLUSION

Digesta from hydrolysis of guar gum by β-mannanase had 16.1% efficiency in binding to aflatoxin (a positive control had 91.2% binding efficiency). Pigs fed NC had decreased (P<0.05) weight gain, tended to have reduced (P=0.090) feed intake, and tended to have decreased (P=0.052) feed efficiency compared with pigs fed PC. Growth performance of pigs fed MAN was not different from PC nor from NC. Pigs fed NC had increased (P<0.05) monocyte count, immunoglobulin G, and immunoglobulin M than pigs fed NC. Pigs fed MAN had a reduced (P<0.05) immunoglobulin G and tended to have a decreased (P=0.055) monocyte count than pigs fed NC. In conclusion, dietary supplementation of β-mannanase can hydrolyze mannans and galactomannans in feed providing substrates for binding to aflatoxin contaminated in feed which in turn help pigs to partially overcome negative effects of aflatoxin in diets on growth and health of pigs.

KEYWORD : Aflatoxin, β-mannanase, Enzyme, Pig

O-18-5

Efficacy of Supplemental Bacteriophages on Growth and Gut Health of Pigs

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OBJECTIVE

This study was conducted to determine the effects of supplemental bacteriophages (BP) targeting 4 pathogenic bacteria including Salmonella and e Coli on growth performance and gut health of nursery pigs with or without antimicrobial growth promoter (AGP).

METHODOLOGY

120 pigs at 6 wk of age were purchased from a commercial farm and allotted to 4 treatments in a 2 x 2 factorial arrangement with AGP and BP as 2 factors based on a randomized completed block design (10 pens/treatment and 3 pigs/pen) and fed experimental diets for 47 days. Growth performance, morbidity, systemic and gut health parameters were measured. Data were analyzed using Procedure Mixed of SAS.

RESULT AND CONCLUSION

AGP increased ($P<0.05$) and BP tended to increase ($P=0.055$) weight gain (WG) of pigs whereas feed intake was not affected during 47-d. Feed efficiency (G:F) was improved ($P<0.05$) by AGP without BP effects. AGP tended to decrease ($P=0.058$) monocyte counts without BP effects. AGP tended to decrease relative weight of pancreas to body weight ($P=0.089$), jejunal TNF-a concentration ($P=0.056$), and colon thickness ($P=0.095$) without BP effects. However, BP decreased ($P<0.05$) serum TNF-a without AGP effects. Concentrations of immunoglobulin G, malondialdehyde, and 8-OH-dG both in serum and junual mucosa as well as jejunal morphology were not affected by 2 factors. For 6-13 week old pigs obtained from commercial farm with a history of typical bacterial infections, supplemental bacteriophage successfully enhanced growth of pigs similar to the benefits from the use of AGP, whereas AGP further enhanced feed efficiency. All pigs used in this study were healthy without morbidity based on hematological evaluation and serum assays. The beneficial effects of bacteriophage were not be affected by AGP. Use of bacteriophage did not influenced immune response of pigs, gut morphology, health status of vital organs.

KEYWORD : Bacteriophage, Growth performance, Gut health, Pigs, Antimicrobial growth promoter

O-18-7

THE EFFECTS OF GROUND RAW ALEURITES MOLUCCANA L. (Willd) KERNEL ON PERFORMANCE OF LAYING HENS

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Aleurites moluccana, from the Euphorbieaceae family, is a common spice used in cooking in the Asian region, and is known to contain high proportions of unsaturated fatty acids such as omega-3 (alpha linolenic acid), omega-6 (linoleic acid) and omega-9 (oleic acid). It can be used in poultry diets as an alternative source of protein and energy to improve growth performance and egg quality in laying hens. The objective of this study is to determine the effect of A. *moluccana* kernels on egg production and quality in laying hens. One hundred Isa Brown layer hens, 20 weeks of age, were randomly allocated into four treatment groups, each replicated five times, consisting of 10 birds per replicate. From 20 to 30 weeks of age, the birds were fed a basal layer diet supplemented with either 0, 1, 3 or 5 % ground raw A. moluccana kernel (AMK). All birds were fed *ad libitum*. Drinking water was made available at all times. Data on feed intake and egg production were measured on a weekly basis. Samples of eggs were analysed for egg mass, shell thickness and saponin contents. All the data were subjected to one way ANOVA. Dietary supplementation of ground raw AMK significantly decreased feed intake, percent egg production, egg mass, shell thickness and increased FCR per dozen of eggs. The nutrient content of eggs were not influenced by the supplementation except for the saponin content which increased with increasing AMK. The saponin contents of eggs in treated groups were significantly higher ($p<0.05$) than those in control groups. It can be concluded that supplementation of layer diets with raw AMK even at 1% can affect egg production in laying hens, possibly due to their saponin contents.

INTRODUCTION

Modification of poultry diets by supplementing products from herbs and spices such as black cumin, anise and turmeric has been shown to improve growth performance, nutrient digestibility, reduce the amount of abdominal fat in broilers, and in layers improve fertility and immune system, improved egg production and quality (Guler, 2006; Hashemi *et al*., 2012). *Aleurites moluccana sp.* is an another crop of the *Euphorbieaceae* family, the fruit of which is a common spice in India and the Asian region, known to contain high proportions of unsaturated fatty acids such as omega-3 (alpha linolenic acid), omega-6 (linoleic acid) and omega-9 (oleic acid) (Martin *et al*., 2010). It can be added in the diet as an alternative of protein and energy to improve growth performance and egg quality in laying hens. The objective of this experiment was to determine the effects of supplementation of ground raw A. *moluccana* kernel at different levels in laying hens diet on growth performance and egg nutrient content.

MATERIALS AND METHODS

One hundred laying hens, Isa Brown, 20 weeks of age, purchased from a local layer farm, weighed, wing-banded and allocated randomly in individual battery cages with stainless steel wire floor. The birds were allocated into four treatment groups, each replicated five times, consisting of 10 birds in each replicate, in a Completely Randomized Design (CRD). From 20 to 30 weeks of age, the birds were fed either one of the four dietary treatments, namely: (T1) Basal layer diet containing no supplement (control); (T2) Basal diet supplemented with 1% ground raw A. *moluccana* kernel; (T3) Basal diet supplemented with 3 % ground raw A. *moluccana* kernel; and (T4) Basal diet supplemented with 5 % ground raw A. *moluccana* kernel. All birds were fed *ad libitum* and given free choice drinking water. Eggs were collected everyday. All the data were analyzed prior to ANOVA using SAS 9.2.

RESULTS AND DISCUSSION

Supplementing of 1 to 5% of ground raw A. *moluccana* kernel in the diets significantly affected the performance in laying hens with decreasing feed intake, egg production, egg mass, shell thickness and increased FCR per dozen of eggs (Table 1). However, at birds fed diet T2 (1%) had higher body weights (1.86 ± 43.3 kg) than birds fed control diet (1.79±18.1 kg). Overall, increasing levels of A. *moluccana* supplementation decreased body weight gains, feed intakes, egg production and egg mass. The nutrient content of eggs were not influenced by the supplementation except for the saponin contents which were significantly higher in eggs from birds fed the supplemented diets

(Table 2). The saponin contents of eggs increased with increasing A. *moluccana* supplementation (12.65±0.439%, 12.40±0.262% and 11.57±0.452%, for treatments T4, T3 and T2, respectively. The presence of saponin in A. *moluccana* kernel was thought to be the main cause of the reduction in egg production and egg quality in laying hens. This was in agreement with Jenkins and Atwal (1994) who reported that Quillaja saponin fed at 0.9% in feed reduced weight gain in chickens.

CONCLUSION

The supplementation with *A. Moluccana* kernel in laying hens diets adversely affected the production and egg quality in laying hens. Saponins in untreated seeds of A. *moluccana* may contribute to the poor performance of laying hens.

KEYWORD : Aleurites moluccana, laying hens, egg quality, saponin

Table 1. Performance of laying hens fed different levels of ground raw A. *moluccana* kernel at 30 weeks of age (Mean+SE)

Parameters	Dietary Treatments				P Sig
	T1 (0%)	T2 (1%)	T3 (3%)	T4 (5%)	
Body weight (kg)	1.79±18.1^{ab}	1.86±43.3^{a}	1.70±15.9^{bc}	1.69±37.4^{c}	*
Feed intake (g/b/d)	123.33±3.2^{a}	108.33±2.3^{b}	102.00±1.7^{bc}	95.67±2.9^{c}	*
Egg production (%)	93.3±2.50^{a}	65.5±4.30^{b}	56.6±5.03^{bc}	51.2±4.27^{c}	*
Egg mass (g)	54.306±3.46^{a}	36.759±2.02^{b}	33.616±2.46^{b}	28.263±3.10^{b}	*
FCR per dozen	1.59±0.064^{b}	2.01±0.157^{ab}	2.20±0.229^{a}	2.28±0.215^{a}	*
Shell thickness (mm)	0.30±0.015^{a}	0.29±0.006^{b}	0.32±0.005^{b}	0.30±0.007^{b}	*
Liver weight (%)	29.380±2.95^{b}	38.720±2.17^{a}	32.200±2.35^{ab}	33.320±0.70^{ab}	*

^{abc}Means within a row with different superscripts differ significantly (P<0.05)
*:Significant

Table 2. Nutrient content of eggs of laying hens fed different levels of ground raw A. *moluccana* kernel at 30 weeks of age (Mean+SE)

Parameters	Dietary Treatments				P
	T1 (0%)	T2 (1%)	T3 (3%)	T4 (5%)	
Moisture (%)	4.98±0.676	4.87±0.031	3.39±1.002	3.36±0.599	NS
Dry Matter (%)	96.64±0.676	96.61±0.031	95.13±0.002	95.03±0.599	NS
Crude Protein (%)	49.17±0.603	49.67±0.694	49.13±1.176	50.30±0.647	NS
Crude Fat (%)	33.39±0.087	34.16±0.712	33.69±0.514	33.22±0.472	NS
Saponin (%)	9.92±0.315^{a}	11.57±0.452^{b}	12.40±0.262^{b}	12.65±0.439^{b}	*

^{ab}Means wthin a row with different superscripts differ significantly (P<0.05)
NS: Non significant; *: Significant

REFERENCES

Guler, T., Dalkilic, B., Ertas, O. N. and Ciftci, M. 2006. The effect of dietary black cumin seeds (Nigella *sativa* L.) on the performance of broilers. Asian-Australian Journal of Animal Science. 19: 425 - 430.

Hashemi, S. R., Zulkifli, I., Davoodi, H., Zunita, Z. and Ebrahimi, M. 2012. Growth performance, intestinal microflora, plasma fatty acid profile in broiler chickens fed herbal plant (Euphorbia *hirta*) and mix of acidifiers. Animal Feed Science and Technology. 178: 167 - 174.

Jenkins, K. J. and Atwal, A. S. 1994. Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick. *Journal of Nutrition and Biochemistry*. 5(3): 134 - 137.

Martin, C., Moure, A., Martin, G., Carillo, E., Dominguez, H. and Parajo, J. C. 2010. Fractical characterization of jatropha, neem, moringa, trisperma, castor and candlenut seeds as potential feedstocks for biodiesel production in Cuba. Biomass and Bioenergy. 34: 533 - 538.

O-18-8

HAEMATOLOGICAL AND PERFORMANCE PARAMETERS OF BROILER SUPPLEMENTED BY Tinospora crispa L. Miers EXTRACT

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INTRODUCTION

Broiler meat was one of any livestock product which has high nutritive value, expecialy protein. Broiler excellence was rapid growing and has high meat yield productivity. The rapid growing of broiler should be supported by enough nutrition and good animal health management. The chemical antibiotic in ration was one of another ways that broiler producers use to maximize broiler quality and productivity. Besides that, the function of antibiotic was as immune agent and antistress on broiler. The use of chemical antibiotics have high side effect on hormonal system demage and immunity system (Cao *et al.* 2004).

As the alternative to overcome that's effect was the using of local material with potential as feed additive such as Brotowali (*Tinospora crispa L. Miers*). Brotowali known as traditional herbage medicine with abundant chemical compound that's effective to be human medicine for clinical diseases such as diabetic, malaria, and hepatitis. Brotowali also containing anti microbial compound *i.e.* berberine, columbine, alkaloid, soft resin, starch, glycoside, picroretocid, bitter substance, tynocrisposid, palmatin, and caoculin (Lantern and Kresnadi, 2003). The usage of Brotowali to be expected could increase production and health status performance of broiler.

MATERIALS METHODE

The research was conducted for 35 day with 200 broilers DOC, basal feed, and Brotowali extract. Research design was complete randomized design with 4 treatments and 5 replications, with 10 birds on each replication. The treatments were Brotowali extract adding on broiler feed with levels as T0: 0%, T1: 0.25%, T2: 0.50%, T3: 0.75%, and T4: 1.00%. Basal feed ration was use formulation according Rahadi *et al*. (2011) as follow; 35% commercial concentrate CAB® by Charoend Phokphan, 45% corn yellow, 13% rice bran, and 7% sago waste. Feed and water were *ad libitum* added for this research. Brotowali extract was obtained from fresh stem according Rahardi *et al*. (2015).

The research parameters in this study were feed consumption (FC), organic matter digestibility (OMD), nitrogen retention (NR), average daily gain (ADG), feed conversion ratio (FCR), erythrocyte count (EC), leukocyte count (LC), and hemoglobin (Hb). Obtained data were analyzed using Anova, and the significant different between treatment were analyzed DMRT post hoc (Steel and Torrie, 1991).

RESULT AND DISCUSSION

Brotowali extract adding on feed effect on production performance of broiler shown in Table 1.

The result showed that BE usage on level 0 and 1% didn't give significant effect ($p>.05$) on broiler FC. This result indicated that using BE until 1% level didn't affected on feed palatability and broiler feed appetite. Unaffected of BE adding on feed consumption probable of broiler energy needed has been fulfilled by their feed, even as daily maintenance or productivity/ growing. Thermostatic theory explain that animal could be increase or decrease their consumption based on their energy needed, and beside that, Brotowali contain tannin compound which was soluble on digestion track, so its caused the feed consumption decreasing (Widodo, 2005; Abun 2012). Even though the feed consumption decreasing in this research was not more than T0, or it's mean there was no significant decreasing. .

Even BE adding didn't affected on broiler FC, it's give significant effect ($p<.05$) on organic matter digestibility (OMD). There occur decreasing of OMD in this research on T0 until T4. The possibility of decreasing OMD was caused by tannin and saponin compound on BE which bounded the feed nutrient, that caused undigested and throughout on excreta. The highest average of OMD was 91.21% (T0) if compare with T4 (85.12%) showed that BE could be affect on nutrient absorption and body metabolism system.

The nitrogen retention (NR) have significant affected ($p<.05$) by BE adding on broiler feed. The average NR was

increasing by the increase of BE adding on feed. Increasing of feed NR on broiler body could be caused by feed consumption, protein consumption, and protein quality (Abun, 2008). The highest NR value was 73.50% (T4) compare the lowest one was T0 (63.75%), and it means that BE were affect on nitrogen absorption process on body system, broiler can be efficiently on nitrogen use. Based on Wahyu (1997), the retention of protein on broiler was 67%, and it was used as tissue growing per day, feather production, and replace the lost endogenous. These explain the broiler's protein usage efficiency.

The ADG parameter was not affected (p>.05) by BE adding on broiler's feed. These indicated that BE adding until 1% level still cannot increase broiler ADG. Tannin and saponin compound on Brotowali plant allegedly as main factor caused the decreasing of ADG. Saponin compound was frothy solute and it was glycoside tryterpenoid which has cell cyto toxic, mainly on developed cell. The high of tannin compound has negative effect on feed nutrition. Tannin could be bound feed protein, carbohydrate, specific amino acid, and phosphorus (Kumar *et al*.2005). The broiler ADG on this research were around 39.45 until 42.47 gr/bird. Based on NRC (1994) the normal broiler ADG was 38.4 - 45.02 gr/ bird.

Effect of BE adding on broiler hematological was shown in Table 2.

The result showed that BE adding on all levels until 1% didn't give significant effect (p.>.05) on broiler erythrocyte count. These indicated that this treatments were unaffected erythrocyte production and still on normal range of its count. Erythrocyte count on T1 until t4 tended to decrease, even on T3 the erythrocyte count have little increasing. Factors that affected on erythrocyte count were; age, species, feed consumption, and the availability of erythrocyte production material (Schalm *et al*., 1986). Based on Mangkoewidjojo and Smith (1988), erythrocyte count of normal broiler was 2.0-3.2 $x10^6/mm^3$. Compare with the research result was around 2.21-2.99 $x10^6/mm^3$, and that mean BE adding until 1% level on broiler feed still produce normal erythrocyte count.

Leukocyte count in this research was be significant affected (p<.01) by BE on broiler feed. The adding of BE on 0.25 - 1% on feed tended decrease of broiler leukocyte count if compare with control (0% BE). Setiadi and Sudarman (2005) reported that stressed broiler due to its cage density could be increase leukocyte count, and its decrease while given by herbalists anti stress. Susanti (2010) also reported that heat stress have huge effect on total leukocyte count especially on lymphocyte. Heterophile/ Lymphocyte Ratio (H/L) can be used as poultry stress standard cause on environment effect (Post *et al*. 2003).

Broiler's leukocyte count in this research tended to decrease but still on normal range. The leukocyte count on normal broiler was 12 - 30 $x10^3/mm^3$ (Jain, 1986; Adipratama, 2009), and the leukocyte count on this research was 13.48 - 23.91 $x10^3/mm^3$.

The leukocyte count on T1 until T4 was suspected not as environment stress, but tended on tannin and berberin active compound of BE. Tannin suspected can increase cells body immune differentiation by protected the receptor cell from pathogen (Nurhayati *et al*., 2006), this make increasing of leukocyte count due to phagositic reaction (Anderson and Siwicki, 1993) and continue by decreasing of leukocyte count if the pathogen was removed. The berberine active compound could be act as antimicrobial which protect the agent from negative microbes attack from body inside or outside. Pathogens microorganism could be pressed and its make leukocyte ability to be lower and leukocyte count on normal point, it's indicated that the broiler was in normal physiology status or on health condition.

The BE adding on broiler feed have significant effect (p<.05) on blood hemoglobin content. This indicated that BE adding on broiler feed was affected hemoglobin content on broiler erythrocyte. The result showed hemoglobin content of T3 was significant higher if compare with T0, T1, and T4, but it's not significant different with T2. Meanwhile hemoglobin content of T0, T1, T2 and T4 was not showing differences. That explained that BE adding on 0.75% level was the optimum level to produce hemoglobin. Based on Jain (1987) the normal chicken hemoglobin was 7.0-13.0 gr/dl, and the hemoglobin content in this research was around 7.27-9.55 gr/dl, and these means that BE adding on broiler feed up to 1% level still have normal range of hemoglobin content. The BE annding as phytobiotics on broiler feed can be recommended to apply and can be use as natural antibiotics to replace the usage of chemical antibiotics.

CONCLUSION

Adding 0.50% extract adding was give best effect on haemoglobin count (9.55 g mm^{-3}), meanwhile, erythrocyte didn't affected. *Tinospora crispa L. Miers* extract decrease OM digestibility on 0.75% and 1.00% levels. The N retention was increasing respectively on increase of extract levels. *Tinospora crispa L. Miers* extract at 0.50% levels was recommended to be natural feed additive on broiler.

KEYWORD : Phytobiotic, Haematological, Performance, Broiler

Tabel 1. Effect Brotowali Extract on Production Performance

Treatment	FC (g/bird/day)^{ns}	OMD (%)	NR (%)	ADG (g/bird/day)^{ns}	FCR
T0	103.75±0.20	91.21± 1.85^{b}	63.75±2.06^{a}	42.08±1.60	2.47±0.10
T1	104.50±1.67	91.85±1.40^{b}	67.25±3.86^{ab}	41.00±4.48	2.57±0.29
T2	104.28±2.40	90.23±2.02^{b}	71.25±7.40^{b}	42.47±2.92	2.46±0.18
T3	105.88±0.69	86.05±0.30^{a}	71.50±1.20^{b}	40.78±2.52	2.60±0.18
T4	105.12±0.80	85.12±0.55^{a}	73.50±2.40^{b}	39.45± 2.09	2.66±0.14

Number followed by different alphabetic in same variable's row indicate significant different (p<.05), ns: non significant (p>.05). T0: Basal Feed (BF) + 0% Brotowali Extract (BE), T1: BF + 0.25% BE, T2: BF + BE 0.50%, T3: BF + BE 0.75%, and T4: BF + BE 1.00%.

Tabel 2. Broiler Hematological Profile

Treatment	Erytrocyte Count (10^6/mm^3)^{ns}	Leucoyite Count (10^3/mm^3)	Haemoglobin (gr/dl)
T0	2.94 ± 0,65	23.91 ± 3.8^{b}	7,43±0,50^{a}
T1	2.99 ± 0,87	15.91 ± 2.9^{a}	8,15±0,77^{a}
T2	2.70 ± 0,46	13.48 ± 2.3^{a}	8,40±0,42^{ab}
T3	2.79 ± 0,55	14.37 ± 2.7^{a}	9,55±0,97^{b}
T4	2.21 ± 0,23	14.25±3.33^{a}	7,27±1,22^{a}

Number followed by different alphabetic in same variable's row indicate significant different (p<.05), ns: non significant (p>.05). T0: Basal Feed (BF) + 0% Brotowali Extract (BE), T1: BF + 0.25% BE, T2: BF + BE 0.50%, T3: BF + BE 0.75%, and T4: BF + BE 1.00%.

REFERENCES

Abun, Wayuni, and Mayasari. 2006. Pengaruh Penggunaan Ekstrak Kulit Jengkol (*Pithecellobiumjiringa (Jack) Prain*) dalam Ransum terhadap Nilai Hematologi Ayam Broiler. Universitas Padjadjaran. Bandung.

Adipratama, D.N. 2009. Pengaruh Ekstrak Etanol Temulawak (*curcuma xanthorhizza roxb*.) Terhadap Jumlah Total dan Diferensiasi Leukosit pada Ayam Petelur (*Gallus Gallus*) Strain Isa Brown. Skripsi Sarjana, Fakultas Kedokteran Hewan, Institut Pertanian Bogor. Bogor.

Anderson, D.P dan A. Siwicki. 1993. Basic Hematology and Serology for Fish Health Programs. Second Symposium on Disease in Asia Aquaculture "Aquatic Animal Health and Environment". Asia Fisheries Society.

Cao, J., K. Li, X. Lu and Y. Zhao. 2004. Effects of florfenical and chromium (III) on humoral immune response in chicks. Asian-Aust. J. Anim. Sci. 17: 366-370.

Jain, N.C. 1986. Schalm's Veteriner Hematology. 4th Ed. Philadelphia. Lea & Febiger.

Kumar, V, A. V. Elangovan, and A. B. Mandal. 2005. Utilization of reconstitued high-tanin sorghum in the diets of broiler chicken. J. Anim. Sci. 18 (4): 538-544.

Lentera, T dan Kresnadi, B. 2003. Khasiat dan Manfaat Brotowali Si Pahit yang Menyembuhkan. PT. AgromediaPustaka. Jakarta.

Mangkoewidjojo, S. dan J. B. Smith. 1988. Pemeliharaa, Pembiakan dan Penggunaan Hewan Percobaan di Daerah Tropis. Universitas Indonesia. Jakarta.

NRC. (ed.), 1994. Nutrient reguirements of poultry. 9th ed. National Acedemy of Science. Wahington, DC.

Nurhayati, S., Kuswardani, Y dan Hadiroseyani, S. 2006. Pengaruh Pemberian Resin Lebah terhadap Gambaran Darah Ikan Koki (*Carassius auratus*) yang terinfeksi bakteri Aeromonas hydropilla. *Jurnal Akuakultur Indonesia, 5 (2): 191-199.*

Post, J., J.M.J. Rebel and A.A.H.M. Ter Huurne. 2003. Automated Blood Cell Count : A Sensitive and Reliable Method to Study Corticosrterone-Related Stress in Broiler. *Journal Poultry Science. 82 : 591-595*.

Rahadi, S., L.M. I. Qalbi, K. Mualimin, Muchlis. 2011. Implementasi Ampas Sagu Fermentasi dalam Ransum Ayam Broiler. in: *Prosiding Seminar Nasional dan Kongres I ISAA*. Semarang, 11-12 Oktober 2011.

Rahadi, S., A. Indi, Rahman, Y. Yadi, W. Kurniawan, and Masur. 2015. The supplementation effect of turmeric (Curcuma domestica Vahl.) and Brotowali (Tinospora crispa L. Miers) Extract on broiler meat quality. In Proceeding of International Seminar Imroving Tropical Animal Production for Food Security. Unhalu Press. pp 185-188.

Schalm, O.W., G.J. Jain and E.J. Carrol. 1986. Veterinary Hematologi. 3th Ed. Lea and Febriger. Philadelphia.

Setiadi, D dan Sudarman A. 2005. Ekstrak Daun Beluntas (*Pluchea indica Less*.) sebagai Obat Antistres pada Ayam Broiler. *Media Peternakan (46-51).*

Steel, R.G.D. and Torrie, J.H. 1991. Prinsip dan prosedur statistic suatu pendekatan biometrik. Terjemahan oleh Sumantri, B. Gramedia Pustaka Utama. Jakarta.

Susanti, A.R. 2010. Interaksi antara Pemberian Jintan Hitam (*Nigella sativa*) dengan Paparan Heat Stress terhadap Jumlah dan Hitung Jenis Leukosit Ayam Pedaging. *Artikel Ilmiah*. Fakultas Kedokteran Hewan. Universitas Airlangga. Surabaya.

Wahju, J., 1997. Ilmu Nutrisi Unggas. Cetakan keempat. Universitas Gadjah Mada Press. Yogyakarta.

Widodo, W., 2005. Tanaman Beracun Dalam Kehidupan Ternak Edisi Pertama. Universitas Muhammadiyah, Malang.

O-18-10 SULFADIAZINEE RESIDUE IN THE LIVER, BREAST MEAT, AND PERFORMANCE OF BROILER SUPPLEMENTED BY TURMERIC (Curcuma domestica Valet) MEAL

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INTRODUCTION

Nowadays, the use of antibiotic as a growth promoter of broiler chicken has been banned because of the fear of its residue in broiler tissue and organs. However, antibiotics are still being widely used by breeders because it has provided economic benefits for breeders. Besides that, the impact on consumer health does not perceived directly.
Sulfadiazine is one of antibiotic that breeders used to prevent and treat diseases or the digestive and respiratory tract. The use of Sulfadiazine is typically combined with trimetoprin with a particular formulation. If it used excessively without regard to the rules of use, the antibiotics will leave residue in the meat, dairy, or eggs. National Standards Council (DSN) stating that drug residue or chemicals is the accumulation of drugs or chemicals and or its metabolites in the tissues or organs of animals after the using of drugs or chemicals for the purpose of prevention or treatment or as a feed prefix to stimulate growth.
Various research reports that the use of antibiotics in broiler chicken and laying tend to overuse regardless of rules of correct usage. The content of residual antibiotics that over the limit of maximum residue will cause the produced meat, milk and eggs are not safe to consume because it can cause allergenic reactions, toxicity, resistance to certain microbes or resulting physiological disorders in humans (Bahri, et al, 2000).
Reduction of antibiotic residue in food has attempted some sort of way of which is the use of the feed additive that can reduce antibiotic residues. Feed additives used have to be derived from natural ingredients so does not cause any side effect. Turmeric is one of the natural ingredients which is commonly used as feed additives (Napirah et al., 2014). Turmeric price is inexpensive and can be found at the local markets. Turmeric contains curcumin, a substance that can provide a positive impact for livestock. Wijayakusuma (2005) states that turmeric could reduces antibiotic residue in meat and has the immune stimulant effect.
Based on the explanation before, this research was conducted to analyze the effect of turmeric in broiler feed on performance (daily weight gain) and residue of sulfadiazine on liver and chicken breast meat broiler.

MATERIAL AND METHODS

This study was conducted at the Animal Production Technology and Science Laboratory, Animal Science Faculty, Universitas Halu Oleo. Two hundred one-day old (SR 707) broiler chicks were allocated randomly based on completely randomized design (CRD) with five dietary treatments and five replications. The chicks were reared during 5 weeks. The experimental diets were as follow: basal diet (commercial feed produced by JAPFA Comfeed) as control (T1), basal diet + 0.5% turmeric meal (T2), basal diet + 1.0 % turmeric meal (T3), basal diet + 1.5 % turmeric meal (T4), and basal diet + 1.0 % turmeric meal (T5). The chemical composition of the basal diets is presented in Table 1. Feed and water were provided ad-libitum, and the applying of experimental diets started after 7 days of rearing. Each chicken was given a total of 147.15 mg sulfadiazine during 5 weeks of rearing.
The parameters measured were sulfadiazine residue on broiler liver and breast meat, and the broiler daily weight gain. The measurement of sulfadiazine residue used HPLC. The data obtained was analyzed using analysis of variance and continued using least significant different (LSD) test.

RESULT AND DISCUSSION

Sulfadiazine residue in broiler liver

The effect of turmeric meal on sulfadiazine residue in broiler liver was shown in Table 2. The residue levels of sulfadiazine in broiler liver given turmeric meal were ranging from 0.000 - 3.980 ppm. Statistical analysis showed that the addition of turmeric meal had no significant effect on sulfadiazine residue in broiler liver. However, the addition of turmeric meal on broiler feed tends to affect the sulfadiazine residue in broiler liver. Table 2 shows us that the addition 0.5, 1, 1.5 and 2% of turmeric meal in feed have lower sulfadiazine residue than control feed (0% turmeric meal). The addition of 1.5% turmeric meal as feed additive showed the lowest sulfadiazine residue

level in broiler liver. The addition of 1.5% turmeric meal has liver sulfadiazine residue which is 51.90% lower than control feed.

Sulfadiazine residue in broiler breast meat

The effect of turmeric meal on broiler breast meat sulfadiazine residue was shown in table 3. The residue levels of sulfadiazine in broiler breast meat given turmeric meal were ranging from 0.000 - 2.350 ppm. Statistical analysis showed that the addition of turmeric meal had no significant effect on sulfadiazine residue in broiler breast meat.

Even there were no any significant effect, the addition of turmeric meal tend to decrease the level of sulfadiazine residue in broiler breast meat. Table 3 show that the addition of 1,5% turmeric meal could decrease 59.6% sulfadiazine residue in broiler breast meat if compared with control feed (0% turmeric meal).

Daily weight gain of broiler

The effect of turmeric meal on broiler daily weight gain was shown in Table 4. Statistical analysis shows that addition of turmeric meal did not give any significant effect on the daily weight gain of broiler. The daily weight gain of broiler among treatments was almost in the same level.

Discussion

Turmeric is known as one of the medical plant that contains some bioactive compounds such as curcumin (Li et al., 2011) and essential oil (Chattopadhyay et al., 2004). These bioactive compounds have some biological activities such as antioxidant (Araujo and Leon, 2001), anti bacterial (Kumar et al., 2001), hepatoprotector (Pavuluri et al., 2011) and immune modulator (Napirah et al, 2013).

Turmeric contains curcumin, the important bioactive compound responsible for the biological activity of turmeric. Some studies reported that curcumin could keep the liver health. Bao et al. (2010) reported that curcumin pretreatment could significantly ameliorate the negative effect of ethanol on liver and could prevent alcoholic liver disease. This condition probably also happen if broiler liver was induced by sulfadiazine contained in feed.

In this study, the addition of turmeric meal in broiler feed did not affect the sulfadiazine residue in liver and breast meat. This is probably caused by the decrease of curcumin and essential oil contained in turmeric meal during storage. Turmeric essential oil is volatile so that it can be reduced during storage. As described by Zaibunnisa (2009) that the oleoresin of turmeric is so sensitive to the change of light, heat, oxygen, and has short life storage.

Residual level of sulfadiazine in broiler liver was higher than in broiler breast meat. This difference is caused by liver function which acts as an antidote to poisons or substances that are toxic. The toxic substances are first broken down in the liver before entering bloodstream and enter other tissues.

Some studies also reported that turmeric could optimize the growth of poultry. Nourizan et al. (2011) and Al-Kasie et al. (2011) reported that addition of turmeric in broiler feed could optimize the broiler productivity, such as optimize the weight gain and reduce the feed conversion ratio. Pavuluri et al. (2011) explained that curcumin and essential oil in turmeric could increase the digestion process by stimulate the secretion of digestive enzymes, such as intestinal lipase and pancreatic lipase in mice.

In this study, the addition of turmeric in feed also did not affect the daily weight gain of broiler. This condition probably caused by the decrease of curcumin during feed storage. Previous study on poultry also reported the same condition. Napirah et al. (2014) reported that addition of turmeric in quail feed did not affect quail weight gain because the curcumin contained in turmeric meal was low.

CONCLUSION

The addition of turmeric meal until 2% in broiler feed did not significantly affect the sulfadiazine residue in broiler' s liver and breast meat. The addition of turmeric meal also did not significantly affect the daily weight gain of broiler. However, in this study, addition of 1.5% turmeric meal in broiler feed tend to reduce sulfadiazine residue in both liver and breast meat of broiler and it was indicated a tendency for better performance with better daily weight gain.

KEYWORD : turmeric, sulfadiazine, feed additive, broiler

Table 1. Nutrient composition of broiler commercial feed as basal diet

Nutrient	Percentage (%)
Water	12.5
Crude protein	18-20
Crude fat	4.0
Crude Fiber	5.0
Ash	7.0
Calcium	0.7-1.1
Phosphorous	0.6-1.0

Table 2. The level of sulfadiazine residue in broiler liver (ppm)

Replication	Turmeric meal levels (%)				
	0.0	0.5	1.0	1.5	2.0
1	0.000	1.070	2.500	1.080	2.230
2	1.410	0.980	1.210	1.190	1.560
3	1.160	1.100	1.280	0.000	0.970
4	0.000	0.000	1.460	0.880	0.000
5	3.980	1.500	1.020	0.000	0.000
Average	1.310	0.924	1.494	0.630	0.952

Table 3. The level of sulfadiazine residue in broiler breast meat (ppm)

Replication	Turmeric meal levels (%)				
	0.0	0.5	1.0	1.5	2.0
1	1.450	0.000	0.910	0.000	1.180
2	1.290	1.270	1.000	1.060	0.000
3	2.350	1.360	1.220	0.900	1.010
4	1.250	0.920	1.660	0.000	0.000
5	0.920	0.000	1.870	0.970	1.010
Average	1.452	0.710	1.132	0.586	0.640

Table 4. Average of broiler daily weight gain (g)

Replication	Turmeric meal levels (%)				
	0.0	0.5	1.0	1.5	2.0
1	56.637	52.255	46.347	54.041	58.013
2	46.939	46.917	53.857	50.018	47.912
3	51.235	54.299	51.299	57.256	59.024
4	55.456	59.534	52.556	51.392	54.228
5	51.750	54.375	56.552	60.807	50.823
Average	52.403	53.476	52.122	54.703	54.000

REFERENCES

Al-Kasie, G.A.M., A. M. Mohseen, and R.A. Abd-Al-Jaleel. 2011. Modification of productive performance and physiological aspect of broilers on the addition of a mixture of cumin and turmeric to the diet. Res. Opinion Anim. Vet. Sci. 1: 31-34.

Araujo, C.A.C. and L.L. Leon. 2001. Biological activities of *Curcuma longa* L. Memorias do Instituto Oswaldo Cruz, Rio de Janeiro. 96: 723-728.

Bahri, S., A. Kusumaningsih, T.B. Murdiati, A. Nurhadi, dan E. Masbulan, 2000. Analisis Kebijakan Keamanan Pangan Asal Ternak (Terutama Ayam Ras Peterlur dan Broiler). Laporan Penelitian. Pusat Penelitian dan Pengembangan Peternakan. Bogor.

Bao, W., K. Li, S. Rong, P. Yao, L. Hao, C. Ying, X. Zhang, A. Nussler, and L. Liu. 2010. Curcumin alleviates ethanol-induced hepatocytes oxidative damage involving heme oxygenase-1 induction. Journal of Ethnopharmacology. 128 (2): 549-553. [Abstract].

Chattopadyay, I., K. Biswas, U. Bandyopadhyay and R.K. Banerjee. 2004. Turmeric and curcumin: biological actions and medicinal applications. Current Sci. 87: 44-53.

Kumar, S., U. Naraian, S. Tripathi and K. Misra. 2001. Synthesis of curcumin bioconjugates and study for their antibacterial activities against β -lactamase producing microorganism. Bioconjugate Chem. 12: 464-469.

Li, M., W. Yuan, G. Deng, P. Wang, P. Yang, and B.B. Aggarwal. 2011. Chemical composition and product quality control of turmeric (Curcuma longa L.). Pharmaceutical Crops. 2: 28-54.

Napirah, A., Supadmo, and Zuprizal. 2013. The effect of turmeric (*Curcuma domestica* Valet) meal supplementation on hematological parameter of quail (*Coturnix-coturnix japonica*) blood. Bulletin of Animal Science. 37(2): 114-119.

Napirah, A., Supadmo, and Zuprizal. 2014. The effect of turmeric (*Curcuma domestica* Valet) meal supplementation on growth performance and meat fat and cholesterol content of male quails (*Coturnix-coturnix japonica*). Bulletin of Animal Science. 38(2): 78-82.

Nourizan, R., S.A. Tabeidian, M. Toghyani, G. Ghalamkari and M. Toghyani. 2011. Effect of turmeric meal on performance, carcass traits, humoral immune responses, and serum metabolites in broiler chicken. J. Anim. Feed Sci. 20: 389-400.

Pavuluri, G., S. Kumar, Hareesha, K. Madhuri and K.V. Swathi. 2011. Curcumin: the spice for life. Int. J. Pharmaceutical Chem. Biol. Sci. 1: 48-56.

Zaibunnisa, A.H., Norashikin, S. Mamot, and Osman. 2009. Stability of curcumin in turmeric oleoresin- β -cyclodextrin inclusion complex during storage. The Malaysian Journal of Analytical Science. 13(2): 165-169.

O-20-1

Effect of Eggshell Levels in Layer Diet on Productive Performance, Egg Quality and Plasma Calcium Concentration

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Introduction

Eggshell is considered a waste produced from hatcheries and poultry farm. Shell membrane contains 69.2% protein, 2.7% fat, 1.5% moisture and 27.2% ash. Moreover, eggshell contains 98.2% Ca as calcium carbonate, 0.9% Mg and 0.9% P as phosphate (Romanoff et al., 1949; MacNeil, 1997; Kingori, 2011). In chicken diet, major of Ca and P source are limestone and oyster shell (Ahmad and Balander, 2003). Absorption of Ca from eggshell is approximately 90% which it is possibly as natural Ca source. Eggshell can clean by boiling in water for 5-10 minute (Bee, 2011; Kingori, 2011). Hence, this objective of this study was to examine the effect of eggshell level (0, 50, 100%) as a calcium source in the diet of laying hens.

Material and Methods

Eggshell preparation

Eggshell was obtained from Chaveevan farm, Chonburi Province. Impurity and interference materials were removed from eggshell. The eggshell was rinsed or washed several times with water. After that the eggshell was sun dried with sunshine and oven dried at 60-70°C for 8-10 hr. Then, the eggshell was ground to particle size no greater 420 microns and later used to substitute calcium source (coarse and fine limestone).

Animal and feeding trial

Ninety laying hens (ISA-Brown strain) 28 weeks of age were assigned into a completely randomized design (CRD) with three dietary treatments, three replications per treatment and ten hens per replication. Each treatment contains different of eggshell level (0, 50 and 100%) (Table 1). All birds were fed with diets containing crude protein 17% and gross energy 2,750 kcal/kg of laying hens diet following nutrient requirements of poultry according to ISA Brown guide (2010). The birds were housed in individual battery cage (50 x 40 x 40 cm) under photoperiod throughout experiment. The birds were reared for 2 periods and 28 days per period making up a total of 56 days. Dietary treatments were maintained at 110 g/h/d throughout the study (54 days) but drinking water was offer *ad libitum* to the bird. Limitation on the experimental diets given was because heavy weight might affect egg production.

Egg productive performance

Egg production, egg weight and feed intake were recorded twice a day at 7.00 am and 1.00 pm. Hen-day egg production, average egg weight, egg mass [(Average Egg Weight x Hen-day production) x 100] were calculated according to the method by Uuganbayar et al. (2005) whereas the cost per egg production (FCR x Cost 1 kg of feed) were calculated as described by Chinrasri (2003). FCR (kg of feed needed to produce a kg of eggs) was calculated according to Yang et al. (2006).

Egg quality

During the last five days of each experimental period (28 days), eggs were collected daily and five eggs were randomly selected from each treatment to determine egg characteristic. Egg weight was measured after washing and drying with cool air to remove contaminants from shell. Egg yolk was separated from the albumen and weighed. Shell weight was measured after removal of remaining albumen with water. The weight of albumen was calculated by subtracting the weights of yolk and shell from the weight of whole egg. Thickness of the shell was obtained by averaging measurement from three areas; blunt end, pointed end and middle part of the egg using a digit meter as described by Hatice and Muhlis (2012). Color of eggshell and yolk were measured. The thickness of the albumen was measured on glass plate with an auto tri-pod micrometer (Chatcharee, 2003). Haugh unit was calculated from the thickness albumen and weight of egg using the following formula proposed by Haugh (1937); H.U. = 100 log [Albumen height in millimeter + 7.57 x 1.7 Weight of egg in gram0.37] according to the method described by Ragabe et al. (2012) using the Eggware software program.

Concentration of plasma calcium and phosphorus

During the last five days of each experimental period (28 days), birds were sampling for blood collection (1 bird/ replicate). Plasma was analyzed calcium and phosphorus using Cobus C501 (Tokyo, Japan)

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the general liner models procedure (Monchai, 2001). Differences among treatment means were compared using Duncan's multiple range test (DMRT) as described by Steel and Torrie (1992). A significance levels P

Results and Discussion

Productive performance

Chemical composition analysis of eggshell shows that eggshell contains 2.14% crude protein, 97.11% ash, 0.76% calcium and 0.16% phosphorus. In addition, dietary treatment contains 12.55-13.86% crude protein and 3,789.68-3,861.81 kcal/kg gross energy which meet nutrients requirement of poultry according to ISA Brown guide (2010). In the first period (week 28-31), hen day production, average egg mass of chicken fed with 100% eggshell were significantly lower than eggshell free diet (0% eggshell) and 50% eggshell ($P<0.05$) (Table 2). The hen day production was 86.73, 83.83 and 79.38%, respectively. Moreover, FCR and FCG of chicken fed with 100% eggshell were significantly higher than other treatments ($P<0.05$). However, in second period (week 32-35) and overall, productive performance was not significantly different among treatments ($P<0.05$) except FCR. FCR of chicken fed eggshell free diet was lower than other treatments ($P<0.05$) in week 32-35. The FCR was 2.17, 2.20 and 2.22, respectively. Similarly, previous studied of Gongruttananum (2011) reported that hen fed with eggshell (0, 50 and 100%) had no effect on egg production and egg weight at week 28-38. The egg production was 61.18, 69.24 and 68.28%, respectively. Scheideler (1998) also reported that egg production was not significantly affect by dietary calcium (limestone, oyster shell and ground eggshell) which hen day production had more than 90%.

Egg quality

In first period (week 28-31), egg quality was not significantly different among treatments ($P>0.05$) except albumen height, egg yolk color and Haugh unit (Table 3). Albumen height and Haugh unit of chicken fed with eggshell free diet were higher than other treatments ($P<0.05$). The Haugh unit was 89.88, 84.69 and 86.08, respectively. In second period (week 32-35), egg quality was significantly different among treatments ($P<0.05$) except whole egg weight, eggshell thickness, shell and albumin weight. Yolk weight, albumin height and Haugh unit of chicken fed with 50% eggshell were significant lower than other treatments ($P<0.05$). The Haugh unit was 89.31, 82.86 and 87.62, respectively. In overall, egg quality was not significantly among treatments ($P<0.01$) except albumin height and Haugh unit. The albumin height was 8.14, 7.15 and 7.71 mm, respectively. The Haugh unit was 89.59, 83.77 and 86.85, respectively. Previous studied of Scheideler (1998) reported that albumen weight was not significantly affect by dietary calcium (limestone, oyster shell and ground eggshell). Moreover, Gongruttananum (2011) also found that reported that hen fed with eggshell (0, 50 and 100%) had no effect on yolk weight, yolk color, albumin weight, albumen height, shell weight and thickness. The albumen weight was 65.98, 65.79 and 65.65%, respectively.

Concentration of calcium and phosphorus in plasma

In plasma, concentration of calcium and phosphorus were not significantly different among treatments ($P>0.05$) (Figure 1). The calcium concentration was 20.43, 23.43 and 22.20%, respectively. In addition, the phosphorus concentration was 2.16, 3.26 and 3.40 %, respectively. Gongruttananum (2011) reported that concentration of plasma Ca and P were not significantly different diet free eggshell and eggshell (50 and 100%). The total Ca and P range was 16-19 mg/dL and 8-9 mg/dL, respectively.

Conclusion

Eggshell is considered a waste produced from hatcheries and poultry farm. Eggshell is high Ca (98.2%) and absorption (90%). It was concluded that chicken fed with eggshell (0, 50 and 100%) had no effect productive performance and egg quality. Hence, eggshell is suitable to use as alternative calcium source in diets of laying hen up to 100% replacement levels without any negative effect on productive performance and egg quality at week 32-35.

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KEYWORD : Eggshell, Egg performance, Egg quality, Calcium

Table 1 Ingredient compositions of dietary treatment

Ingredients	Replacement levels of eggshell		
	0%	50%	100%
Corn	49.15	49.15	49.00
Broken rice	10.53	10.53	10.50
Full fat soybean	3.01	3.01	3.00
Soybean meal	24.07	24.07	24.00
Palm oil	1.00	1.00	1.80
Salt	0.38	0.38	0.38
Premixed	0.50	0.50	0.50
DL-methionine	0.32	0.32	0.32
Coarse limestone	5.52	5.52	-
Fine limestone	5.52	-	-
Eggshell	-	5.52	10.80
Total			
Calculate analyses			
ME (kcal/kg)	2,770.00	2,770.00	2,770.00
CP (%)	17.70	17.70	17.70
Ca (%)	3.95	3.95	3.86
Available P (mg/g)	0.21	1.64	0.21

Table 2 Effect of eggshell levels on productive performance of in laying hens

Productive performance	Replacement levels of eggshell			SEM
	0%	50%	100%	
Period 1 (week 28-31)				
Average egg weight (g)	59.02	57.95	59.17	0.29
Hen day production (%)	86.73^{a}	83.83^{a}	79.38^{b}	0.74
Average egg mass (g)	51.19^{a}	48.55^{b}	46.97^{c}	0.26
FCR (Feed intake/Egg mass)	2.15^{b}	2.27^{a}	2.34^{a}	0.01
FCG per 1 kg of egg (Bath)	28.83^{c}	29.95^{b}	31.52^{a}	0.16
Period 2 (week 32-35)				
Average egg weight (g)	58.71	57.94	58.76	0.18
Hen day production (%)	86.42	86.30	84.69	1.65
Average egg mass (g)	50.73	49.98	49.76	0.91
FCR (Feed intake/Egg mass)	2.17^{b}	2.20^{ab}	2.22^{a}	0.04
FCG per 1 kg of egg (Bath)	29.14	29.11	29.81	0.50
Overall (week 28-35)				
Average egg weight (g)	58.86	57.94	58.96	0.27
Hen day production (%)	86.54	85.06	82.04	1.33
Average egg mass (g)	50.94	49.27	48.37	0.68
FCR (Feed intake/Egg mass)	2.16	2.23	2.28	0.03
FCG per 1 kg of egg (Bath)	29.01	29.55	30.63	0.32

^{a,b,c} Values on the same row with different superscripts differ significantly ($P<0.05$), SEM= Standard Error of Mean.

Table 3 Effect of eggshell levels on egg quality of in laying hens

Productive performance	Replacement levels of eggshell			SEM
	0%	50%	100%	
Period 1 (week 28-31)				
Whole egg weight (g)	59.01	59.08	60.38	0.70
Shell weight (g)	10.03	7.82	7.97	0.40
Yolk weight (g)	14.26	14.25	14.71	0.14
Albumen weight (g)	37.62	37.05	37.51	0.41
Albumen height (mm)	8.18^{a}	7.26^{b}	7.56^{ab}	0.12
Eggshell color	14.18	13.52	14.23	0.25
Egg yolk color	4.83^{b}	5.17^{a}	5.04^{ab}	0.04
Haugh unit	89.88^{a}	84.69^{b}	86.08^{ab}	0.07
Eggshell thickness (mm)	0.37	0.36	0.36	<0.01
Period 2 (week 32-35)				
Whole egg weight (g)	60.50	60.24	61.40	0.45
Shell weight (g)	7.99	8.00	8.17	0.11
Yolk weight (g)	14.61^{a}	10.20^{b}	14.79^{a}	0.76
Albumen weight (g)	37.57	37.19	38.13	0.37
Albumen height (mm)	8.10^{a}	7.04^{b}	7.85^{a}	0.08
Eggshell color	13.71	13.78	14.02	0.29
Egg yolk color	4.83^{a}	4.51^{b}	4.47^{b}	0.04
Haugh unit	89.31^{a}	82.86^{b}	87.62^{a}	0.64
Eggshell thickness (mm)	0.37	0.38	0.38	<0.01
Overall (week 28-35)				
Whole egg weight (g)	59.75	59.66	60.89	0.40
Shell weight (g)	9.01	7.91	8.07	0.24
Yolk weight (g)	14.43	14.44	14.75	0.08
Albumen weight (g)	37.59	37.12	37.82	0.24
Albumen height (mm)	8.14^{c}	7.15^{e}	7.71^{d}	0.07
Eggshell color	13.95	13.65	14.12	0.17
Egg yolk color	4.83	4.84	4.76	0.07
Haugh unit	89.59^{c}	83.77^{d}	86.85^{e}	0.45
Eggshell thickness (mm)	0.37	0.37	0.37	<0.01

^{a,b} Values on the same row with different superscripts differ significantly (P<0.05), ^{c,d,e} Values on the same row with different superscripts differ significantly (P<0.01), SEM= Standard Error of Mean.

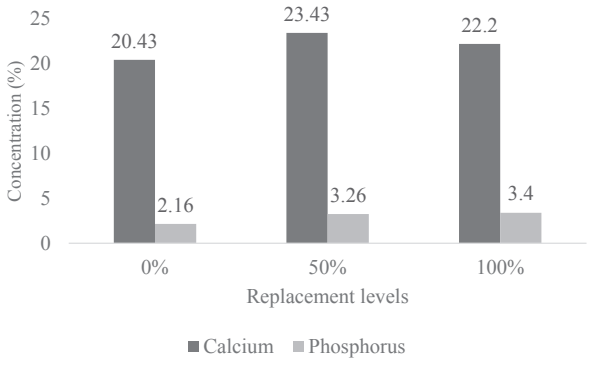

Figure 1 Concentration of calcium and phosphorus in plasma

REFERENCES

Ahmad, H.A. and Balander, R.J. 2003. Alternative feeding regimen of calcium source and phosphorus level for better eggshell quality in commercial layers. The Journal of Applied Poultry Research. 12: 509-514.

Bee, W. 2011. How to make calcium from egg shells. www. Healing naturally by Bee. Downloaded from the internet on 30/09/2011.

Chatcharee, H. (2003) QCD/EQM and QCM+ Eggware software manual. 1st ed., Bangkok. 31 p.

Chinrasri, O. (2003) Poultry production technology. 1st ed., Mahasarakham University. Mahasarakham, Thailand. 206 p.

Gongruttananun, N. 2011. Effects of eggshell calcium on productive performance, plasma calcium, bone mineralization, and gonadal characteristics in laying hens. Poultry Science. 90 :524–529.

Hatice, K. and Muhlis, M. (2012) Effect of inclusion of garlic (Allium sativum) powder at different level and copper into diets of hens on performance egg quality traits and yolk cholesterol content. International Journal of Poultry Science 10(1): 12-18.

Haugh, R.R. (1937). The Haugh unit for measuring egg quality. U.S. Egg and Poultry Magazine 43: 552-572.

Kingori, A.M. 2011. A Review of the uses of poultry eggshells and shell membranes. International Journal of Poultry Science 10 (11): 908-912.

MacNeil, J., 1997. Separation and utilization of waste eggshell. International egg commission annual production and marketing conference. Toronto, Ontario, September.

Monchai D. (2001). SAS enterprise guide for analysis. Nanawittaya publishing company, Khon Kaen.

Ragab, H. I., Abdel Ati, K. A., Kijora C., and Ibrahim, S. (2012) Effect of difference level of the processed Lablab purpureus seed on laying performance, egg quality and serum paramaters. International Journal of Poultry Science 11(2): 131-137.

Romanoff, A.L. and Romanoff, A.J. 1949. The avian egg. John Wiley & sons, Inc. New York, NY.

Scheideler, S.E. 1998. Eggshell Calcium Effects on Egg Quality and Ca Digestibility in First- or Third-Cycle Laying Hens. The Journal of Applied Poultry Research. 7: 69-74.

Steel, R. G. D. and Torrie, J. H. (1992) Principles and rocedure statistic. 2nd ed., McGrew-Hill Book Co., Inc., Singapore.

Uuganbayar, D., Bea I. H., Choi K. S., Shin I. S., Firman J. D., and Yang, C. J. (2005) Effect of green tea powder on laying performance and egg quality in laying hens. Asian Australasian Journal Animal Science 18: 1769-1774.

Yang, Y. X., Kim, Y. J., Jin, Z., Lohakare, J. D., Kim, C. H., Ohh S. H., Lee, S. H., Choi, J. Y., and Chae, B. J. (2006) Effects of dietary supplementation of astaxanthin on production performance, egg quality in layers and meat quality in finishing pigs. Australasian Journal Animal Science 7: 1019 - 1025.

O-20-2

Beneficial effect of AZOMITE® as a source of natural minerals on growth performance of broilers

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Abstract

The aim of present study was to investigate the feasibility of using AZOMITE®, a natural mineral product, in the diet of commercial broilers to observe their growth performance and carcass yields. To achieve the goal, three independent feeding trials were carried out. In trial 1, AZOMITE® was fed to the commercial broilers at five different dietary levels; namely 0, 0.25, 0.50, 0.75 or 1.0% as partial supplementation of macro and micro minerals with complete feed. In trial 2, AZOMITE® was fed at 0.5% with or without a mycotoxin binder to evaluate the efficacy of AZOMITE® as toxin binder in mixed feed. Finally in trial 3, AZOMITE® was fed at 0.5% to the broilers with or without antibiotic growth promoter to investigate the efficacy of AZOMITE® as a viable alternative to antibiotic growth promoter. Cobb-500 day-old broiler chicks were used as experimental birds in all the feeding trials. In each trial, chicks were equally divided and distributed into different dietary groups. Broiler diets were formulated for two phases, namely starter (0-21 d) and grower (22-end). Data records were kept on body weight, feed intake and mortality while weight gain, feed conversion ratio (FCR adjusted and actual), survivability, EEF and gain cost were calculated based on the recorded data. Edible meat characteristic records were also kept. Results showed that in trial 1 inclusion of AZOMITE® at 0.50%, 0.75% or 1.0% had significant effect on growth performance ($p<0.01$) and carcass yields of commercial broilers. In trial 2, dietary treatments however had no significant effect on growth performance of the experimental birds. In trial 3, on the other hand, supplementation of AZOMITE® with or without antibiotic had significant effect on BW and ADG ($p<0.05$) in AZOMITE® supplemented groups over the control. In all feeding trials, supplementation of AZOMITE® had no significant effect on feed intake and survivability. Based on the results, it could be concluded that inclusion of AZOMITE® at 0.50% in the diet may improve the growth performance, carcass yields and anti-microbial function of commercial broilers.

Introduction

Although the mineral constituents in poultry diet, both macro and micro minerals, do not yield energy for the birds however they have pivotal roles in many biological activities in the body (Malhotra, 1998). Therefore every form of living matter requires these inorganic elements or minerals for their normal life (Hays and Swenson, 1985). Unlike other nutrients, mineral elements cannot be synthesized by living organisms (McDowell, 2003); thus these elements must supply from the exogenous sources through diets. Minerals have four broad functions like structural, physiological, catalytic and hormonal or regulatory.

Based on the dietary requirement minerals are classified as essential major or macrominerals (calcium, phosphorus, potassium, sodium and magnesium) and trace or microminerals. Nielsen (1985) stated that during the period of 1970 to 1985, at least 11 elements were added to the list of mineral elements essential in animal nutrition. The newly proposed elements were arsenic, boron, bromine, cadmium, fluorine, lead, lithium, nickel, silicon, tin and vanadium. Estimated dietary requirements for these elements are usually less than 1 mg/kg (parts per million). They have been also designated as the ultratrace minerals. Chromium has been legally approved in several countries in the world (@ 200-400 parts per billion additions to the diet may be representative) for some animal species and purposes (Qinghua, 1996). Uthus and Seaborn (1996) also suggested three other minerals such as aluminum, rubidium and germanium as essential for the birds. Tungsten, in the form of tungstate, exhibits a significant antihyperglycemic effect on both type 1 and 2 diabetic animals (Liu *et al*., 2004). Nielsen (1996) suggested that the term ultratrace elements could be applied to at least 20 elements that have established estimated or suspected requirements or have beneficial effects to birds and animals.

Dietary minerals have been scrutinized with regard to their efficacy for providing essential elements (for example, bioavailability and metabolic utilization), toxicity and also environmental friendliness. As a result, more natural and organically certified mineral products have come to market and are being welcomed for use in the diets of broilers and layers. One of such mineral sources is AZOMITE®, a volcanic ash harvested in USA, could be considered as natural mineral sources in poultry diet in Bangladesh. The product typically contains analyzable

concentrations of all known essential elements for plants and animals. Out of the 92 naturally occurring elements on Earth, approximately 87% are available in the AZOMITE®. Although the AZOMITE® is considered as a potential source of natural minerals and the product is commercially used in birds and animals in the developed countries particularly in USA, its feasibility has yet not been examined in Bangladesh. Thus, potentiality of the ingredient could be examined in local environment as mineral supplements to improve production performance and carcass yields of commercial broilers.

Secondly, contamination of poultry feeds with mycotoxins is a major concern in the present poultry industry. Presence of mycotoxins in poultry feed is a natural phenomenon, not an exception. Mycotoxins may cause decreased performance, lower feed intake, poor feed conversion, diminished body weight gain, immune suppression, reproductive disorders, and retention in harmful residues in food products (Akande, 2006; Galvano *et al*., 2001; Huwig *et al*., 2001). In order to avoid mycotoxicosis, several feed additives like antioxidants, sulphur-containing amino acids, vitamins, and trace elements are useful as detoxicants (Nahm, 1995). Further, addition of adsorbents such as hydrated sodium calcium aluminosilicates (HSCAS), zeolites, bentonites, activated charcoals, probiotics etc. can be used (Dawson *et al.,* 2001; Smith *et al.,* 2001; Raju and Devegowda, 2002). Reports suggest the toxin binding properties of AZOMITE® in the mixed feed. Because of the chemical properties, AZOMITE® is also considered to be a hydrated sodium calcium aluminosilicate (HSCAS). In present study, therefore the efficacy of AZOMITE® as toxin binding agent was also investigated. Thirdly, published reports indicated that the rare earth elements have potential functions to prevent growth and development of harmful microorganisms (He and Rambeck, 2000; He *et al.,* 2010). Since the test ingredient AZOMITE® contained some earth elements, it could be assumed that the products might have anti-minrobial properties as well.

Keeping all the above views in mind, present study was designed to determine appropriate inclusion level of AZOMITE® in the diet of commercial broiler as potential source of natural minerals. Further, efficacy AZOMITE® as mycotoxin binder and its effectiveness as an alternative to traditional harmful antibiotic growth promoter (AGP) was also investigated.

Materials and Methods

In experiment 1, AZOMITE® was fed at five dietary levels, namely 0 (control), 0.25, 0.50, 0.75 and 1.0% to investigate its suitability as a partial mineral supplement in commercial broilers. AZOMITE® was manufactured by one of the American Company named "AZOMITE® Mineral Products, Inc., Kansas City". A total of 1020 day old broiler chicks were equally divided and distributed into five different dietary treatment groups having 204 chicks per treatment. Each treatment again contained six replications with 34 birds in each. The aim of experiment 2 was to evaluate the efficacy of AZOMITE® as a mycotoxin binder to reduce the toxicity of mycotoxins in commercial broilers. A total of 544 day old Cobb-500 broiler chicks were randomly allocated to 4 dietary treatments having 4 replications per treatment and 34 chicks in each replicate group. The four dietary treatments were: a corn soybean meal- based basal diet as control, basal diet plus 0.5% AZOMITE® (AZO), basal diet plus a commercial mycotoxin binder (MTB, @ 2.5kg/1000kg) and basal diet plus AZO and MTB. In experiment 3, the efficacy of AZOMITE® was evaluated as an alternative to antibiotic growth promoter in commercial broilers. A total of 816 day old Cobb-500 broiler chicks were randomly allocated into 4 dietary treatments having 6 replications per treatment and 34 day-old chicks in each replicate group. The four dietary treatments that considered in the experiment were a corn soybean meal-based basal diet treated as control, basal diet plus AZOMITE® (AZO, @ 0.5%), basal diet plus antibiotic growth promoter (Lincoplex, @ 200g/1000kg) and basal diet plus AZO and AGP.

Results

Live weight was significantly higher in the broilers fed AZOMITE® at 0.75% (1628.15g/b), followed by 0.50% (1607.52g/b), 1.00% (1582.61g/b) and 0.25% (1545.35g/b) levels. The lowest body weight (1206.48g/b) was however observed in broilers fed diet without AZOMITE® (Table 1). Likewise the feed intake, feed conversion ratio, European efficiency factor, and livability were significantly improved in the broilers fed diet supplemented with 0.5, 0.75 or 1.0% levels of AZOMITE®. Most of the parameters in edible meat yields were also gave better results after inclusion of AZOMITE® (Table 2). When AZOMITE® was fed to broilers with or without a toxin binder, no significant effect was observed in growth performance and carcass yield characteristics. Chemical analysis of experimental diets for common mycotoxins is shown in Table 3 (a, b). Results showed that the natural contamination of six common mycotoxins occurred in both starter and grower diets, although the presence of all mycotoxins (Aflatoxin B1, Ochratoxin A, Citrinin, Trichothecene, Zearalenone and Deoxynivalenol) was within the

permissible limits set by European Food Safety Authority, and consequently there were no significant differences among the dietary groups (p>0.05). The level of stressor (natural contamination of mycotoxins) used in present study might not be enough to understand the efficacy of AZOMITE® on toxin binding ability. Thus further study can be designed by inclusion of artificial toxin in the diet as positive control.

Antimicrobial properties of AZOMITE® in commercial broilers have been examined. Results indicates that the highest live weight (1408.6g/b) was found in broilers fed 0.50% AZOMITE®, followed by AGP+AZO (1402.56g/b) and AGP (1396.67g/b) groups (Table 4). The lowest body weight was found in control (1358.84g/b). No significant differences however were found in average feed intake, feed conversion ratio, survivability, European efficiency factor and gain cost among the treatment groups.

Conclusion

Based on the results of current study, it can be concluded that test ingredient AZOMITE® may be included at 0.50% level in the diets of commercial broilers as partial supplementation of micro and macro minerals and also can be considered as viable alternative to harmful antibiotic growth promoter.

KEYWORD : Azomite, Commercial broiler, Toxin binder, Growth performance, Antibiotic

Table 1. Production performance of the broiler supplemented with different levels of AZOMITE®

Parameter	Age (day)	Dietary levels of AZOMITE® (%)					Level of Sig.
		0.00 (Control)	0.25	0.50	0.75	1.00	
LBW (g)	0	46.66 ± 0.09	46.59 ± 0.08	46.7 ± 0.07	46.47 ± 0.10	46.56 ± 0.10	NS
	0-21	823.64 ± 9.35	817.97 ± 8.78	842.1 ± 8.61	839.22 ± 6.95	828.14 ± 10.79	NS
	22-32	1206.48^{c} ± 22.70	1545.35^{b} ± 25.90	1607.52^{ab} ± 24.32	1628.15^{a} ± 19.48	1582.61^{ab} ± 8.56	**
	0-32	1206.48^{c} ± 22.70	1545.35^{b} ± 25.90	1607.52^{ab} ± 24.32	1628.15^{a} ± 19.84	1582.61^{ab} ± 8.56	**
LWG (g)	0-21	763.46 ± 8.43	765.44 ± 11.14	783.05 ± 11.02	792.75 ± 6.92	776.34 ± 13.10	NS
	22-32	378.13^{b} ± 23.46	722.84^{a} ± 22.33	765.43^{a} ± 20.92	776.69^{a} ± 15.80	748.88^{a} ± 9.84	**
	0-32	1134.44^{c} ± 24.96	1481.48^{b} ± 32.16	1532.85^{ab} ± 21.31	1569.44^{a} ± 15.30	1518.01^{ab} ± 19.73	**
ADG (g)	0-21	37 ± 0.45	36.73 ± 0.42	37.88 ± 0.41	37.75 ± 0.33	37.22 ± 0.52	NS
	22-32	34.8^{c} ± 2.13	66.13^{b} ± 1.79	69.58^{ab} ± 1.90	71.72^{a} ± 1.66	68.59^{ab} ± 0.90	**
	0-32	36.25^{c} ± 0.71	46.84^{b} ± 0.81	48.78^{ab} ± 0.76	49.43^{a} ± 0.62	48.0^{ab} ± 0.27	**
Avg. FI (g)	0-21	1280.44 ± 13.90	1263.29 ± 8.46	1277.78 ± 5.59	1289.51 ± 5.49	1277.72 ± 9.04	NS
	22-32	1406.92^{b} ± 35.42	1588.88^{a} ± 28.03	1649.55^{a} ± 13.70	1648.09^{a} ± 14.78	1644.43^{a} ± 15.24	**
	0-32	2690.32^{b} ± 44.78	2855.16^{a} ± 31.82	2927.32^{a} ± 12.47	2940.93^{a} ± 17.93	2922.16^{a} ± 19.70	**
FCR Adj.	0-21	1.68 ± 0.02	1.65 ± 0.02	1.63 ± 0.02	1.63 ± 0.01	1.65 ± 0.02	NS
	22-32	3.78^{a} ± 0.20	2.2^{b} ± 0.04	2.16^{b} ± 0.05	2.13^{b} ± 0.03	2.2^{b} ± 0.02	**
	0-32	2.38^{a} ± 0.06	1.93^{b} ± 0.03	1.91^{b} ± 0.02	1.88^{b} ± 0.01	1.93^{b} ± 0.02	**

Table 2. Edible meat yield characteristics of broilers fed diets supplemented with increasing levels of AZOMITE®

Parameter	Dietary levels of AZOMITE® (%)					Level of Sig.
	0.0 (control)	0.25	0.50	0.75	1.00	
Live body wt (g/B)	1404.67^{c} ± 30.24	1547.42^{b} ± 11.95	1627.83^{a} ± 11.61	1636.58^{a} ± 11.67	1596.08^{a} ± 4.70	**
Dressing wt (g/b)	948.2^{d} ± 17.85	1060.65^{c} ± 9.21	1119.09^{ab} ± 13.19	1128.79^{a} ± 8.99	1084.92^{bc} ± 9.12	**
Dressing wt (%)	67.73 ± 1.53	68.56 ± 0.48	68.75 ± 0.63	68.98 ± 0.42	67.98 ± 0.58	NS
Thigh wt (%)	10.25 ± 0.31	10.65 ± 0.12	10.42 ± 0.16	10.58 ± 0.11	10.49 ± 0.19	NS
Drumstick wt (%)	8.7 ± 0.20	8.43 ± 0.07	9.01 ± 0.13	8.92 ± 0.12	8.68 ± 0.18	NS
Breast wt (%)	19.35 ± 0.62	18.79 ± 0.41	19.41 ± 0.46	18.38 ± 0.44	18.59 ± 0.46	NS
Wing wt (%)	7.23^{ab} ± 0.12	7.03^{b} ± 0.13	7.47^{a} ± 0.12	7.51^{a} ± 0.14	7.47^{a} ± 0.12	*
Head wt (%)	2.4^{b} ± 0.04	2.31^{b} ± 0.04	2.33^{b} ± 0.03	2.37^{b} ± 0.05	2.57^{a} ± 0.06	**
Neck wt (%)	2.36^{a} ± 0.12	2.0^{b} ± 0.06	1.97^{b} ± 0.08	1.95^{b} ± 0.04	1.85^{b} ± 0.05	**
Liver wt (%)	2.32^{ab} ± 0.10	2.37^{a} ± 0.12	2.02^{c} ± 0.06	2.08^{bc} ± 0.05	2.1^{bc} ± 0.06	*
Gizzard wt (%)	2.17^{a} ± 0.08	1.96^{b} ± 0.05	1.94^{b} ± 0.08	1.83^{b} ± 0.06	1.86^{b} ± 0.09	*
Heart wt (%)	0.42^{b} ± 0.02	0.4^{b} ± 0.01	0.4^{b} ± 0.01	0.44^{ab} ± 0.01	0.47^{a} ± 0.02	*

NS= Non-significant, Values indicate average ± Standard Error Mean (SEM).
^{a,b,c} Means bearing dissimilar superscript in a row differ significantly, **=(P<0.01), *=(P<0.05),

Table 3 (a) Naturally occurring mycotoxins in broiler starter diet

Parameters	Unit	Treatment				Maximum Permissible Limits (EFSA*)
		BD	BD+AZO	BD+MTB	BD+MTB+AZO	
Mold	cfu/g	11000	5000	9000	14000	-
Enterobacteriaceae	cfu/g	<100	<100	<100	<100	-
Aflatoxin B1	ppb	16	24	8	16	20 ppb
Ochratoxin A	ppb	<8	<8	<8	<8	40 ppb
Citrinin	ppb	<8	<8	<8	<8	100 ppb
Trichothecene (T2)	ppb	<8	<8	<8	<8	200 ppb
Zearalenone (ZON)	ppb	<100	<100	<100	<100	400 ppb
Deoxynivalenol (DON)	ppb	<100	<100	<100	<100	5000 ppb

(b) Naturally occurring mycotoxins in broiler grower diet

Parameters	Unit	Treatment				Maximum Permissible Limits (EFSA*)
		BD	BD+AZO	BD+MTB	BD+MTB+AZO	
Mold	cfu/g	6000	5000	7000	1000	-
Enterobacteriaceae	cfu/g	<100	<100	<100	800	-
Aflatoxin B1	ppb	<8	8	12	<8	20 ppb
Ochratoxin A	ppb	<8	<8	<8	<8	40 ppb
Citrinin	ppb	<8	<8	<8	<8	100 ppb
Trichothecene (T2)	ppb	<8	<8	<8	<8	200 ppb
Zearalenone (ZON)	ppb	<100	<100	<100	<100	400 ppb
Deoxynivalenol (DON)	ppb	<100	<100	<100	<100	5000 ppb

Table 4 Production performance of the broiler supplemented with AZOMITE® with or without AGP

Parameter	Age (day)	Dietary treatments				Level of Sig.
		BD	BD+AZO	BD+AGP	BD+AGP+AZO	
AVG BW (kg)	0	46.15 ± 0.05	46.17 ± 0.04	46.17 ± 0.03	46.2 ± 0.07	NS
	0-21	533.82^{b} ± 6.98	558.61^{a} ± 5.28	558.42^{a} ± 4.66	557.74^{a} ± 9.50	*
	22-35	1358.84^{b} ± 12.25	1408.6^{a} ± 13.41	1396.67^{a} ± 8.53	1402.56^{a} ± 15.28	*
	0-35	1358.84^{b} ± 12.25	1408.6^{a} ± 13.41	1396.67^{a} ± 8.52	1402.56^{a} ± 15.28	*
AVG BWG (kg)	0-21	467.26 ± 7.19	497.69 ± 9.63	486.76 ± 5.92	497.42 ± 10.57	NS
	22-35	803.55 ± 16.14	814.27 ± 18.89	819.53 ± 15.44	822.8 ± 16.84	NS
	0-35	1231.03 ± 14.27	1280.14 ± 33.33	1254.03 ± 15.1	1287.2 ± 20.18	NS
ADG (g)	0-21	23.22^{b} ± 0.34	24.4^{a} ± 0.25	24.39^{a} ± 0.22	24.36^{a} ± 0.45	*
	22-35	58.93 ± 0.67	60.71 ± 0.94	59.88 ± 0.79	60.35 ± 0.76	NS
	0-35	37.5^{b} ± 0.35	38.93^{a} ± 0.38	38.59^{a} ± 0.24	38.75^{a} ± 0.44	*
AVG FI (g)	0-21	877.32 ± 8.11	901.55 ± 7.88	900.36 ± 5.85	900.62 ± 12.31	NS
	22-35	1495.56 ± 23.37	1539.87 ± 26.60	1530.9 ± 17.83	1523.91 ± 33.23	NS
	0-35	2381.25 ± 26.78	2454.53 ± 26.90	2444.92 ± 12.77	2433.3 ± 39.05	NS
FCR Adj.	0-21	1.88 ± 0.03	1.81 ± 0.04	1.85 ± 0.03	1.81 ± 0.02	NS
	22-35	1.86 ± 0.01	1.89 ± 0.01	1.87 ± 0.02	1.85 ± 0.02	NS
	0-35	1.94 ± 0.01	1.93 ± 0.04	1.95 ± 0.02	1.89 ± 0.02	NS

BD=Basal Diet, AZO= AZOMITE®, AGP=Antibiotic Growth Promoter, AVG BW=Average Live Weight, AVG BWG=Average Live Weight Gain, ADG=Average daily gain, AVG FI=Average Feed Intake, FCR Adj.=Feed Conversion Ratio Adjusted, g=Grams, NS=Non-significant, Values indicate average ± Standard Error Mean (SEM). ^{a,b,c,d} Means bearing uncommon superscripts in a row differ significantly. ** = (P<0.01), *= (P<0.05).

REFERENCES

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE 2006: Nutritional and health implications of mycotoxins in animal feeds: a review. *Pakistan Journal of Nutrition* 5 398-403.

Dawson K, Evans J, Kudupoje M 2001: Understanding of adsorption characteristics of yeast cell wall preparations associated with mycotoxin binding. In: Science and Technology in the Feed Industry, Proceedings of Alltech's 17th Annual Symposium. Nottingham University Press (Ed. T. P. Lyons and K. A. Jacques), UK, Page: 169-182.

Galvano F, Piva A, Ritieni A, Galvano G 2001: Dietary strategies to counteract the effects of mycotoxins: a review. *Journal of Food Protection* 64 120-131.

Hays VW, Swenson MJ 1985: Minerals and Bones. *Dukes' Physiology of Domestic Animals* (Tenth Edition) Page: 449-466.

He ML, Wehr U, Rambeck WA 2010: Effect of low doses of dietary rare earth elements on growth performance of broilers. *Journal of Animal Physiology and Animal Nutrition* 94 86-92

Huwig A, Freimund S, Kappeli O, Dutler H 2001: Mycotoxin detoxification of animal feed by different adsorbents. *Toxicology Letters* 122 179-188.

Liu HK, Green BD, McClenaghan NH, McCluskey JT Flatt PR 2004: Long-term beneficial effects of vanadate, tungstate and molybdate on insulin secretion and function of cultured beta cells. *Pancreas* 28(4) 364-368.

Malhotra VK 1998: Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.

McDowell LR 2003: Minerals in Animal and Human Nutrition. Elsevier, Amsterdam, Page: 2.

Nielsen FH 1985: The importance of diet composition in ultra-trace element research. *Journal of Nutrition* 11 1239-1247.

Qinghua V 1996: Winning world-wide Feeding Times. Dublin, Ireland. 1(3) 6-7.

Raju MVLN, Devegowda G 2002: Esterifiedglucomannan in broiler chicken diets-contaminated with aflatoxin, ochratoxin and T2 toxin: Evaluation of its bingding ability (in vitro) and efficacy as immunomodulator. Asian-Aust. *Journal of Animal Science* 15(7) 1051-1056.

Smith TK, MacDonald EJ, Haladi S 2001: Current concepts in feed-borne mycotoxins and the potential for dietary prevention of mycotoxicoses. In: Science and Technology in the feed industry, Proceedings of Alltech's 17th Annual Symposium. Nottingham University Press (Ed. T. P. Lyons and K. A. Jacques), UK, Page: 183-190.

Uthus EO, Seaborn CD 1996: Deliberations and evaluations of the approaches, endpoints and paradigms for dietary recommendations of the other trace elements. *Journal of Nutrition* 126 2452-2459.

O-20-4

Use of Blue Swimming Crab Waste as Alternative Animal Feedstuff

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Introduction

The blue swimming crab (*Portunus pelagicus*) is one type of water animal with high economic importance in Thailand. Their main habitats usually include sandy beaches, coastal mud and sandy mud areas. In Thailand, this water animal can be found along the coastal areas of the Andaman Sea, the Gulf Coast of Thailand and the surrounding mouth of many rivers. These crabs normally live in areas with bodies of water of not more than 30 meters deep and could grow well in salty areas of 29-32 ppt with temperatures from 30 to 32°C (Thien Ratsamee, 2001). The blue swimming crab is a kind of water animal that has become very interesting especially from government and non-government units as a very popular consumption product in general and as an important export commodity. In 2013, (together with crabs reared in coasts), export value reached the highest amount of 3,561.7 million baht from crab yield including those caught by natural means at about 25,712 tons (Department of Fisheries, 2013). Crabs are processed in a variety of ways as food products. Crab crusts could be used as fertilizer or for extraction of chitosan and chitin which are beneficial substances in many ways such as in the environment including waste water management in different industrial factories. Nowadays, blue swimming crab raising is being promoted in the country with the main purpose of producing varietal offspring for sale to farmers to raise as soft-shelled crab and as commercial crab meat production to the food processing factories. When crab yield is sufficient to meet the ever increasing demands of the consumers, the impact would mainly consist of the increasing amount of remaining crusts from the production and consumption. Westendorf (2000) reported that crab used for meat production was only 10-15% and the remaining 85-90% were discards thrown as waste, thus leading to the increase in the amount of crab shells from production and hiring of labor for meat extraction from local communities. Most often crab discards are thrown to the waters making the waters dirty while creating bad smell and providing impact to the ecosystem and producing odor pollution along the coastal environments (Figure 1).

Most of the provinces in the eastern region have long coastal areas along the Gulf of Thailand while the central parts are near high mountain ranges. Most of the population work as fishermen or as farmers growing fruits. If there left-overs from fish products such as shells from shrimp or crabs, people used them as feed for animals thus increasing the value of the waste from seafood and at the same time, as a way to reduce pollution caused by thrown discards from seafood processing. It would then decrease the problems of pollution due to stinking odor from seafood left-overs caused by marine tourism and which were found to affect physical and mental health of both tourists and people in local communities because dirt is the main source of bacteria and germs that cause diseases and ugly sights. These aforementioned problems currently exist along the Bang Phra beach communities in Sriracha district, Cholburi province. Most of the local people are fishermen and are involved in one way or the other in the processing of seafood products. Based on the problems of the communities as initially mentioned, the concept of this research was the application of technology that could reduce the amount of discards that mainly consist of shell crust of crabs by reducing their size in order to be able to use it easily for other purposes and needs such as being alternative raw feed materials (Department of Fishery Products, Faculty of fisheries, Kasetsart University, 2015).

Generally, the grinding of materials is done by using many existing machines such as hammer mill, roller mill, attrition or burr or disc or plate mill, and pin mill, all of which have their own advantages and disadvantages depending on the working purpose and suitability of that job. As such, the hammer is considered the most popular (Bochart et al., 2015) particularly in the animal feed industry. This type of grinder consists of a hammer in a rotor set up attached to the drive shaft. The hammers rotate inside the grinding chamber with a screen that covers or line the sides and with the use of initial power which might be from electric rotor or engine as a driving mechanism for rotation or for grinding the materials that would then reduce their size. One advantage of the hammer mill is the convenience in using the tool especially in specifying the size of the particle after grinding based on the size of the holes in the screen. The working principle is quite simple and is capable of grinding many

kinds of raw materials. Inaddition, the care and maintenance of this machine is more easy and simple as compared with other machines particularly in terms of removing the parts for cleaning or repair. This research, therefore, was aimed to study the grinding of blue swimming crab waste left as discards from production so as to be able to utilize them in many other ways with emphasis on their use as an alternative raw material for animal feed.

Material and Methods

Physical Properties of Blue Swimming Crab Waste

The waste of the blue swimming crab in this study came from discards resulting from the work of the villagers as hired labor in cracking the crust in tambon communities around Bang Phra, Sriracha district in Cholburi province. The crab crusts used in this study were complete and had no cracks or breaks and could be measured by Vernier caliper at 50x109x25 mm (width x length x thickness) (n=30). Moisture was applied by hot air oven method and showed that initial value was 52.5 + 5.61% (w.b.) and later was reduced to 7.30-8.94% (w.b.) (n=30). Meanwhile, strength or hardness was measured by Texture Analyzer (Stable Micro Systems brand of TA.XT Plus) pressing the ball system (sms p/0.25s) with initial strength of 3.741 ± 0.074 kgf and which was then reduced to 2.684 ± 0.030 kgf when sun dried for 5 days (n=30).

Design and Fabrication of the Blue Swimming Crab Waste Grinding Machine

The design and fabrication of the blue swimming crab waste grinding machine (Figure 2) was patterned after the hammer mill using an electric motor of 2.2 kW (3 hp) as initial power drive. It mainly consisted of an engine frame, grinding set and grinding chamber, feeding setand cover, and others.

1. Grinding set and grinding chamber

The grinding set consisted of a rotor shaft and beaters. The beater had a thickness of 5 mm and a width x length of 40x135 mm and a total of 24 fins. The underneath area was an iron screen of the size, 400x530x200 mm (w x l x d) with holes of about 1 mm.

2. Feeding set and cover

The feeder and cover functioned as a lock to prevent danger arising from the rotational beating of the shaft to the materials that get inside the grinding chamber and also controlled the materials from being mixed with impurities with open-close slots. These door and cap were made of iron and with size of 4 mm.

Experimental Design

Since the limitations involved mainly the grinding machine, a study by Fang et al. (1997) found that rate of impurities did not show any effect to the capacity of grinding hard red winter wheat, thus this particular research was conducted as a one-way experiment. The first study was concerned on the effect of moisture in blue swimming crab on the grinding performance at 3 levels: 8.94 ± 0.46, 7.90 ± 0.59 and 7.30 ± 0.43% w.b. when sun dried for 3, 4 and 5 days, respectively, in the month of November 2014 at the Rajamangala University of Technology Tawan-ok, Bang Phra, Sriracha district in Chonburi province, with grinding speed of 1054 rpm. The second study was on the effect of grinding speed at 3 levels: 843, 1054 and 1405rpm using the waste of blue swimming crab with suitable moisture resulting from the first study in a Randomized Complete Block Design (RCBD) with 3 replications at 5 kg each. During the entire study, recording was done every time on the weight of crab waste after grinding and electrical wattage used during grinding in order to calculate the grinding performance, workload and energy, respectively, following the method of Bochat et al. (2015). Afterwards, the blue swimming crab particles after grinding were used to determine the average grinding size and consistency with hot screen method of Henderson and Perry (1976) with ASTM E11 screen 3/8" (9.50 mm), 4 (4.75 mm), 8 (2.36 mm), 14 (1.40 mm), 20 (0.85 mm), 30 (0.60 mm), 50 (0.30 mm), 100 (0.15 mm) and pan, respectively.

Test at Appropriated Grinding Conditions

In this study, the moisture of blue swimming crab and the speed of grinding as mentioned in #3, served as the criteria for appropriated grinding conditions in 3 replications at 15 kg each. During and after the study, measurements were done similarly as in #3.

Chemical Content Analysis

The evaluation of animal feed values was done using the proximate analysis method in order to determine moisture, ash, crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen free extract (NFE) based on AOAC method (1995). The analysis to measure the amount of calcium and phosphorus including total energy was done with the use of Bomb calorimeter (IKA ® Calorimetery System C500, Germany) and was conducted in the Faculty of Animal Science and Technology, Maejo University, Sansai, Chiang Mai, Thailand.

Statistical Analysis

Grinding performances was subjected to analysis of variance (ANOVA) at significant levels of 0.05 and 0.01. For significant difference, mean comparisons among grinding performances were performed by Duncan\'s new multiple range test at significant level of 0.05. All statistical analyses of the experimental results were carried out using Minitab 16 (©2013 Minitab Inc.).

Result and Discussion

Effect of Initial Moisture Content of Crab Waste on Grinding Performance

The results of sun drying of fresh blue swimming crab wastes for 3, 4 and 5 days with initial moisture of 8.94 ± 0.46, 7.90 ± 0.59 and 7.30 ± 0.43% w.b., respectively, and with the grinding speed of 1054 rpm (Figure 3), are shown in Table 1, which indicated that sun drying for 4 and 5 days were not significantly different in statistics although grinding capacity was increased as moisture of crab waste decreased. At the same time, the size of the particles after grinding, power used during grinding and energy required for grinding were reduced in direct proportion to moisture content, as reported by Fang et al. (1997) who stated that power used in grinding hard red winter wheat was reduced in direct proportion to moisture of materials being subjected to grinding. But Probst et al. (2013) found that moisture did not significantly affect grinding performance for corn seeds but grinding of corn cobs was significantly different in statistics in moisture of ~10-15% (w.b.) with ~20% (w.b.). Moisture showed effect on the power used in grinding corn seeds and corn cobs with a significant increase in grinding corn seeds at a moisture of ~10-15% (w.b.). Energy used in grinding corn cobs was 30 times higher than in grinding corn seeds at a moisture level of ~10-20% w.b., which might be due to the hardness of the materials and the natural characteristics of corn cobs that consisted mainly of strands.

Effect of Grinding Rotational Speed on Grinding Performances

Based on the study to determine the speed suitable for grinding by changing the pulley of grinding shaft of 6, 8 and 10 inches and instead, using a pulley of rotor shaft fixed at 3 inches that caused the grinding speed equivalent to 1405, 1054 and 843 rpm, respectively, and using the moisture of crab waste after sun drying for 5 days, results as shown in Table 2, indicated that the grinding speed of 1054 rpm caused the highest grinding performance but the size of the particles that resulted after grinding was not different as in 1405 rpm while grinding power and required grinding energy were the lowest. This was in conformity with the findings of Patil et al. (2005) who found that mathematical average size of particles after grinding was reduced when grinding speed was increased. For Bochat et al. (2015), it was reported that grinding performance was directly proportional to grinding rotational speed while grinding energy was reduced in polynomial function. Thus, the grinding of crab waste must use a speed of 1054 rpm because the grinding performance was more efficient to two levels and the resulting materials were much finer.

Tests at Appropriated Grinding Conditions

Testing of the grinding of crab waste on a long term basis by using the best appropriate conditions, namely: approximate crab waste moisture at 7.13 ± 0.40 % (w.b.) sun dried for 5 days with grinding speed of 1054 rpm in 3 replications at 15 kg each, showed that grinding performance was 18.34 ± 1.53 kg/hr with size of particles after grinding at 0.77 ± 0.09 mm while grinding power was 1.38 ± 0.01 kW and required grinding energy was 0.075 ± 0.006 kW.hr/kg.

Chemical Composition

BSCW consisted of moisture, ash, crude protein, crude fiber, ether extract, calcium, phosphorus, nitrogen free extract (NFE) and energy at 2.65, 57.27, 22.67, 11.58, 1.54, 17.53, 0.58, 4.29 % (air dry basis) and 1,767 cal/g, indicating that blue swimming crab waste contained an equivalent high amount of protein and calcium. The study of Ferris et al. (1995) with analysis of food waste from 2 areas, showed ash content at about 2.52-3.63% and protein 11.88-19.65% from the university dining center, and ash value at 2.94-4.56% and protein at 15.24-19.77% from two military facilities, making it suitable for animal feeding. Meanwhile, BSCW could be used as animal feed as a source of protein and minerals and most especially as raw feed ingredient for poultry layers that need high amount of calcium which might cause an effect in the yield and quality of chicken in most particular to the strength or hardness of the egg shell.

Conclusion

The research on the grinding of the blue swimming crab waste discarded after production for various beneficial purposes as needed and with the focus of this study on the application as raw materials for animal feed production. It was found that grinding of crab waste of low initial moisture (7.30 ± 0.43% w.b.) and a grinding

speed of 1054 rpm, resulted to a good grinding capacity in terms of high grinding performance, finer size of the materials after grinding and low work power and required grinding energy. Analysis of the animal feed value by proximate analysis showed that blue swimming crab waste contained high values of ash, protein and calcium thus could be considered as good source of protein and minerals and most particularly, as raw feed material for layers which need a high level of calcium thus positively affecting the yield and quality of eggs and especially the strength of egg shell.

KEYWORD : Waste, Blue swimming crab, Animal feed, Grinding

Table 1 Effect of initial moisture content of crab waste according to days of sun drying on grinding performance at fixed 1,054 rpm grinding speed

Days of sun drying	Moisture content (% w.b.)	Grinding capacity (kg/hr)	Particle size (mm)	Power required (kW)	Energy required (kW.hr/kg)
3	8.94±0.46 a	15.59±0.63 a	0.93±0.02 a	1.59±0.02 a	0.100±0.003 a
4	7.90±0.59 b	18.57±0.57 b	0.84±0.03 b	1.46±0.03 b	0.079±0.004 b
5	7.30±0.43 b	20.36±0.51 c	0.78±0.03 c	1.32±0.03 c	0.065±0.003 c

Pairs of values in the same column followed by the same letter are not significantly different ($P \geq 0.05$); values after ± are standard deviations; n = 3.

Table 2 Effect of grinding rotational speed on grinding performances at initial moisture content of waste of fixed7.30±0.43 % wet basis

Grinding speed (rpm)	Grinding capacity(kg/hr)	Particle size (mm)	Power required (kW)	Energy required (kW.hr/kg)
843	16.53±0.50 a	0.93±0.02 a	1.26±0.06 a	0.076±0.003 a
1054	20.36±0.51 b	0.73±0.03 b	1.32±0.03 a	0.065±0.003 b
1405	19.02±0.44 c	0.75±0.06 b	1.48±0.04 b	0.078±0.003 a

Pairs of values in the same column followed by the same letter are not significantly different ($P \geq 0.05$); values after ± are standard deviations; n = 3.

(a) Steamed crab (b) Local processing (c) Waste left as discards

Figure 1 Blue swimming crab

Figure 2 Illustration of the crab waste grinding machine

Figure 3 Grinding test (left) and ground blue swimming crab waste (right)

REFERENCES

Amornthewaphat, N. 2010. Animal feed production. Bangkok: Odeon Store Publisher, 232. (in Thai)

AOAC. 1995. Official method of analysis of the Association of Official Analytical Chemistry. 16th ed. Washington DC.

Bochat, A., L. Wesolowski and M. Zastempowski. 2015. A comparison study of new and traditional designs of a hammer mill. Trans. *ASABE* 58(3): 585-596

Department of Fisheries. 2013. Fisheries Statistics of Thailand 2013. Information Technology Center, Department of Fisheries, Ministry of Agriculture and Cooperatives (in Thai)

Department of Fishery Products. 2015. Lectures on fishery product science and technology.. Faculty of Fisheries, Kasetsart University. 2015. Bangkok: Kasetsart University Press, 385 (in Thai)

Fang, Q., I Bölöni, E. Haque and C.K. Spillman. 1997. Comparison of energy efficiency between a roller mill and a hammer mill. *Applied Engineering in Agriculture* 13(5): 631-635

Ferris, D.A., R.A. Flores, C. W. Shanklin and M. K. Whitworth. 1995. Proximate analysis of food service wastes. *Applied Engineering in Agriculture* 11(4):567-572

Henderson, S. M. and R. L. Perry. 1976. *Agricultural Process Engineering*, 3rd Ed. Westport, Connecticut, U.S.A.: AVI Publishing Co, Inc.

Mozart, Y. 2013. Animal feed analysis. Bangkok: Chulalongkorn University Press, 294. (in Thai)

Patil, R.T., J. de Berrios, J. Tang, J. Pan and B. Swanson. 2005. Milling and particle size characterization of pin milled legumes. ASAE Paper No. 056077. St. Joseph, Mich.: ASAE

Probst, K.V., R.P. Kingsly Ambrose, R.L. Pinto, R. Bali, P. Krishnakumar and K.E. Ileleji. 2013. The effect of moisture content on the grinding performance of corn and corncobs by hammer milling. *Trans. ASABE* 56(3): 1025-1033

Thien Ratsamee, B. 2001. "Blue crab production: a new choice for farmers in 2000". *Aquatic Life Magazine* 12(145): 163-168 (in Thai)

Westendorf, M.L. 2000. Food waste to animal feed. Iowa; Iowa State University Press, 298

O-20-6

UTILIZATION OF gGOROHO h BANANA STEM (Musa acuminafe,sp) MEAL AS AN ALTERNATIVE FEED ON BROILER PERFORMANCE

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Abstract

A research evaluating the effects of utilization of "goroho" banana (*Musa acuminafe,sp*) stem meal as an alternative feed on broiler performance. "Goroho" banana stem was collected from Kawangkoan, Minahasa region in North Sulawesi Indonesia. 100 of one week old chick broilers strain CP 707 were used. Completely randomized design (CRD) with 4 treatments and 5 replications was used and each replication consisted of five birds. The treatments were R_0 = 0% of "goroho" banana stem meal in the ration; R_1 = 2.85 % of "goroho" banana stem meal in the ration ('goroho' banana stem substituting 5% corn); R_2 = 5.70 % "goroho" banana stem meal in the ration ('goroho' banana stem substituting 10% corn), and R_3 = 8.55 % "goroho" banana stem meal in the ration ('goroho' banana stem substituting 15% corn). Variables measured were ration consumption, average body gain, feed conversion, carcass percentage and percentage of abdominal lipid. Analysis of variance showed that there were no significant effects ($P>0.05$) on ration consumption and percentage of abdominal lipid; however, there were significant effects ($P<0.05$) on average body gain, feed conversion, and carcass percentage. Tukey test showed that average body gain and feed conversion of R_0 (863.39 g and 2.14) and R_1 (844.39 g and 2.19) were significantly higher compared to R_2 (733.66 g and 2.53) and R_3 (670.66 g and 2.71). While carcass percentage of R_3 (77.72%) was lower compared to R_0 (86.70 %), R_1 (84,12 %) and R_2 (82,40 %). It can be concluded that "goroho" banana stem meal can be utilized up to 2,85% (substituting 5% corn) in broiler ration.

INTRODUCTION

"Goroho' banana (*Musa acuminafe,sp*) is endemic banana in North Sulawesi, Indonesia. This species of banana has a special characteristic which the pill is still green although the fruit already ripe (Tasirin, 2012). The largest portion of banana tree component is the stem (60 %) compared to leaves (10%) and fruit (30%) (Munadjim, 1983). Its nutrient contents are : crude protein (4.81% %), crude fiber (27.73%), lipid (11.23 %), lignin (9.92%), and ash (23.12 %) (Hashrida, 2011); while Wina (2001) reported that nutrient compositions of banana stem are: crude protein 2.4 - 8.30%, crude fiber 13.40 - 31.70%, lipid 3.20 -8.10%, and ash 18.24 -24.76%. On the other hand, banana stem also contains lignin and tannin (Qotimah, 2000) which act as inhibitors to decrease dry matter and organic matter digestibility by binding protein (Zimmer and Cordesse, 1996). Tidi *et al*. (2011) also reported that tannin is a fenol compound that reduce organic matter digestibility especially protein by binding protein. Santoso (1987) reported that tannin affected digestibility in poultry by reducing amino acid digestibility due to depressing nitrogen retention.

Water content in banana stem ranged from 80 to 90%, so that it is easy to decay. Banana stem can be used as in-conventional feed (Mathius and Sinurat, 2001).

The utilization of banana stem in ruminant's diet had no negative effect on young sheep performance and showed increasing in N retention compared to control ration (Mathias *et al*, 2001). Only a few research had been conducted in utilizing banana stem in the diets on broiler performance.

The objective of this research was to evaluate the effects of utilization of "goroho" banana stem meal as an alternative feed on broiler performance.

MATERIALS AND METHODS

This research was conducted in Manado, North Sulawesi, Indonesia; used 100 one week old unsexed broilers strain CP 707. Completely Randomized Design (CRD) with 4 treatments and 5 replications was applied to this experiments. The treatments were : R0 = diet without 'goroho' banana stem meal; R1 = diet with 2.85% 'goroho' banana steam meal (substitute 5% corn); R2 = diet with 5.70% 'goroho' banana stem meal (substitute 10% corn); R3 = diet with 8.55% 'goroho' banana stem meal (substitute 15% corn). The chicken had been raised for four weeks (up to 35 days old). Variable measured were ration consumption, average body gain, ration conversion, carcass percentage, and abdominal lipid percentage. Data were analyzed by Analysis of variance (Anova), and the

differences among means by Tukey test (Steel and Torrie, 1990).
Table 1. showed nutrient compositions and metabolic energy of ingredients and Table 2 showed composition and nutrients of the rations.

RESULTS AND DISCUSSION

Data of variables measured of the utilization of 'goroho' banana stem meal as an alternative feed on broiler performances were showed in table 3.

The data showed that averages of feed consumption in this experiment ranged from 1812.97 to 1849.69 grams/head. There were no significant effects (P>0.05) on feed consumption by utilization of 'goroho' banana stem meal in the ration. It is assumed that because of metabolic energy content of the four treatments was so close, therefor no significant effects on feed consumption due to feed consumed was to fulfill metabolic energy needs.
Averages of body gain ranged from 670.66 to 863.39 grams/head. Utilization of 'goroho' banana stem meal in the ration significantly (P<0.05) affected averages of broiler body gain. Tukey test showed that there were no significant different (P>0.05) between R0 and R1, and between R2 and R3; however, R0 and R1 were higher in average body gain compared to R2 and R3 (P<0.05). It is assumed that the inhibitor agents such as tannin in banana stem bound protein and minerals that caused decreased the availability of protein and minerals. Wina (2001) reported that liquid of banana stem contained 0.14 - 4.96 of condensed tannin; so that by increasing banana stem in the ration could decrease body gain of the chickens.
Averages of feed conversion and carcass percentage in this experiment were 2.14 - 2.71 and 77.72 - 86.70%, respectively. There were significant effects of utilization of banana stem in the ration (P<0.5) on feed conversion and carcass percentage.
Tukey test showed that feed conversion of R0 was no significantly different (P>0.05) compared to R1; however, feed conversion of R0 and R1 were lower (P<0.5) than R2 and R3. There was no significant difference (P>0.05) of carcass percentage among R0, R1, and R2, however, carcass percentage of R0, R1, and R2 were higher compared to R3. As in body gain, tannin had negative effects on feed conversion and carcass percentage. Increasing of banana stem portion in the ration increased feed conversion, on the other hand, increasing banana stem in the ration decreased carcass percentage of the chickens. This result was supported by Zimmer and Cordesse (1996), who reported that tannin could decrease dry matter and organic matter digestibility. Tidi *et al*. (2011) also reported that tannin is a fenol compound that reduce organic matter digestibility especially protein by binding protein. Santoso (1987) reported that tannin affected digestibility in poultry by reducing amino acid digestibility due to depressing nitrogen retention. So that increasing banana stem meal in the ration increased tannin content and this condition could suppress digestibility, on return, caused in increasing feed conversion and decreasing carcass percentage.
Averages of abdominal lipid percentage ranged from 1.90 to 2.26 %. There was no significant effect of utilizing banana stem meal in the rations on abdominal lipid percentage. The abdominal lipid percentages in this research were in the range of Becker et al (1979) report that abdominal lipid percentage of five broiler strains were 0.73 - 3.78%.

CONCLUSION

'Goroho' banana (*Musa acuminafe,sp*) stem meal as an alternative feed could be utilized up to 2,85 % (substituting 5 % corn) in broiler ration.

KEYWORD : Banana, stem, performance, broiler

Table 1. Nutrients Composition and Metabolic Energy of Ingredients Used in the Experiment of Utilization of 'Goroho Banana Stem Meal as an Alternative Feed on Broiler Performance

Ingredients	Crude Protein	Lipid	Crude Fiber	Ca	P	ME
Corn *	9.42	5.17	2.15	0.22	0.60	2983.50
Rice bran *	13.44	6.07	6.35	0.19	0.73	2695.50
Coconut meal *	24.74	9.36	15.02	0.11	0.47	3279.75
Fish meal*	55.59	12.10	0.017	5.10	2.08	3470.40
Soybean meal*	40.38	9.91	6.56	0.24	0.58	2540.00
Banana stem meal**	2.53	1.49	23.48	0.85	0.14	2792.00
Coconut oil**		100.00				8812.00
Mineral mix**				5.38	1.44	

Sources : *) Dengah, *et al*, 2016
**) Makanan Ternak Laboratory, Padjadjaran University, 2015

Table 2. Ingredients and Nutrients Composition of the Ration

Ingredients	R0	R1	R2	R3
Corn	57	54.15	51.3	48.45
Rice bran	5	5	5	5
Coconut meal	9	9	9	9
Fish meal	12	12	12	12
Soybean meal	15	15	15	15
Banana stem meal	0	2.85	5.7	8.55
Coconut oil	1	1	1	1
Mineral mix	1	1	1	1
Total	100	100	100	100
Nutrient composition	R0	R1	R2	R3
Crude Protein	21.00	20.80	20.60	20.41
Lipid	8.03	7.93	7.82	7.72
Crude Fiber	3.88	4.49	5.10	5.70
Ca	0.85	0.86	0.88	0.90
P	0.77	0.76	0.75	0.73
ME	3016.12	3010.66	3005.21	2999.76

Calculated based on Table 1.

Table 3. Averages of Feed Consumption, Body Gain, Feed Conversion, Carcass Percentage and Abdominal Lipids during the Experiment of Utilization of 'Goroho' Banana Stem Meal as an Alternative Feed.

Parameter	Treatments			
	R_0	R_1	R_2	R_3
Feed Consumption *(gram/head/)*	1849,69	1838.03	1843.49	1812.97
Body gain *(gram/head)*	863.39 ^{a}	844.39 ^{a}	733.66 ^{b}	670.66 ^{b}
Feed Conversion	2,14^{a}	2,19 ^{a}	2,53 ^{b}	2,71 ^{b}
Carcass Percentage	86,70^{a}	84,12^{a}	82,40^{a}	77,72^{b}
Abdominal lipid Percentage	2,08	2.20	2.26	1.90

Different Superscripts in the same row showed there were significant differences (P< 0.05).

REFERENCES

Amoah, J. K., E. A. Martin, A. J. Barroga, E. P. Garillo and I. Domingo. 2012. Calcium and phosphorus requirements of Japanese quail layers.. Journal of Applied Biosciences 54: 3892– 3900.ISSN 1997–5902

Association of Official Analytic Chemist (A.O.A.C). 1995. Official Method of Analysis. 16th Ed. The Association of Official Analytic Chemist Inc, Washington, DC.

Bagau, B, 2010. Pengolahan Limbah Padat Ikan Cakalang dengan HCl dan NaOH. UNPAD. Bandung.

Bagau, B, 2012a. Analisa Berbagai Komponen Limbah Padat Ikan Cakalang. Fapet UNPAD.

Bagau, B, 2012b. Bioavailabilitas Kalsium dan Fosfor special bone meal Produk Hidrolisis Alkali Tulang ikan cakalang (*Katsuwonus pelamis* L) pada Ayam Broiler. Disertasi Pasca Sarjana Unpad.

Bagau, B, 2012c. Special Bone Meal. Aplikasi Alkali Alami dan Sintetik Tulang Ikan Cakalang. Buku, Unpad Press. ISBN 978-602-8743-85-3

Cheng, T.K. and C.N. Coon, 1990. Effect on layer performance and shell quality of switching limestones with different solubilities. Poult. Sci., 69:2199-2203.

Florescu, S., S. Paraschiv and A. Florescu, 1986. Utilization of calcium from different sources in feeding laying hens. Rev. de Cres. Anim., 36: 45-51.

Makled, M. N. and O.W. Charles, 1987. Eggshell quality as influenced by sodium bicarbonate, calcium source, and photoperiod. Poul, Sci., 66: 705-712.

Oliveira, J. D. F., D. L. Oliveira and A. G. Bertechini, 1997. Calcium level, particle size and feeding form of Limestone on the performance and egg quality of laying hens in the second laying cycle. Ciencia-EAgrotecnologia, 21: 502-510.

Steel, R.G.D and J.H. Torrie. 1990. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Ed. McGraw-Hill Book Co., New York.

NRC, 1994. Nutrition Requirement of Poultry, (9th Rev.Eds), National Academy Press, Washington DC.

Rabon, H.W., D.A. Roland, M. Bryant, D.G. Barnes, and S. M. Laurent, 1991. Influence of sodium zeolite A with and without pullet-sized limestone or oyster shell on eggshell quality. Poult. Sci., 70: 1943-1947.

Raymund, A. L 2003. Project on quail raising shows promising results Patrick Raymund A. Lesaca. Bureau of Agricultural Research http://www.bar.gov.ph/news/quail_raising.asp

Richter, G., G. Kiessling, W.I. Ochrimenko and H. Ludke, 1999. Influence of particle size and calcium source on limestone solubility *in vitro*, performance and eggshell quality in laying hens. Archiv. Fur. Geflu., 63: 208-213.

Scheideler, S. E., 1998. Eggshell calcium effects on egg quality and Ca digestibility in first-or third-cycle laying hens. J. Appl. Poult. Res., 7: 69-74.

Sultana F, M.S. Islam, and M.A.R. Howlider. 2007. Effect of dietary calcium sources and levels on egg production and egg quality. International Journal of Poultry Science. 6(2): 131-136.

O-20-8

Effects of Different Feed Processing Methods on Growth Performance in Broiler chickens

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OBJECTIVE

Improvement of feed utilization as well as reduction of wasted feed have been the crucial factor for reducing production cost in broiler diets.

The physical form (mash vs pellet) of feed is a crucial factor in meat yield of broiler. Proper feed processing such as pelleting has commonly employed in poultry industry to reduce feed wastage, improve performance and reduce overall feed cost (Corzo et al., 2011). It has been well documented that feed pelleting reduces feed waste, avoids ingredient segregation and improves diet flowability compared to mash diet.

Therefore, the aim of this study was to investigate growth performance and gut microbiology of broiler chickens fed diets with different feed forms and different processed pellet.

METHODOLOGY

This experiment was designed to evaluate the interaction of feed processing on the growth performance of broilers. A total of 360 broilers (Ross 308; average BW 541 ± 5.7 g) were randomly assigned based on BW and sex to 3 dietary treatments including mash, simple pellet (SP) and expanded pellet (EP) forms. Each treatment had 6 replicate pens with 20 broilers (10 males and 10 females) per pen. Prior to the experiment, the birds were fed a standard broiler starter diet and management from d 1 to 14. Isocaloric and isonitrogenous diets were prepared three different diets form (Mash, SP and EP).

The mash diet was formulated to contain 13.18 MJ/kg of ME, 210.0 (gr/kg diet) CP, and 11.0 (gr/kg diet) total lysine; supplemented with vitamins, minerals and AA to meet or exceed the nutrient requirements (Table 1) listed in Ross 308 nutrition specification (Aviagen, 2014). For the SP diet, the mash diet was steam conditioned to 75℃ and pelleted using a 220 hp pellet mill (Model; 12 type, Matador, Denmark) with a 2.8 mm die in diameter. The EP was produced by subjecting the mash diet to a 300 hp expander (Model M12, Matador, Denmark) with 180 amperes, a gap opening of 39% and temperature of 105 ℃ . The expanded diet was further processed through a pellet mill similar to the one used for the SP diet. The experiment duration was 21 d, and the final BW was approximately 1.5 kg.

The birds were housed in rice hull-covered floor pens. Each pen was provided with a self-feeder and hanging bell drinker to allow free access to feed and water. The house temperature was 23℃ and lighting was provided for 23 h/d.

The birds were individually weighed at the start of the trial and on d 35. Feed that was not consumed was weighed at end of experiment and feed intake was calculated for d 14-35. Body weight (BW) gain, feed intake, and feed efficiency (G:F) were corrected for the weight of dead birds. Nutrient balance trials were conducted during the last week of the feeding trial to determine retention of dry matter (DM), crude protein (CP) and gross energy (GE). From d 28 onwards, two birds from each replicate were allocated in individual cages (one bird/cage) to facilitate the collection of excreta samples. The diets containing 2.5 g/kg chromium as an indigestible marker was given from d 28 onwards. Excreta samples (about 100 g/d per bird) were collected from each bird during d 33-35. The excreta samples were dried in a forced air drying oven at 60℃ for 72 h and ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas scientific, Swedesboro, NJ, USA) using a 1-mm screen. The nutrient retention was calculated as: nutrient retention (%) = 100 − [100 × (% Cr in feed/% Cr in excreta) × (% nutrient in excreta/% nutrient in feed)].

On the last day of experiment, 72 birds (2 birds per replicate) were randomly selected and slaughtered by cervical dislocation. Samples of digesta from the ileum and caeca of each of the two birds were collected and stored at -20℃ for the analyses of monosaccharaides. Two Eppendorf tubes were filled (1.5 g) with ileal digesta and centrifuged (4℃ , 3,500 × g, 10 min) and the viscosity of the supernatant (1 mL) was measured at 25℃ using a digital viscosimeter (Brookfield Digital DV-III Ultra Programmable Rheometer) as indicated by Lazaro et al. (2003).

A subsample of the ileal digesta was dried at 60℃ and ground for chemical analyses.
Experimental diets and excreta samples were analyzed in triplicate for DM (Method 930.15), crude protein (CP; method 990.03) and calcium, and phosphorus (method 985.01) according to AOAC (2007). Gross energy of diets and excreta were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL). Chromium concentration was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979). Amino acid composition of feed samples and ileum contents were determined by HPLC (Waters 486, Waters Corp., Milford, MA, USA) after acid hydrolysis (Knabe et al., 1989). The methionine and cysteine were determined following oxidation with performic acid (Moore, 1963).
The microbiological assay of gizzard, duodenum, jejunum, ileum and cecum chyme were carried out by the procedure suggested by Lee et al. (2014). In short, of 1 g of mixed content was diluted with 9 ml of Butterfields phosphate buffer solution, followed by further serial dilutions in butter-fields phosphate buffer dilution solution. Duplicate plates were then inoculated with 0.1 ml sample and incubated. The microbial groups enumerated were *Lactobacillus* spp. (MRS agar + 0.02% NaN3 + 0.05% l-cystine hydrochloride monohydrate) and *Clostridium* spp. (Tryptose sulphite cycloserine agar, Oxoid, Hampshire, UK). The microbial populations were log transformed before statistical analysis. The pH of gizzard, duodenum, jejunum, ileum and cecum chyme was determined by pH meter (Basic pH Meter PB-11, Sartorius, Germany).
The fatty acid profile was measured using the previously reported methods (Kim et al., 2014). In brief, all layers of adipose tissue from the longissimus dorsi were utilized for fatty acid determination. Separation of fatty acid methyl esters was achieved by gas chromatography (Shimdzu, GC-17A, Japan) equipped with 100 m fused-silica capillary column with i.d. of 0.25 mm, a 0.20 m film coating and SPTM-2560 column stationary phase (Sigma-Aldrich Co. LLC) and a flame ionization detector. Oven temperature was maintained at 175℃ for 30 min, increased at 5℃ per min to 215℃, and then increased at 10℃ per min to 235℃. Injector and detector temperature was maintained at 260℃. Methyl ester standards were used to identify sample fatty acid methyl esters.
Data generated in this experiment was subjected to statistical analysis using the GLM- one-way analysis of variance test using the SAS statistical software package version 8.2 (SAS Inst. Inc., Cary, NC, USA), and broiler chickens were experimental units for measuring the digestibility of nutrients, fatty acid profile, and all intestinal sampling. P-values ≤ 0.05 were considered statistically significant.

RESULTS

At 35 days old, the broilers fed the SP and EP diet exhibited higher final body weight (P<0.05) compared with those that received mash diets, which showed increased BW by 6.1 and 6.5%, respectively. The reduced final body weight of the broilers was reflected in decreased weight gain, with the broilers in the SP and EP treatments exhibiting weight gain values that were 3.9 and 4.1% higher (P<0.05) compared with those in the mash treatment. The FI was tended to increase (P=0.065) by the SP and EP treatments. However, there was no difference in G:F (P<0.05) between the broilers among the treatments.
Values for PDI and hardness were higher for EP compared with SP (P<0.01).
Digestibility of DM was unaffected among FP groups (P>0.05). Broiler chickens fed SP diet had higher digestibility of GE (P<0.05) compared with mash diet. The SP and EP treatments reduced the digestibility of CP (P<0.05) by 2.9 and 2.7% for SP and EP, respectively, compared with mash diet. The reduced CP digestibility of the broilers in processed feed was reflected in decreased AA digestibility, with the broilers in the EP treatments exhibiting less digestibility in isoleucine, leucine, lysine, methionine, phenylalanine, threonine, cysteine and glutamine compared with broiler fed mash diet (P<0.05).
Feeding pellets increased (P<0.01) pH in gizzard and duodenum, and decreased pH in ileum (P<0.01). Generally, feed processing did not affect *lactobacillus* spp. population in GI tract of birds except in duodenum (P<0.05), where the population of *lactobacillus* spp. was greater in chickens fed mash diet (P<0.05). Compared to birds fed EP diet, the colonization of Clostridium spp. decreased in birds fed mash diet (P<0.01).
Simple pellet diet increased the ratio of C16:0 in thigh meat, whereas the ratio of linolenic acid decreased (P<0.05). There was also a trend (P=0.087) for decrease in C10:0 and C22:1 with EP diet.

CONCLUSION

In conclusion, feed processing and in particular simple pelleted diet, improved final gain in broiler chickens due to improved digestibility of GE and pellet physical quality. Expansion process improved pellet quality, however, it did not show any performance preference compared with simple pelleted diet. Simple pellet diet decreased the

concentration of linolenic acid in thigh as a nutritious fatty acid in meat. The pH of GI changed when chickens fed processed diet, following change in colonization of *Clostridium* spp.

KEYWORD : Broiler chickens, pellet, mash, expanded pellet

Table 1. Ingredient and chemical composition of basal diet (as-fed basis)

Item	Starter (d 0 - 14)	Finisher (d 15 - 35)
Ingredients (%)		
Corn	55.40	55.19
Wheat	2.00	5.00
Soya bean meal (45% CP)	24.78	23.37
Fish meal	5.00	-
Corn gluten	4.76	6.89
Rapeseed meal	2.00	2.00
Animal fat	2.85	3.31
Dicalcium phosphate	1.26	1.69
Limestone	0.62	0.92
Vitamin premix[1]	0.10	0.10
Mineral premix[2]	0.10	0.10
Salt	0.20	0.20
$_{L}$-Lysine (78%)	0.48	0.75
Threonine (98.5 %)	0.06	0.06
$_{DL}$-Methionine (98%)	0.29	0.32
Choline chloride (25%)	0.10	0.10
Chemical composition, calculated		
Metabolic energy (kacl/kg)	3,100	3,150
Crude protein (%)	23.00	21.00
Calcium (%)	0.95	0.92
Available phosphorus (%)	0.45	0.41
Lysine (%)	1.25	1.10

[1] Supplied per kg diet: 9,600IU vitamin A, 1,800IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid,18 mg ethoxyquin.
[2] Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

Table 2. Effect of processing feed on growth performance pellet quality and apparent fecal digestibility of broilers.

Items	Feed processing[a]			SEM[b]	*P*-value
	M	SP	EP		
Growth performance					
Intial weight, g	541	542	541	1.7	0.84
Final weight, g	1429[b]	1488[a]	1491[a]	17.2	0.028
Weight gain, g	888[b]	946[a]	950[a]	17.6	0.033
Feed intake, g	2033	2152	2145	38.4	0.065
G:F, g/kg	437	440	443	6.15	0.81
Pellet quality					
PDI %[c]	-	89.3	91.9	0.49	<0.01
Hardness	-	2.35	2.95	0.12	<0.01
Digestibility, %					
Dry matter	73.2	73.8	73.9	0.49	0.531
Gross energy	75.5[b]	77.1[a]	76.0[ab]	0.42	0.034
Crude protein	69.1[a]	67.1[b]	67.2[ab]	0.55	0.027

[a]Feed Processing: M = mash, SP = simple pellet, EP = expanded pellet.

[b]Standard error of means.

[c]PDI: Pellet durability index.

[ab]Values with different superscripts of the row significantly differ (P<0.05).

Table 3. Effect of feed processing on apparent ileal amino acid digestibility in broilers.

Items	Feed processing[a]			SEM[b]	*P*-value
	M	SP	EP		
Indispensable					
Arginine	66.2	64.8	64.2	0.68	0.116
Histidine	61.8	61.7	60.9	0.66	0.57
Isoleucine	63.8[a]	62.8[a]	60.4[b]	0.67	<0.01
Leucine	70.7[ab]	71.0[a]	69.2[b]	0.47	0.029
Lysine	70.5[a]	69.1[ab]	67.4[b]	0.52	<0.01
Methionine	72.6[a]	71.0[ab]	65.4[b]	1.62	0.01
Phenylalanine	65.6[a]	64.8[ab]	61.9[b]	0.83	0.011
Threonine	47.0[a]	45.3[ab]	43.2[b]	0.77	<0.01
Valine	52.3	52.2	51.6	0.67	0.732
Dispensable					
Alanine	61.4	61.5	60.2	0.53	0.187
Asparagine	59.2	59.5	59.3	0.49	0.917
Cysteine	58.1[a]	56.9[ab]	55.8[b]	0.53	0.02
Glutamine	68.2[a]	68.1[a]	65.5[b]	0.54	<0.01
Glycine	50.8	51.4	49.9	0.54	0.201
Serine	59.5	59.4	59.7	0.57	0.937
Tyrosine	56.8	56.9	56.8	0.37	0.957

[a]Feed Processing: M = mash, SP = simple pellet, EP = expanded pellet.

[b]Standard error of means.

[ab]Values with different superscripts of the row significantly differ (P<0.05).

Table 4. Effect of feed processing on pH value and bacterial count in different section of digestive tract of broilers.

Items	Feed processing[a]			SEM[b]	*P*-value
	M	SP	EP		
pH					
Gizzard	3.52[b]	4.07[a]	4.01[a]	0.051	<0.01
Duodenum	6.30[b]	6.53[a]	6.63[a]	0.042	<0.01
Jejunum	6.54	6.38	6.43	0.047	0.055
Ileum	7.48[a]	7.28[b]	7.25[b]	0.041	<0.01
Cecum	6.26	6.2	6.27	0.056	0.632
Lactobacillus spp. (log^{10} cfu/g)					
Gizzard	7.21	7.08	7.06	0.079	0.359
Duodenum	7.4[a]	7.31[ab]	7.22[b]	0.042	0.015
Jejunum	7.62	7.57	7.54	0.054	0.563
Ileum	8.2	8.11	8.02	0.085	0.349
Cecum	8.53	8.41	8.41	0.053	0.269
Clostridium spp. (log^{10} cfu/g)					
Gizzard	4.17[b]	4.38[a]	4.35[a]	0.04	<0.01
Duodenum	4.58	4.63	4.64	0.057	0.664
Jejunum	5.58	5.5	5.52	0.083	0.75
Ileum	6.20[b]	6.31[ab]	6.39[a]	0.053	0.058
Cecum	6.61	6.62	6.59	0.075	0.952

[a]Feed Processing: M = mash, SP = simple pellet, EP = expanded pellet.

[b]Standard error of means.

[ab]Values with different superscripts of the row significantly differ (P<0.05).

Table 5. Effect of feed processing on fatty acid profile of thigh meat in broilers.

Items (%)	Feed processing[a]			SEM[b]	*P*-value
	M	SP	EP		
Octanoic (C8:0)	0.1	0.1	0.11	0.008	0.362
Decanoic (C10:0)	0.07	0.07	0.06	0.006	0.087
Lauric (C12:0)	0.28	0.3	0.28	0.009	0.394
Myristic (C14:0)	1.34	1.34	1.31	0.026	0.519
Palmitic (C16:0)	21.7[b]	23.6[a]	23.5[a]	0.42	<0.01
Palmitoleic (C16:1c)	5.55	5.68	5.81	0.131	0.384
Stearic (C18:0)	8.32	8.25	8.32	0.162	0.951
Oleic (C18:1c9)	37.9	37.7	37.9	0.4	0.901
Linoleic (C18:2n-6)	22.8	22.9	22.1	0.59	0.521
Linolenic (C18:3n-3)	0.97[a]	0.90[b]	0.93[ab]	0.019	0.035
Arachidonic (C20:4n-6)	0.1	0.09	0.1	0.005	0.556
Behenic (C22:0)	0.12	0.1	0.1	0.009	0.355
Erucaic (C22:1)	0.07	0.09	0.07	0.006	0.06
Lignoceric (C24:0)	0.43	0.41	0.4	0.053	0.938

[a]Feed Processing: M = mash, SP = simple pellet, EP = expanded pellet.

[b]Standard error of means.

[ab]Values with different superscripts of the row significantly differ (P<0.05).

REFERENCES

AOAC., 2007. Official Methods of Analysis of the Association of Official Analytical Chemists International, 18th ed. Gaithersburg, MD, USA.

Aviagen, R., 2014. Ross Broiler Management Manual. http://pt. aviagen. com/assets/Tech_Cen ter/Ross_Broiler/Ross_Broiler_Manual_. 9, 350-364.

Corzo, A., Mejia, L., Loar R. E., 2011. Effect of pellet quality on various broiler production parameters. J. Appl. Poult. Res. 20, 68-74.

Fenton, T.W., Fenton, M., 1979. An improved method for chromic oxide determination in feed and feces. Can. J. Anim. Sci. 59, 631–634. Hadley, E.B., Hancock, R.E., 2010. Strategies for the discovery and advancement of novel cationic antimicrobial peptides. Curr. Top. Med. Chem. 10, 1872–1881.

Kim, J.S., Ingale, S., Lee, S.H., Choi, Y.H., Kim, E.H., Lee, D.C., Kim, Y.H. Chae, B.J., 2014. Impact of dietary fat sources and feeding level on adipose tissue fatty acids composition and lipid metabolism related gene expression in finisher pigs. Anim. Feed Sci. Technol. 196, 60-67.

Knabe, D.A., LaRue, D.C., Gregg, E.J., Martinez, G.M., Tanksley Jr., T.D., 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67, 441–458.

Lee, S.H., Ingale, S.L., Kim, J.S., Kim, K.H., Lokhande, A., Kim, E.K., Kwon, I.K., Kim, Y.H. Chae, B.J., 2014. Effects of dietary supplementation with Bacillus subtilis LS 1–2 fermentation biomass on growth performance, nutrient digestibility, cecal microbiota and intestinal morphology of weanling pig. Anim. Feed Sci. Technol. 188, 102-110.

Moore, S., 1963. On the determination of cystine as cysteric acid. J. Biol. Sci. 38, 235–237.

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THE EFFECTS OF UTILIZATION OF DIFFERENT PROCESSING TECHNIQUES OF SKIPJACK BONE MEAL IN THE RATION ON EGG PRODUCTION OF QUAIL

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ABSTRACT

A research was conducted to evaluate the effects of utilization of different processing techniques of skipjack fishbone (*Katsuwanus pelamis L*) meal in the diets on Japanese quail (*Coturnix coturnix japonica*) performance. The present study has been conducted for six weeks using 120 five weeks old quail birds. Experimental design used was a Completely Randomized Design with six treatments and four replications. Treatments were: (T_0) = *commercial bone meal* ; (T_1) = *Green fish bone meal* ; (T_2) = *Raw fish bone meal* ; (T_3) = *Steam fish bone meal* ; (T_4) = *Calcinated fish bone meal* ; and (T_5) = Special fish bone meal. The rations were formulated iso-protein and iso-energy. Variables measured were: feed consumption, average daily gain, feed efficiency ratio, hen day production, and egg weight. Research results showed that there were no significant effects (P>0.05) on feed consumption, average daily gain, hen day production, and feed efficiency ratio; but there was a significant effect (P<0.05) on egg weight. Feed consumption during the research were 944.07-951.51 g, daily gain were 49.71 - 50,90 g; hen day production were 42.86 - 46.79%; feed efficiency ratio were 0.53 - 0.56; and egg weight were 9.07 - 9.51 grams. Egg weight in T5 (special fishbone meal) was higher (9.51 g) compared with T_0 (9.07 g), and there were no significant different (P>0.05) among T5 and T_1, T_2, T_3, and T_4 . It can be concluded that different processing methods of fishbone as calcium sources in the diets did not influence feed consumption, daily gain, feed conversion ratio, hen day production, but T_5 (special fishbone meal) increased egg weight of Japanese quails.

INTRODUCTION

Laying hens or quail birds should be given proper calcium rich feed in their diet not only for the formation of egg shell but also for the high quality of egg shell, necessary for the prevention of breakage during handling and hatching. Quail birds require high calcium (Ca) and phosphorus (P) in the diet in order to provide enough Calcium and Phosphorus stored to be mobilized when needed at the peak egg production period. As in the case of hen, a deficiency of calcium in the diet of quails cause decline in egg production. One of the best calcium sources is fishbone as a waste product of canned fish in North Sulawesi, Indonesia (Bagau, 2012b).

Most of the canned fish produced in North Sulawesi, Indonesia are originated from *pelagis* fish (tuna, skipjack, and deho), left quite enough waste products. Solid waste which is consisted mostly of bone, produced about 8,2% from total fish canned (Bagau, 2012b). It is hipothesized that eventhough the fishbone is processed with different methods, the quality is still not much affected or still remain the same (Bagau, 2012c).

Raising quails provides livelihood and opportunity for income generation (Raymund, AL 2003). One of the areas that need particular attention is nutrition of the birds. The Japanese quail (Commix coturnix japonica) is the predominant breed being raised, largely for the production of table eggs (Amoah, *et al*., 2012).

Yet, little is known about the effects of a varying fishbone processing methods on bioavailability of Ca and P of quail birds. The present study was carried out to elaborate the effect of different processing techniques of skipjack (*Katsuwanus pelamis L*) fihbone in the diets on egg production of Japanese quails (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

A total of 120 Japanese quail (*Coturnix coturnix japonica*) at an initial laying phase, aged 5 weeks were randomly divided into 24 experimental units of 5 chicks each and each diet was offered at random in each pen. Fresh water and feed were provided *ad libitum* throughout the experimental period. Birds were housed in an experimental cage for quails, with trough feeders and drinkers, grouped in galvanized wire cages with dimensions 33 × 33 × 14 cm. The lighting program was of 17 hours, provided as natural plus artificial illumination.

The dietary treatments were in a Completely Randomized Design with 6 treatments and 4 replications (Steel and Torrie, 1990). Each treatment was replicated four times. Each replicate had five birds. Treatments were skipjack (*Katsuwanus pelamis L*) fishbone meal from "PT. Nichindo Manado Suisan" Amurang, South Minahasa Regency, processed and arranged into each treatment as follow: T_0 = commercial bone meal (as a control); T_1 = green

fishbone meal; T_2 = raw fishbone meal; T_3 = steam fishbone meal; (T_4) = calcinated fishbone meal; and T_5 = special fishbone meal.

Diets (Table 1) were formulated to meet the requirement of quails according to the National Research Council (NRC, 1994). The birds were raised under standard management conditions and feed and water were supplied *ad libitum* throughout the experimental period.

Feed intake was determined from the weight difference obtained between the amounts of feed provided at the beginning and leftovers at the end of 42 days period. Egg production was calculated by the ratio of number of eggs produced by the number of birds housed in the period, multiplying the value by one hundred. Feed conversion was calculated by dividing the feed intake (kg/bird) by the number of eggs produced.

Data collection

The performance characteristics monitored were: feed intake, body weight gain, and feed conversion ratio (FCR), hen day production, and egg weight. Feed intake and weight gain were recorded at the end of the experiment and feed conversion ratio (FCR) was calculated.

Eggs were weighed individually in a digital three-digit scale (0.001g) (Shimadzu, model BL-320H) and the values obtained were used for the calculation of the average egg weight.

Statistical analysis

The experimental feed samples were analyzed as described by AOAC (2005). The results were analyzed by General Linear Model (GLM) and Tukey's significant difference test was used to compare means (Steel and Torrie, 1990).

RESULTS AND DISCUSSION

The data obtained during the experimental period for different parameters were analyzed and the results were presented in Table 3. These findings indicated that improvement in performance of quails in both early and post peak production period can be achieved with dietary calcium level higher than the 2.5% recommended by the NRC (1994). The treatment diets in the present study have a calcium level of 2,12% (Table 2), which were slightly below the above recommended level.

The data presented in Table 3 showed that different fishbone meal sources in the diets did not influence ($P>0.05$) daily feed consumption, daily weight gain, feed conversion ratio, and hen day production. Egg weight was the only parameter measured that gave a significant different ($P<0.05$) among treatments. T5 (special fishbone meal) significantly ($P<0.05$) gave the highest egg weight compared with other treatments. As proposed at the beginning of the study that different methods of fishbone meal processing will result in the quality that is not much affected.

Feed consumption and feed conversion ratio of quails were both statistically similar and averaged 952.35 g/day and 0.54, respectively. Hen day production of quails was also similar and averaged 44.52%. The results also indicated that there was no significant ($P>0.05$) effect of different methods of fishbone processing irrespective of calcium sources on hen day production per day. The similarity of egg production revealed that different methods of fishbone processing as calcium sources in quails diets had no effect on hen day egg production. This result is in agreement with some previous research findings. Scheideler (1998), Makled and Charles (1987) observed that egg production did not significantly ($P>0.05$) differ due to various calcium sources. Florescu *et al*. (1986) supplied dietary calcium from various sources and found no significant different in egg production.

Average daily gain body weight of quails were statistically similar and averaged 50.18 g/day during trial period. At the end of the experiment result showed that body weight gain gave non significant ($P>0.05$) increased for every treatment irrespective of different fishbone processing methods. Cheng and Coon (1990), supplied dietary calcium from various sources and found no significant different in body weight and egg production. Oliveira *et al.* (1997), supplied dietary calcium from different sources and found no significant different in egg production and body weight.

Egg weight on different treatments (Table 3) was significantly ($P<0.05$) different. Egg weights on different fishbone processing methods were 9.07 g for T_0 (commercial bone meal); 9.33 g for T_1 (green fishbone meal); 9.37 g for T_2 (raw fishbone meal); 9.33 g for T_3 (steam fishbone meal); 9.36 g for T_4 (calcinated fishbone meal); and 9.51 for T_5 (special fishbone meal). Egg weight in the current finding is in agreement with some researchers. Sultana, *et al*., (2007) reported that giving different sources of calcium in quail diets and found that egg weight was ranged from 9.74 - 10.24 g.

Lack of influence on dietary calcium sources on egg weight obtained in current finding is in agreement with some

researchers. Richter *et al.* (1999) used dietary calcium from various source and found no significant difference in egg weight. Scheideler (1998) and Rabon *et al.* (1991) also observed that egg weight did not significantly differ due to various calcium sources.

Egg weight in the present study was significantly different (P<0.05) amongst treatment or amongst different fishbone processing methods. T5 (special fishbone) gave the highest (P<0.05) egg weight compared with other treatments. It is proposed that high egg weight in T5 (special fishbone) due to processing method employed on this treatment. Special fishbone was hydrolyzed by using NaOH 4% for 48 hours (as in Bagau, 2010 and 2012 procedures) to remove all collagen in the fishbone, so that calcium is in the form of available calcium for better used in quail diets.

CONCLUSION

Different processing methods of fishbone as calcium sources in the diets did not influence feed consumption, daily gain, feed conversion ratio, hen day production, but T_5 (special fishbone meal) increased egg weight of Japanese quails.

Table 1. Nutrient composition of feedstuffs used in the diets

Feedstuffs	Protein (%)	Crude Fiber (%)	Ether extract (%)	Ca (%)	P (%)	Energy (kcal/kg)
Yellow corn	9.42	2.15	5.17	0.22	0.60	2.983
Soybean meal	40.38	6.56	9.91	0.24	0.58	2.540
Copra meal	24.74	15.02	9.36	0.11	0.47	3.279
Fish meal	58.52	2.95	13.90	7.04	3.67	3.851
Rice bran	13.44	6.35	6.07	0.19	0.73	2.695
Commercial fishbone (T_0)				26.21	10.79	
Green bone meal (T_1)				22.99	10.14	
Raw bone meal (T_2)				24.61	10.05	
Steam bone meal (T_3)				29.12	12.17	
Calcinated bone (T_4)				30.89	13.14	
Special bone meal (T_5)				29.83	12.12	

Table 2. Ingredients and Nutrients Composition of the Ration

Ingredients	T0	T1	T2	T3	T4	T5
Yellow corn	53	53	53	53	53	53
Soybean meal	10	10	10	10	10	10
Copra meal	9	9	9	9	9	9
Fish meal	15	15	15	15	15	15
Rice bran	9	9	9	9	9	9
Fishbone meal	3	3	3	3	3	3
Topmix**)	1	1	1	1	1	1
Total	100	100	100	100	100	100
Nutrient composition	T0	T1	T2	T3	T4	T5
Crude protein (%)	21.24	21.24	21.24	21.24	21.24	21.24
Ether extract (%)	7.20	7.20	7.20	7.20	7.20	7.20
Crude fiber (%)	4.16	4.16	4.16	4.16	4.16	4.16
Ca (%)	2.01	1.91	1.96	2.10	2.15	2.12
P (%)	1.36	1.34	1.34	1.40	1.43	1.39
Metabolizable Energy (kcal/kg)	2950.80	2950.80	2950.80	2950.80	2950.80	2950.80

*) Calculated based on Table 1.

Table 3. Performance characteristics of Japanese quails fed six different fishbone meal in the diets

Parameters	Treatments					
	T0	T1	T2	T3	T4	T5
Feed consumption (g)	951.51	951.09	947.94	959.89	944.07	959.61
Daily gain (g)	49.88	49.85	50.02	50.72	49.71	50.90
Feed conversion ratio	0.53	0.55	0.55	0.54	0.56	0.56
Hen day production (%)	44.29	44.29	45.36	43.57	42.86	46.79
Egg weight (g)	9.07^{a}	9.33^{ab}	9.37^{b}	9.33^{ab}	9.36^{ab}	9.51^{b}

$^{a, b,}$ Means on the same row with different superscripts differ significantly ($P < 0.05$)

KEYWORD : fish, bone, egg, production, quail

REFERENCES

Amoah, J. K., E. A. Martin, A. J. Barroga, E. P. Garillo and I. Domingo. 2012. Calcium and phosphorus requirements of Japanese quail layers.. Journal of Applied Biosciences 54: 3892– 3900.ISSN 1997–5902

Association of Official Analytic Chemist (A.O.A.C). 1995. Official Method of Analysis. 16th Ed. The Association of Official Analytic Chemist Inc, Washington, DC.

Bagau, B, 2010. Pengolahan Limbah Padat Ikan Cakalang dengan HCl dan NaOH. UNPAD. Bandung.

Bagau, B, 2012a. Analisa Berbagai Komponen Limbah Padat Ikan Cakalang. Fapet UNPAD.

Bagau, B, 2012b. Bioavailabilitas Kalsium dan Fosfor special bone meal Produk Hidrolisis Alkali Tulang ikan cakalang (*Katsuwonus pelamis* L) pada Ayam Broiler. Disertasi Pasca Sarjana Unpad.

Bagau, B, 2012c. Special Bone Meal. Aplikasi Alkali Alami dan Sintetik Tulang Ikan Cakalang. Buku, Unpad Press. ISBN 978-602-8743-85-3

Cheng, T.K. and C.N. Coon, 1990. Effect on layer performance and shell quality of switching limestones with different solubilities. Poult. Sci., 69:2199-2203.

Florescu, S., S. Paraschiv and A. Florescu, 1986. Utilization of calcium from different sources in feeding laying hens. Rev. de Cres. Anim., 36: 45-51.

Makled, M. N. and O.W. Charles, 1987. Eggshell quality as influenced by sodium bicarbonate, calcium source, and photoperiod. Poul, Sci., 66: 705-712.

Oliveira, J. D. F., D. L. Oliveira and A. G. Bertechini, 1997. Calcium level, particle size and feeding form of Limestone on the performance and egg quality of laying hens in the second laying cycle. Ciencia-EAgrotecnologia, 21: 502-510.

Steel, R.G.D and J.H. Torrie. 1990. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Ed. McGraw-Hill Book Co., New York.

NRC, 1994. Nutrition Requirement of Poultry, (9th Rev.Eds), National Academy Press, Washington DC.

Rabon, H.W., D.A. Roland, M. Bryant, D.G. Barnes, and S. M. Laurent, 1991. Influence of sodium zeolite A with and without pullet-sized limestone or oyster shell on eggshell quality. Poult. Sci., 70: 1943-1947.

Raymund, A. L 2003. Project on quail raising shows promising results Patrick Raymund A. Lesaca. Bureau of Agricultural Research http://www.bar.gov.ph/news/quail_raising.asp

Richter, G., G. Kiessling, W.I. Ochrimenko and H. Ludke, 1999. Influence of particle size and calcium source on limestone solubility in vitro, performance and eggshell quality in laying hens. Archiv. Fur. Geflu., 63: 208-213.

Scheideler, S. E., 1998. Eggshell calcium effects on egg quality and Ca digestibility in first-or third-cycle laying hens. J. Appl. Poult. Res., 7: 69-74.

Sultana F, M.S. Islam, and M.A.R. Howlider. 2007. Effect of dietary calcium sources and levels on egg production and egg quality. International Journal of Poultry Science. 6(2): 131-136.

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Effects of porcine C8a-b polymorphisms on hemolytic complement activity

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Background

The complement system is a highly regulated complex cascade of proteins, which plays an important role in innate immune defence of host. Most of the complement components circulating in the blood plasma are produced in liver. Complement activation of three pathways (classical, alternative and lectin pathway) results in the generation of MAC, an assembly of distinct soluble plasma proteins C5b, C6, C7, C8 and C9, which allows killing of invading pathogens. Many reports indicated that the complement activation depends on various modes such as antibody-antigen complex (Janeway et al. 2001), lipid A (Galanos et al. 1971; Cooper and Morrison 1978), O-specific antigen (Pluschke and Achtman 1984), porin (Galdiero et al. 1984; Latsch et al. 1990), or lipopolysaccharide (Galanos et al. 1971; Morrison and Kline 1977).

C8 is an oligomeric serum protein containing three non-identical polypeptide chains C8a, C8b and C8g (Ng et al. 1987; Kaufman and Sodetzd 1994; Dewald et al. 1996; Plumb and Sodetz 2000). The C8a and C8g subunits are bound covalently through a disulfide linkage, whereas the C8b is associated via weaker, non-covalent bonds (Alper et al. 1983). Furthermore, human C8a-b genes were assigned in close proximity on chromosome 1 (Rogde et al. 1986 Theriault et al. 1992; Michelotti et al. 1995 ;Platteborze et al. 1996). Correspondingly, porcine *C8a-b (pC8a-b)* located on the same chromosome 6 by using IMpRH mapping (Khoa and Wimmers 2014). There were 3 SNPs detected in the pC8a (Nakajima et al. 1998). C8 deficiencies were associated with hemolytic complement activity (Alper et al. 1983), recurrent neisserial infections (Densen et al. 1983; Kaufmann et al. 1993) or *Xeroderma pigmentosum* (Giraldo et al. 1977).

Due to the important role of C8 in the complement system and in innate immune response mechanism, this study will, therefore, focus on screening SNPs within pC8a-b cDNA sequence, which may influence on hemolytic complement activity in pigs.

Materials and methods

Animals are from the porcine breeds such as Hampshire (n=1), Duroc (n=1), German Landrace (n=30), Pietrain (n=30), Vietnamese Muong Khuong (n=25), Berlin Miniature Pig (n=1) and F2 (Duroc x Berlin Miniature Pig, DUMI) (n=417) (Hardge et al. 1999 Janeway et al. 2001). Designing primers were based on nucleotide sequences on NCBI's GenBank database such as Clone xx-1c1 (DNA for both genes), DQ333200 (pC8a cDNA) and DQ333201 (pC8b cDNA) (Table 1). DNA and cDNA were extracted and synthesized from ear cuts and liver tissues, respectively (Janeway et al. 2001). PCR were performed in 20 ml total volume containing 50 ng of cDNA or 100 ng of genomic DNA, 0.2 mM of each primer (forward or reverse primer), 50 mM of each dNTP (Roth, Karlsruhe), 0.5 U of Taq polymerase (Sigma-Aldrich, Taufkirchen) in 1xTaq buffer and 1.5 mM of $MgCl_2$. PCR program was set up with an initial denaturation step of 94℃ for 4 min followed by 35-40 amplification cycles (denaturation at 94°C for 30 s, annealing at 54, 56 or 60°C for 30 s, elongation at 72°C for 1 min) and termination by an extension at 72°C for 5 min using a T1 Research Thermocycler (Biometra, Göttingen). PCR products were evaluated on 1% agarose TAE gels stained with ethidium bromide and then purified before sequencing using an ABI 3130 DNA Analyzer (Applied Biosystems, Darmstadt). All SNP sites were confirmed by an appropriate PCR/RFLP in presence of restriction enzymes or by resequencing.

Serum CH50 and AH50 were measured before and after inoculating *Mycoplasma hyopneumoniae*, MH (Stellamune, Mycoplasma, Pfizer, Karlsruhe), Aujeszky disease virus, ADV (Porcilis, Begonia Diluvac, Intervet, Tönisvorst), and Porcine Reproductive and Respiratory Syndrome, PRRS (Ingelvac PRRS MLV, Boehringer Ingelheim) vaccines for 417 DUMI animals at 6, 14 and 16 weeks of age, respectively (Wimmers et al. 2003). Enter of candidate genes and with hemolytic complement activity (CH50 and AH50) was, respectively, investigated for SNPs at nucleotide C1544T (pC8a) and C222T (pC8b) in the F2 DUMI population.

The SAS's PROC MIXED procedure combined with REPEATED statement (The SAS software package, release 9.1)

were used for analyzing the variance of experimental dataset to estimate the effect of genotypes of candidate genes on complement activity at eight different time points of vaccination. The analysis model address valid standard errors of the fixed-effect factor estimates in order to identify other significant environmental and genetic effects apart from the factor of genotypes and its interaction by stepwise elimination of non-significant effects.

$y_{ijklmno} = m + sire_i + dam_j + parity_k + treatment_l + genotype_m + time_n + sex_o + ANIMAL_{ijklmno} + (genotype \times time)_{mn} + e_{ijklmno}$

Where $y_{ijklmno}$ is the hemolytic complement activity (CH50 and AH50), m is overall mean, $sire_i$ is the fixed-effect of sire (i = 1 to 3), dam_j is the fixed-effect of dam (j = 1 to 11), $parity_k$ is the fixed-effect of parity (k = 1 to 5), $treatment_l$ is the fixed-effect of treatment-vaccinated group/ unvaccinated group (l = 1 to 2), $genotype_m$ is the fixed-effect of genotype (m = 1 to 3), $time_n$ is the fixed-effect of time points of measurement prior and after vaccinations (m = 1 to 8), sex_o is the fixed-effect of sex (o = 1 to 2), $ANIMAL_{ijklmno}$ is the random effect of animal, $(genotype \times time)_{mn}$ is the interaction between genotype and time point, and $\varepsilon_{ijklmno}$ is the residual error.

Results and discussion

Polymorphisms

Whole cDNA length of pC8a (2,146 nucleotides encoding 598 amino acids, [GenBank DQ333200]) and pC8b (1,987 nucleotides, encoding 611 amino acids, [GenBank DQ333201]) was sequenced. Both genes located on the same chromosome 6q3.1-q3.5 significantly linked to markers SW1069 and SW322 (Khoa and Wimmers 2014) (Fig. 3). An alignment of cDNA sequences to NCBI Map Viewer displayed that the pC8a and pC8b respectively has 11 and 12 exons.

Comparative cDNA sequencing of animals from different porcine breeds revealed 7 SNPs in pC8a and 9 SNPs in pC8b, in which 2 and 8 of them leaded to amino acid exchanges, respectively. For pC8a, there were 2 and 4 SNPs belonging to functional protein domain TSP1 and MACPF, respectively. For pC8b, 5 SNPs, in which SNP with three discriminated alleles was detected at nucleotide A935G935T (Thr303Gly303Ser), were found in MACPF domain (Table 2). Genetic variation in pigs has been indicated in response to pathogens or immune system challenges (Mallard and Quinton 1998; Wilkie and Mallard 1999; Henryon et al. 2002) and to infections with PRRS (Petry et al. 2005). It was implied that genetic variations in functional domains may affect the structure and function of protein as well as activity of the complement components in lysis process of cells (Khoa and Wimmers 2015).

Three of SNPs for each genes were genotyped. Genotypic and allelic frequency at all mutation points fitted to Hardy-Weinberg equilibrium (Table 3). Differentiation of frequencies at some loci (i.e. genotype AA and TT at locus A535G and C1544T for pC8a, respectively genotype CT and AA at locus C1544T and A1244G for pC8b, respectively) was crossing among breeds or between European (German Landrace and Pietrain) and Vietnamese (Muong Khuong) pigs. These may be due to geographical distance (Thuy et al. 2006), common ancestors (Yang et al. 2003), or wild pigs with various origins (Lan and Shi 1993; Huang et al. 1999). Partly, common crossing among high-yield breeds (German Landrace and Pietrain) in pig production industry leads to scattering of original alleles, while specific genotypes due to natural selection are conserved in the small population (i.e. Muong Khuong) living in harsh condition of lack of medicine, vaccine, exotic cross,...in the small population.

Association

In this study, the SNPs C1544T in pC8a and C222T in pC8b segregating alleles in the F2 DUMI population were used to analyze genetic association of the candidate genes with CH50 and AH50. As a result, there was no significant difference among genotypes for both activities, except homozygous genotype TT (68.24 ± 3.29 U/ml) of pC8a showed the highest CH50 value significantly differing from homozygous genotype CC (59.45 ± 4.07U/ml) (P=0.065) (Fig. 1). Actually, during biosynthetic process, C8a contains a unique insertion (residues 189-205) being highly conserved between human and pig but with an exchange of Thr^{193} and Ala^{196}, respectively, where Cys^{194} can form the disulfide bond to C8g due to a complementary binding site on C8g (Plumb and Sodetz 2000) . The bond between C8a-g plays an essential role in binding to C8b. Additionally, although proteins containing MACPF domain play important roles in vertebrate immunity, embryonic development, neural-cell migration as well as formation of pores and disruption of cell membranes (Rosado et al. 2007), MACPF region of C8a is a residence for C8g and C8b in formation process of the C8a-g-b complex (Plumb et al. 1999 Slade et al. 2006) as well as in binding to C9 (Slade et al. 2006). Also the asparaginyl-terminal TSP1 module concerns to co-operative interaction among components of the C8 (Goundi sand Reid 1988). According to Goundis and Reid 1988, TSP1 conserved sequence motif has a key role in mechanisms by which malaria parasites avoid host defenses mediated

by complement. Probably it was not right in this study because invading pathogens are virus (PRRSV, ADV) and bacteria (MH). Moreover, amino-terminal modules in C8a also have a mediated function in binding and self-polymerization of C9 to form a pore-like structure on the membrane of target cells as well as expression of C8 activity (Plumb et al. 1999; Scibek et al. 2002; Slade et al. 2006). Because of these reasons, C8 is not probably important in direct hemolytic process. C8 can be a factor for closely binding complement components in the terminal lytic pathway (C5b-9) together, which results in formation of MAC. Thus, in this study genetic variations in pC8 molecules had no meanful in hemolytic complement activity. In a previous report, it was also indicated that polymorphism in pC8g did not affect hemolytic complement activity in both classical and alternative pathways. Although not directly relating to hemolytic complement activity, pC8g either acts in other mechanisms or interacts to other components of the immune system to support immune defense (Khoa 2010). Once linkage among molecules was closer, hemolytic complement activity of MAC will be increased. In contrast, loose bindings can lead to deficiency of molecules concentration in plasma of blood stream. Clearly, deficiency of human C8 complement component associated with meningitis infection was found in many different populations (Matthews et al. 1980 Kemp et al. 1985 Cooke et al. 1987; Keller et al. 1987; Platonov et al. 1997).

In another sense, the interaction of genotypes and the eight different time points (before and after immunizations) was significantly different among pC8a genotypes for AH50 (P=0.0027) as well as pC8b ones for both CH50 (P=0.0048) and AH50 (P=0.0231) (Fig. 2). For CH50, homozygous genotypes TT of the pC8a and CC of the pC8b always showed the highest value along experiment. For AH50, these genotypes tended to gradually increase from before MH vaccination to after PRRS vaccination, while the remaining genotypes performed continuous alterations at different time points. Generally, most of CH50 and AH50 increased after each vaccination and then they rapidly reached optimal level in immune response to ADV or PRRS vaccines. Levels of lysis in AH50 or CH50 assay is evidence for complement activation (Ahmed and Peter 1995), but not for pC8a-b in this case. Although, the titers of complement components in the bronchial secretions reached the highest level at 6 weeks after infection with MH (Loos and Brunner 1979), immune activation by ADV seemed strongest. The levels of each component or percentage of hemolysis increased with aging (Fukuoka et al. 1982; Mathew et al. 1996; Wimmers et al. 2003). The increase in complement components shortly after infection may represent an early unspecific defense mechanism of the host before the specific immune response becomes effective. The complement system can be activated by MH via the classical as well as the alternative pathway in the absence of antibodies (Loos and Brunner 1979). While initiation of the classical pathway is due to presence of antibody-antigen complex, complement activation via the alternative pathway depends on pathogen surfaces (Janeway et al. 2001). Since the alternative pathway serves as an amplification mechanism for the classical pathway (Mold et al. 1999) by generating more C3-convertase, in this study complement activation via the classical was clearer than via the alternative because of good quality of vaccines.

Implication

The classical pathway may be a major route leading the formation of the MAC and cell lysis in the complement system. Factors concerning to complement activation are aging, genotypes and antigenic characteristics. Innate immune mechanism is complicated. It is due to interaction of multigenes complexes and other factors. In this study, it can demonstrate that responsiveness of host body was due to aging, kind of vaccine (characteristic of antigen) and weak interaction of pC8a-b genetic variation. It was suggested that AVD should be inoculated earlier to activate immune system more early as well as to boost immune response effectiveness of host to inoculations subsequently. The pC8a-b probably function in binding complement component, especially components in MAC, much more than in hemolytic complement activity in the classical and alternative pathways.

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KEYWORD：Porcine C8a-b, Polymorphisms, Hemolytic complement activity

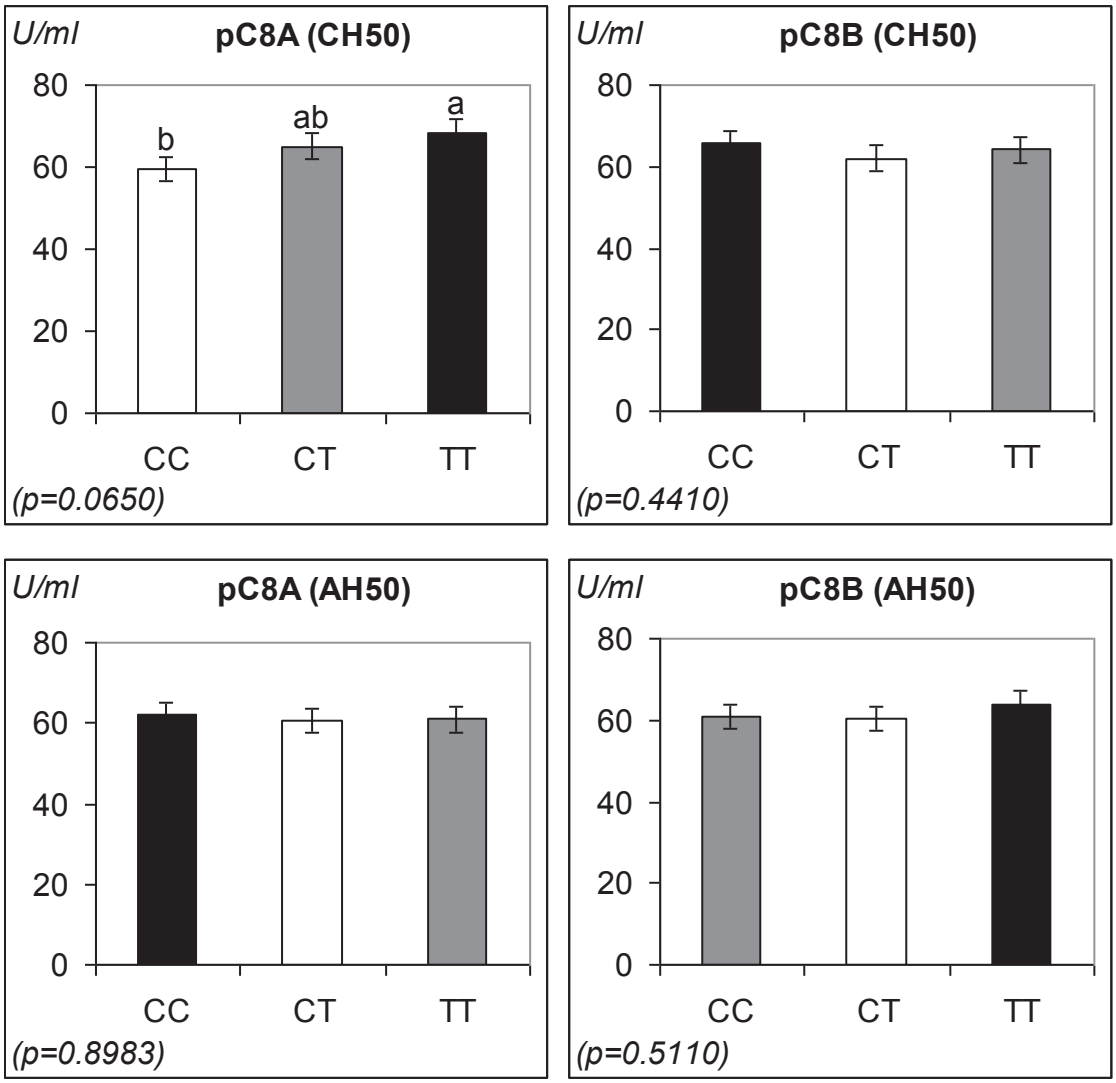

Figure 1. Expression of hemolytic complement activity (CH50 and AH50 depending on genotypes).

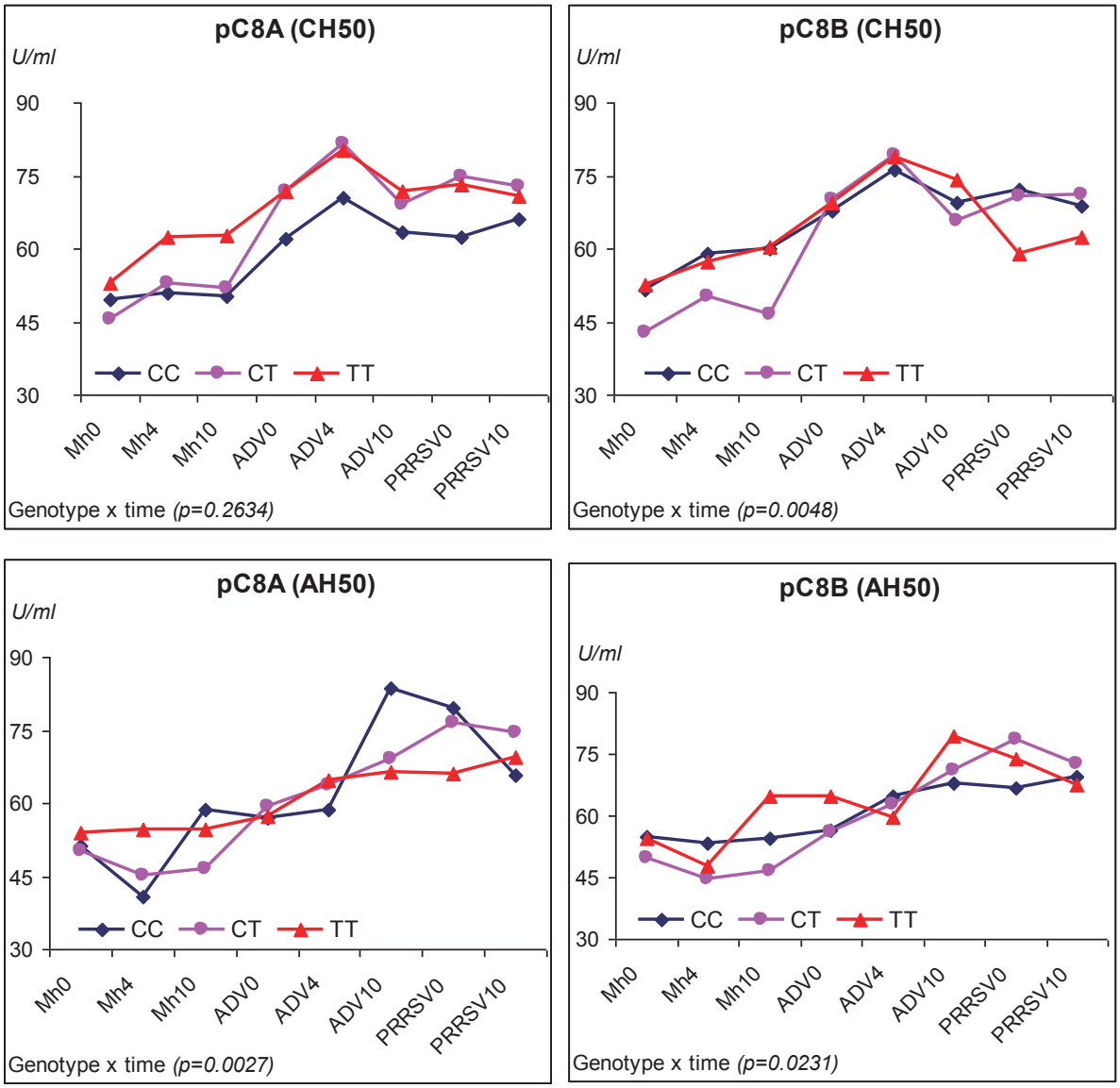

Figure 2. Expression of hemolytic complement activity (CH50 and AH50 along vaccination inthe interaction of genotypes and eight different time points).

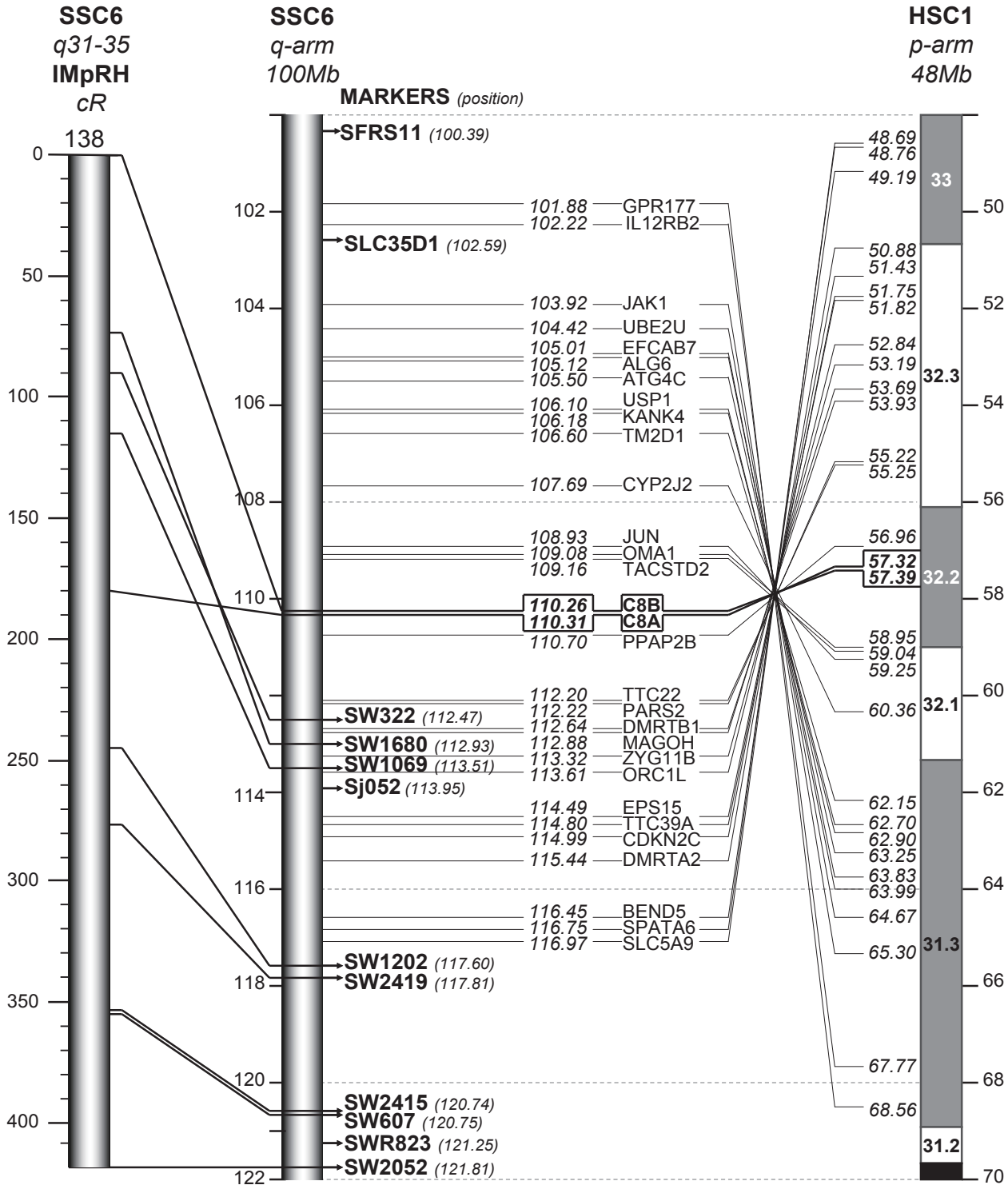

Figure 3. Comparative mapping for C8a-b

Position of C8a-b on physical comparative maps (SSC6 radiation hydrid [17], the telomeric portion of SSC6 q-arm (100-122Mb), and HSC1 p-arm (48-70Mb)). Physical map of SSC6 and HSC1 was formed using genome and microsatellite marker data from www.ncbi.nlm.nih.gov (build 37.1), www.ensembl.org (build 56) and www.thearkdb.org (ENSEMBL56 Sscrofa9 Pig Assembly: 6).

Table 1: Oligonucleotide primer pairs used for sequencing and genotyping

Primer set	Sequence	Position, bp*	Length, bp	Tm, °C
Sequencing and screening SNPs				
pC8a1.cDNA	up 5´- ggtcctggtctgtagacacctc-3´ (5´-UTR)	5-26	501	60
	down 3´-gatcctgacatcttcacagtcatc-5´ (exon 4)	485-505		
pC8a2.cDNA	up 5´-gagtgtctgaggcaagcaca-3´ (exon 3)	365-384	526	60
	down 3´-ttgctgtaaatgggctacctg-5´(exon 6)	870-890		
pC8a3.cDNA	up 5´-aagccctggcagacagtaaa-3´ (exon 6)	753-772	572	60
	down 3´-gtggccacctccaagtttt-5´ (exon 8)	1306-1324		
pC8a4.cDNA	up 5´-catcatgctttggagggtct-3´ (exon 8)	1218-1237	501	60
	down 3´-ccttggctccctctgactt-5´ (exon 10-11)	1700-1718		
pC8a5.cDNA	up 5´-tacgagatactgcgccacac-3´ (exon 10)	1499-1517	490	60
	down 3´-agctggcaggacagagaaaa-5´ (3´UTR)	1969-1988		
pC8b1.cNDA	up 5´-atgaagacctctgggacgtg-3´ (exon 1)	65-84	500	60
	down 3´-tccatttcctgctgacacttt-5´ (exon 4)	544-564		
pC8b2.cDNA	up 5´-atcagtcggatgaggcaaac-3´ (exon 4)	507-526	522	60
	down 3´-tgaccctctgaaggaactcg-5´ (exon 7)	1009-1028		
pC8b3.cDNA	up 5´-agcgcgttacaagctgaaat-3´(exon 7)	967-986	527	60
	down 3´-cggctgtcaccagttcatag-5´ (exon 10)	1474-1493		
pC8b4.cDNA	up 5´-gcagtgcagtacaacccaga-3´ (exon 9)	1427-1446	515	60
	down 3´-gtggaagctggtgtggagat-5´ (3´ UTR)	1922-1941		
Genotyping				
pC8a1.DNA	up 5´-cacctcgtgtgtaacggaga-3´ (exon 4)	434-453	322	56
(SNP: 535A→G)	down 3´-gccaccagcgtatggtattt-5´ (clone xx-1c1)	-		
pC8a2.DNA	up 5´-aagcccatttacgagatactgc-3´(exon 10)	1490-1511	184	60
(SNP: 1544C→T)	down 3´-gtcagtgccctgtgggtt-5´ (exon 10)	1656-1673		
pC8a3.DNA	up 5´-agcacagaggtcttggttgg-3´ (clone xx-1c1)	-	426	56
(SNP: 1768C→T)	down 3´-agctggcaggacagagaaaa-5´(3´UTR)	1969-1988		
pC8b1.DNA	up 5´-tgagaggccacactctcttg-3´ (exon 2)	160-179	148	54
(SNP: 222C→T)	down 3´-cttctgacagggatcacacg-5´(exon 2)	288-307		
pC8b2.DNA	up 5´-caagagcacctgcttccaa-3´(TI 775597740)	481-499	312	56
(SNP: 935A→G→T)	down 3´-cgagttccttcagagggtca-5´ (exon 7)	1009-1028		
pC8b3.DNA	up 5´-ttcttatcatcgggctggtc-3´ (clone xx-1E1)	859-881	528	56
(SNP: 1244A→G)	down 3´-ctgccttgtccttgcttctt-5´ (clone xx-1E1)	915-934		

*_Localization of primer pairs via GenBank DQ333200 (for pC8a), DQ333201 (for pC8b), CT025761 (clone xx-1c1), CT025767 (clone XX-1E1), or TI number775597740 retrieved from GenBank of NCBI homepage using the Trace Archive tool.

Table 2: Characterization of SNPs

SNP	Amino acid exchange	Codon	Exon	Restriction enzyme	Domain
pC8a gene					
358C→T	-	89AG**C**→AG**T**	3	HpyCH4III	TSP1
535A→G	-	148CC**A**→CC**G**	4	TfiI	-
1207C→T	-	372AG**C**→AG**T**	8	BsrI	MACPF
1510A→G	-	473CT**A**→CT**G**	10	Hin6I	MACPF
1544C→T	Arg→Cys	485**C**GC→**T**GC	10	Hin6I	MACPF
1545G→A *	Arg→Cys	485C**G**C→T**A**C	10	HhaI	MACPF
1674A→G	Tyr→Cys	528T**A**C→T**G**C	10	Eco91I	-
1768C→T	-	559GG**C**→GG**T**	11	KnpI	TSP1
pC8b gene					
99C→T	Pro→Leu	24C**C**G→C**T**G	1	HpaII	-
222C→T	Thr→Met	65A**C**G→A**T**G	2	FnuDII	-
935A→G→T	Thr→Gly→Ser	303**A**CA→**G**CA→**T**CA	7	**	MACPF
1244A→G	Ile→Val	406**A**TC→**G**TC	8	MaeII	MACPF
1259C→T	Pro→Ser	411**C**CG→**G**CG	8	HpaII	MACPF
1374C→T	Ala→Val	449G**C**C→G**T**C	9	BseDI	MACPF
1494C→T	Ala→Val	489G**C**G→G**T**G	10	AciI	MACPF
1797C→T	Ala→Val	590G**C**A→G**T**A	12	BbvI	-
1801C→T	-	591G**C**C→G**T**C	12	AluI	-

*_also detected by (Nakajima et al. 1998); **_detected by resequening.

Table 3: Genotypic and allelic frequencies in pig breeds.

Gene: SNPs	LR	%	PI	%	MK	%
pC8a: 535A→G	30		30		25	
AA	2	0.07	9	0.30	0	0.00
AG	10	0.33	15	0.50	5	0.20
GG	18	0.60	6	0.20	20	0.80
A	14	0.23	33	0.55	5	0.10
G	46	0.77	27	0.45	45	0.90
pC8a: 1544C→T	30		30		25	
CC	10	0.33	14	0.47	23	0.92
CT	12	0.40	12	0.40	2	0.08
TT	8	0.27	4	0.13	0	0.00
C	32	0.53	40	0.67	48	0.96
T	28	0.47	20	0.33	2	0.04
pC8a: 1768C→T	30		29		25	
CC	23	0.77	25	0.86	18	0.72
CT	7	0.23	4	0.14	7	0.28
TT	0	0.00	0	0.00	0	0.00
C	53	0.88	54	0.93	43	0.86
T	7	0.12	4	0.07	7	0.14
pC8b: 222C→T (n)	30		30		25	
CC	30	1.00	30	1.00	18	0.72
CT	0	0.00	0	0.00	7	0.28
TT	0	0.00	0	0.00	0	0.00
C	60	1.00	60	1.00	43	0.86
T	0	0.00	0	0.00	7	0.14
pC8b: 935A→G→T *	30		30		25	
AA	7	0.23	0	0.00	0	0.00
AG	6	0.20	0	0.00	0	0.00
AT	13	0.43	9	0.30	2	0.08
GT	2	0.07	4	0.13	7	0.28
TT	2	0.07	17	0.57	16	0.64
A	33	0.50	9	0.15	2	0.04
G	14	0.21	4	0.07	7	0.14
T	19	0.29	47	0.78	41	0.82
pC8b: 1244A→G	30		29		25	
AA	7	0.23	6	0.21	0	0.00
AG	19	0.63	17	0.58	4	0.16
GG	4	0.13	6	0.21	21	0.84
A	33	0.55	29	0.50	4	0.08
G	27	0.45	29	0.50	46	0.92

*_detected by resequencing

Availability of supporting data

Least squares means of the classical complement hemolytic activity for the interaction of different genotypes x time point in porcine C8A gene (LSM±SE) (U/ml)

Vaccination	Blood sampling	CC	CT	TT
Mycoplasma	1	49.51±4.67	45.41±4.01	52.96±3.72
	2	50.95±5.14	52.89±4.10	62.38±3.89
	3	50.39±5.34	51.94±5.26	62.59±4.02
Aujeszky	4	62.06±5.49	71.76±4.30	71.94±4.15
	5	70.59±5.67	81.61±4.37	80.35±4.17
	6	63.46±6.13	69.25±4.49	71.91±4.23
PRRSV	7	62.51±5.66	74.86±4.23	73.00±4.03
	8	66.11±5.80	72.91±4.43	70.78±4.19

Least squares means of the classical complement hemolytic activity for the interaction of different genotypes x time point in porcine C8B gene (LSM±SE) (U/ml)

Vaccination	Blood sampling	CC	CT	TT
Mycoplasma	1	51.54±3.45	42.83±4.02	52.53±5.69
	2	59.06±3.65	50.36±4.14	57.16±6.51
	3	59.91±3.75	46.65±4.27	60.38±6.73
Aujeszky	4	67.91±3.86	70.00±4.25	69.52±6.80
	5	76.15±3.89	79.06±4.33	79.04±7.05
	6	69.27±3.95	65.79±4.47	74.13±7.66
PRRSV	7	71.98±3.73	70.64±4.20	59.09±6.77
	8	68.84±3.89	71.21±4.39	62.28±7.24

Least squares means of the alternative complement hemolytic activity for the interaction of different genotypes x time point in porcine C8A gene (LSM±SE) (U/ml)

Vaccination	Blood sampling	CC	CT	TT
Mycoplasma	1	51.29±5.18	50.06±3.81	54.09±3.45
	2	40.85±4.97	45.31±3.68	54.56±3.35
	3	58.62±4.80	46.60±3.54	54.48±3.16
Aujeszky	4	56.99±4.80	59.39±3.52	57.46±3.23
	5	58.74±5.11	63.67±3.59	64.57±3.40
	6	83.69±7.34	69.07±4.65	66.50±4.42
PRRSV	7	79.50±6.04	76.56±4.15	66.02±3.81
	8	65.87±5.90	74.42±4.06	69.50±3.74

Least squares means of the alternative complement hemolytic activity for the interaction of different genotypes x time point in porcine C8B gene (LSM±SE) (U/ml)

Vaccination	Blood sampling	CC	CT	TT
Mycoplasma	1	54.90±3.45	49.80±3.76	54.52±6.08
	2	53.29±3.27	44.56±3.57	47.81±5.66
	3	54.46±3.09	46.60±3.46	64.73±5.42
Aujeszky	4	56.44±3.11	56.14±3.40	64.55±5.56
	5	64.67±3.30	62.88±3.52	59.53±5.86
	6	67.96±4.24	70.97±4.48	79.46±8.53
PRRSV	7	66.59±3.71	78.58±4.03	73.83±7.01
	8	69.37±3.66	72.82±3.96	67.47±6.95

REFERENCES

Ahmed AEE, Peter JB (1995): Clinical utility of complement assessment [Minireview]. Clinical and Diagnostic Laboratory Immunology 2, 507-517.

Alper CA, Marcus D, Raum D, Petersen BH, Spira TJ (1983): Genetic polymorphism in C8 beta-chains. Evidence for two unlinked genetic loci for the eighth component of human complement (C8). Journal of Clinical Investigation 72, 1526-1531.

Cooke RP, Zafar M, Haeney MR (1987): Recurrent meningococcal meningitis associated with deficiencies of C8 and anti-meningococcal antibody. Journal of clinical and laboratory immunology 23, 53-56.

Cooper NR, Morrison DC (1978): Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. Journal of Immunology 120, 1862-1868.

Densen P, Brown EJ, O'Neill GJ, Tedesco F, Clark RA, Frank MM, Webb D, Myers J (1983): Inherited deficiency of C8 in a patient with recurrent meningococcal infections: further evidence for a dysfunctional C8 molecule and nonlinkage to the HLA system. Journal of Clinical Immunology 3, 90-99.

Dewald G, Cichon S, Bryant SP, Hemmer S, Nothen MM, Spurr NK (1996): The human complement C8G gene, a member of the lipocalin gene family: polymorphisms and mapping to chromosome 9q34.3. Annals of Human Genetics 60, 281-291.

Fukuoka Y, Seino J, Okuda T, Tachibana T (1982): Development complement variation of of a hemolytic assay for mouse components in sera and the their levels with age. The Tohoku Journal of Experimental Medicine 137, 79-90.

Galanos C, Rietschel ET, Liideritz O, Westphal O (1971): Interaction of lipopolysaccharides and lipid A with complement. European Journal of Biochemistry 19, 143-152.

Galdiero F, Tufano MA, Sommese L, Folgore A, Tedesco F (1984): Activation of complement system by porins extracted from Salmonella typhimurium. Infection and Immunity 46, 559-563.

Giraldo G, Degos L, Beth E, Sasportes M, Marcelli A, Gharbi R, Day NK (1977): C8 deficiency in a family with xeroderma pigmentosum: lack of linkage to HLA region. Clinical Immunology and Immunopathology 8, 377-384.

Goundis D, Reid KBM (1988): Properdin, the terminal complement components, thrombospondin and the circumsporozoite protein of malaria parasites contain similar sequence motifs. Nature 335, 82-85.

Hardge T, Koepke K, Reissman M, Wimmers K (1999): Maternal influences on litter size and growth in reciprocol crossed Miniature Pigs and Durocs. Archives Animal Breeding 1, 83-92.

Henryon M, Juul-Madsen HR, Berg P (2002): Genetic variation for total and differential numbers of leukocytes in growing pigs. Proceedings of the 7th WCGALP, INRA. Montpellier, France.

Huang YF, Shi XW, Zhang YP (1999): Mitochondrial genetic variation in Chinese pigs and wild boars. Biochemical Genetics 37, 335-343.

Janeway CA Jr, Travers P, Walport M, Shlomchik MJ (2001): Immunobiology: The Immune System in Health and Disease, 5 ed. Garland Science, New York. http://www.ncbi.nlm.nih.gov/books/NBK27100/

Kaufman KM, Sodetz JM (1994): Genomic structure of the human complement protein C8 gamma: homology to the lipocalin gene family. Biochemistry 33, 5162-5166.

Kaufmann T, Hansch G, Rittner C, Spath P, Tedesco F, Schneider PM (1993): Genetic basis of human complement C8-beta deficiency. Journal of Immunology 150, 4943-4947.

Keller H, Löke S, Hänsch GM, Jentschura D, Gerhard H, Heene DL (1987): Rezidivierende Meningitis bei familiärem Mangel der achten Komponente des Komplementsystems. Journal of Molecular Medicine 65, 387-390.

Kemp AS, Vernon J, Muller-Eberhard HJ, Bau DCK (1985): Complement C8 deficiency with recurrent meningococcemia: Examination of meningococcal opsonization. Journal of Paediatrics and Child Health 21, 169-171.

Khoa DVA (2010): Porcine terminal complement genes C6-9: Molecular characterization of terminal complement genes and their association with hemolytic complement activity in pigs. VDM Verlag Dr. Müller Publisher. ISBN-10 3836494019, ISBN-13 978-3-8364-9401-4, ASIN: 3836494019

Khoa DVA, Wimmers K (2014): Assignment for genes encoding the terminal complement components to porcine chromosome. Biotechnology in Animal Husbandry 30(3), 407-414.

Khoa DVA, Wimmers K (2015): Genetic association of the porcine C9 complement component with hemolytic complement activity. Asian Australasian Journal of Animal Sciences (accepted)

Lan H, Shi L (1993): The origin and genetic differentiation of native breeds of pigs in southwest China: An

approach from mitochondrial DNA polymorphism. Biochemical Genetics 31, 51-60.
Latsch M, Mollerfeld J, Ringsdorf H, Loos M (1990): Studies on the interaction of C1q, a subcomponent of the first component of complement, with porins from Salmonella minnesota incorporated into artificial membranes. FEBS Letters 276, 201-204.
Loos M, Brunner H (1979): Complement components (C1, C2, C3, C4) in bronchial secretions after intranasal infection of guinea pigs with Mycoplasma pneumoniae: dissociation of unspecific and specific defense mechanisms. Infection and Immunity 25, 583-585.
Mallard BA, Wilkie BN, Kennedy BW, Gibson J, Quinton M (1998): Immune responsiveness in swine: eight generations of selection for high and low immune response in Yorkshire pigs. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production. University of New England, Armidale, New South Wales, Australia 257-264.
Mathew AG, Franklin MA, Upchurch WG, Chattin SE (1996): Influence of weaning age on ileal microflora and fermentation acids in young pigs. Nutrition research 16, 817-827.
Matthews N, Stark JM, Harper PS, Doran J, Jones DM (1980): Recurrent meningococcal infections associated with a functional deficiency of the C8 component of human complement. Clinical and Experimental Immunology 39, 53-59.
Michelotti GA, Snider JV, Sodetz JM (1995): Genomic organization of human complement protein C8 alpha and further examination of its linkage to C8 beta. Human Genetics 95, 513-518.
Mold C, Gewurz H, Duclos TW (1999): Regulation of complement activation by C-reactive protein. Immunopharmacology 42, 23-30.
Morrison DC, Kline LF (1977): Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS). Journal of Immunology 118, 362-368.
Musingarimi P, Plumb ME, Sodetz JM (2002): Interaction between the C8 alpha-gamma and C8 beta subunits of human complement C8: role of the C8 beta N-terminal thrombospondin type 1 module and membrane attack complex/perforin domain. Biochemistry 41, 11255-11260.
Nakajima E, Itoh T, Suzuk K, Kawakami K, Takeda K, Onishi A, Komatsu M (1998): Characterization, chromosomal localization, and genetic variation of the [alpha] subunit of porcine eighth component of complement. Animal Genetics 29, 377-380.
Ng SC, Rao AG, Howard OMZ, Sodetz JM (1987): The eighth component of human complement: evidence that it is an oligomeric serum protein assembled from products of three different genes. Biochemistry 26, 5229-5233.
Petry DB, Holl JW, Weber JS, Doster AR, Osorio FA, Johnson RK (2005): Biological responses to porcine respiratory and reproductive syndrome virus in pigs of two genetic populations. Journal of Animal Science 83, 1494-1502.
Platonov AE, Shipulin GA, Shipulina JO, Vershinina IV, Densen P (1997): Heterozygous C8 β complement deficiency does not predispose to meningococcal disease. Clinical & Experimental Immunology 108, 497-499.
Platteborze PL, Hobart MJ, Sodetz JM (1996): Physical linkage and orientation of the human complement C8a and C8ß genes on chromosome 1p32. Human Genetics 98, 443-446.
Plumb ME, Scibek JJ, Barber TD, Dunlap RJ, Platteborze PL, Sodetz JM (1999): Chimeric and truncated forms of human complement protein C8 alpha reveal binding sites for C8 beta and C8 gamma within the membrane attack complex/perforin region. Biochemistry 38, 8478-8484.
Plumb ME, Sodetz JM (2000): An indel within the C8 alpha subunit of human complement C8 mediates intracellular binding of C8 gamma and formation of C8 alpha-gamma. Biochemistry 39, 13078-13083.
Pluschke G, Achtman M (1984): Degree of antibody-independent activation of the classical complement pathway by K1 Escherichia coli differs with O antigen type and correlates with virulence of meningitis in newborns. Infection and Immunity 43, 684-692.
Rogde S, Olaisen B, Gedde-Dahl T, Teisberg P (1986): The C8A and C8B loci are closely linked on chromosome 1. Annals of Human Genetics 50, 139-144.
Rosado CJ, Buckle AM, Law RH, Butcher RE, Kan WT, Bird CH, Ung K, Browne KA, Baran K, Bashtannyk-Puhalovich TA, Faux NG, Wong W, Porter CJ, Pike RN, Ellisdon AM, Pearce MC, Bottomley SP, Emsley J, Smith AI, Rossjohn J, Hartland EL, Voskoboinik I, Trapani JA, Bird PI, Dunstone MA, Whisstock JC (2007): A common fold mediates vertebrate defense and bacterial attack. Science 317, 1548-1551.

Scibek JJ, Plumb ME, Sodetz JM (2002): Binding of human complement C8 to C9: role of the N-terminal modules in the C8 alpha subunit. Biochemistry 41, 4546-14551.

Slade DJ, Chiswell B, Sodetz JM (2006): Functional studies of the MACPF domain of human complement protein C8alpha reveal sites for simultaneous binding of C8beta, C8gamma, and C9. Biochemistry 45, 5290-5296.

Theriault A, Boyd E, Whaley K, Sodetz JM, Connor JM (1992): Regional chromosomal assignment of genes encoding the alpha and beta subunits of human complement protein C8 to 1p32. Human Genetics 88, 703-704.

Thuy NTD, Melchinger-Wild E, Kuss AW, Cuong NV, Bartenschlager H, Geldermann H (2006): Comparison of Vietnamese and European pig breeds using microsatellites. Journal of Animal Science 84, 2601-2608.

Wilkie B, Mallard B (1999): Selection for high immune response: an alternative approach to animal health maintenance? Veterinary Immunology and Immunopathology 72, 231-235.

Wimmers K, Mekchay S, Schellander K, Ponsuksili S (2003): Molecular characterization of the pig C3 gene and its association with complement activity. Immunogenetics 54, 714-724.

Yang SL, Wang ZG, Liu B, Zhang GX, Zhao SH, Yu M, Fan B, Li MH, Xiong TA, Li K (2003): Genetic variation and relationships of eighteen Chinese indigenous pig breeds. Genetics Selection Evolution 35, 657-671.

O-21-2

Genome Wide Association Study of Oleic Fatty Acid in Duroc Swine

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Introduction

Oleic acid is the predominant neutral lipid and monounsaturated fatty acid (MUFA) which is approximately 35.8 percent of the total fatty acid in muscle and fat tissues of swine (Woods et. al., 2008). The saturated fatty acids (SFA) are believed to be the culprit related with heart disease thus lower intake is recommended. On the other hand, polyunsaturated fatty acid (PUFA) is known to lower LDL-cholesterol in human lowering the risk of coronary disease and other heart related diseases, however increasing PUFA is negatively associated to meat quality traits such as flavor and susceptibility to oxidation (Bosi et. al., 2000). Therefore, this emphasizes the potential of MUFA modification in balancing nutritional value of meat and meat quality traits.

Stearic acid, predominant SFA, was converted to oleic acid during fatty acid elongation process. This means that the amount of oleic acid influences other fatty acid such stearic which may change sensory characteristics and health related factors of meat. In addition, Oleic acid shows positive correlation with flavor, flavor liking and overall acceptability of pork (Cameron et al., 2000). Thus, identification of quantitative traits associated to oleic acid can serve as a tool to improve meat quality traits of Duroc swine.

Materials and Methods

Phenotype

Four hundred eighty heads of female Duroc pigs were raised under commercial condition and slaughtered by batch in the same abattoir. Collection of abdominal fat and longissimus dorsi tissues were done immediately after slaughtering and kept frozen at -20 ^{0}C. Oleic acid was analyzed followed to procedure protocol mentioned by Folch et. al., (1957). Gas chromatography (Agillent Technology 6890, USA) was used to process fat.

Genotyping

Genomic DNA extraction was performed using ear tissue of each animal subjected to standard phenol/chloroform method. A total of 559 heads from the F2 generation were genotyped for 61,565 SNPs using Illumina PorcineSNP60K bead chips according to the manufacturer's protocol. Threshold level for genomic quality control were set to >90% call rate, >0.05 minor allele frequency (MAF), and 0.001 Hardy-Weinberg equilibrium (HWE). This was performed using PLINK.

Statistical Analysis

Association test followed genome-wide rapid association using mixed model and regression (GRAMMAR) approach by (Aulchenko et al. (2007)). First, oleic acid was corrected for fixed effect and covariate using the following model:

where is the phenotype of the ith individual, is the value of the *j*th covariate or fixed effect for the individual *i*, is an estimate of the jth fixed effect or covariate, and G_i and e_i are random additive polygenic and residual effects, respectively. Fixed effects accounted farm, and slaughter batch while the covariate was the slaughter age. Residuals from this equation were used as dependent trait in a simple linear regression analysis for each snp.

Where μ is the mean, g is the vector of genotypes, k is the regression coefficient and e is the vector of random residuals. Genome-wide significant threshold was determined based on bonferroni adjustment at 0.05 significance (*P*-values = 1.29 x 10^{-6}). Least square means of candidate genes were calculated using SAS v9.1.

Result and Discussion

Association

In this study statistical analysis of oleic acid showed an average percentage of 41.53 with lowest and highest values of 35.35 and 37.41 %, respectively. Genome-wide associated SNPs were visualized in Manhattan plot illustrated in Figure 1 while SNP details were presented in Table 1. A total of 29 genome-wide significant SNPs were identified in this study located in SSC14 from 120Mb to 124 Mb. Annotation identified plausible genes which were excluded in further investigations and discussion.

Two strong candidate genes directly involved in fatty acid elongation were identified: SCD and ELOVL3 genes. SCD

(stearoyl-CoA desaturase) and ELOVL3 (elongation of very long chain fatty acids-3) genes are involved in fatty acid metabolic process, fatty acid biosynthesis, and lipid biosynthetic process. Thus, these genes play relevant role in quantity and quality of fatty acids in meat.

Genome wide association study in Swine identified SCD gene as important tool in fatty acid manipulation (Uemoto et. al., 2012 and Yang et. al., 2013), thus this result provides further evidence of SCD gene as strong genetic marker that influence oleic acid in Duroc pigs. Moreover, ELOVL3 positioned at 123 Mb on SSC14 this chromosomal segment was identified with highly differentiated region that arises from intensive selection and breeding specific to Duroc swine (Wilkinson et. al., 2013). This study provides informative result showing the potential of ELOVL3 gene as effective genetic marker to improve oleic acid that might be unique in Duroc swine, however investigations on ELOVL3 polymorphisms in Duroc swine that may clarify influence of ELOVL3 in fatty acid composition is still limited.

Least Significant Difference

In this study least significance difference between genotypes were analyze to determine which allele has the advantage in altering oleic acid. ALGA0008191 SNP located nearby SCD gene, and ASGA0066174l SNP positioned within ELOVL3 were used identified. These SNPs represents genotypes of candidate genes. Table 2 shows the least significant difference of candidate genes. Allele A of SCD gene significantly increases oleic percentage with 43.07% for AA genotype compared with 41.64% for GG genotype. Conversely, Allele G of ELOVL3 gene showed statistically higher oleic acid with 43.04 percent than 41.52 percent for allele A. This propose involvement of SCD and ELOVL3 genes in oleic acid biosynthesis. An increase in oleic acid which is elongated using stearic acid might suggest healthier meat as the decrease on saturated fatty acid is recommended to prevent heart related diseases (Jimenez-Colmenero et al., 2001).

Summary and Conclusion

In summary, this study identified significant snp markers located in SSC14 associated to oleic acid. This result gives valuable information on manipulation of fatty acid composition using SCD and ELOVL3 genes as important genomic marker in breeding selection. Further investigation on ELOVL3 polymorphisms are recommended to evaluate its importance as candidate gene that influences oleic acid and fatty acid composition.

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KEYWORD : Oleic acid, genome wide assocciation study, Duroc, ELOVL3

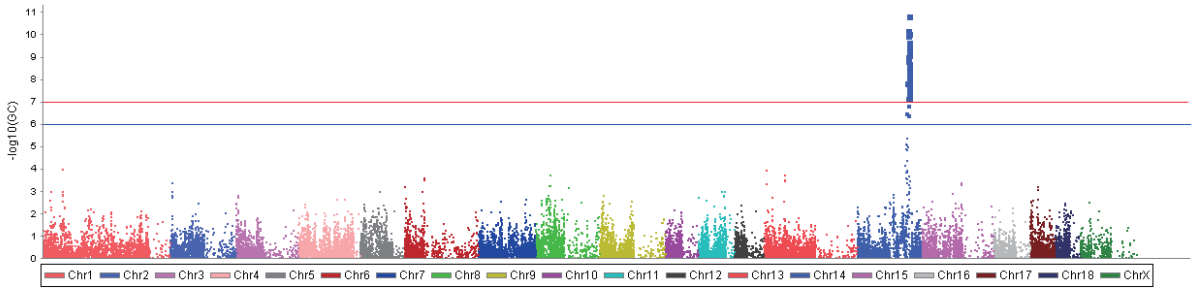

Figure 1. Manhattan plot of genome wide association study (GWAS) for oleic acid. Chromosomes are represented in X-axis while P-values in Y-axis. Bonferroni threshold p-values were at 5% and 1 % were illustrated in blue and red horizontal lines, repectively.

Table 1. Genome-wide significant SNP markers for Oleic acid (18:1)

CHR	SNP	Position[1]	Allele		MAF	Nearest Gene	functional consequnce	*P*-value*
			1	2				
14	ASGA0066120	121515129	G	A	0.34	PAX2	intron	6.00^{-11}
14	ASGA0066177	123214577	A	G	0.38	GBF1	intron	7.67^{-11}
14	ALGA0081091	120986865	A	C	0.34	SCD	5 kb	8.64^{-11}
14	DIAS0004744	123268607	A	G	0.38	CUEDC2 ,NFKB2	intron	2.20^{-10}
14	ALGA0081164	122935134	C	A	0.38	HPS6	missense	3.89^{-10}
14	ALGA0081161	122861163	G	A	0.38	C14H10orf76	intron	3.89^{-10}
14	ASGA0066165	22819384	G	A	0.38	C14H10orf76	intron	4.83^{-10}
14	ASGA0066162	122718232	G	A	0.38	LOC100037948	intron	4.83^{-10}
14	ASGA0066174	123083682	G	A	0.38	ELOVL3	3' utr variant	6.74^{-10}
14	ALGA0081097	121330920	G	A	0.29			8.72^{-10}
14	CASI0010164	121305916	A	C	0.29			8.72^{-10}
14	ASGA0066144	122072563	A	G	0.34		intron	3.21^{-09}
14	H3GA0042098	122667984	G	A	0.38		intron	5.06^{-09}
14	ASGA0066158	122427131	G	A	0.34	FBXW4	intron	7.86^{-09}
14	INRA0046731	122279650	A	G	0.34	BTRC	intron	7.86^{-09}
14	H3GA0042103	123318264	G	A	0.35	LOC102167427, TMEM180	intron, upstream variant 2KB	1.07^{-08}
14	H3GA0042104	123339204	A	C	0.35	ACTR1A	intron	1.31^{-08}
14	INRA0046735	122482603	A	G	0.34	FBXW4, LOC102162056	intron, upstream variant 2KB	1.65^{-08}
14	INRA0046761	123475867	G	A	0.36	SUFU	intron	1.90^{-08}
14	H3GA0042111	123496150	A	G	0.30	TRIM8	intron	3.36^{-08}
14	ASGA0066192	123409339	A	G	0.33	SUFU	intron	4.50^{-08}
14	ALGA0081147	122390223	G	A	0.29	FBXW4	intron	5.59^{-08}
14	ASGA0066098	120474440	A	G	0.28	ABCC2	intron	6.17^{-08}
14	ASGA0066137	122043297	C	G	0.29	LOC102161206	intron	6.59^{-08}

Position[1]: Snp locations were derived in Sus scrofa 10.2 assembly;
*Bonferoni adjustment at 5% ($P=1026x10^{-6}$), at 1% ($P= 3.29 \times 10^{-7}$); MAF=minor allele frequency;

Table 2. Least Square means of genotypes of candidate genes.

	N	Oleic Acid
SCD gene (ALGA0081091)		
AA	50	43.07 ± 0.23^{a}
AC	208	42.56 ± 0.13^{b}
CC	203	41.64 ± 0.14^{c}
ELOVL3 (ASGA0066174 l)		
AA	197	41.51 ± 0.15^{c}
AG	269	42.44 ± 0.13^{b}
GG	72	43.04 ± 0.22^{a}

REFERENCES

Amin, N., C. M. Van Duijn, and Y. S. Aulchenko. 2007. A genomic background based method for association analysis in related individuals. PloS one 2: e1274.

Aulchenko, Y. S., D.-J. De Koning, and C. Haley. 2007. Genomewide rapid association using mixed model and regression: a fast and simple method for genomewide pedigree-based quantitative trait loci association analysis. Genetics 177: 577-585.

Bosi, P. et al. 2000. Effects of dietary high-oleic acid sunflower oil, copper and vitamin E levels on the fatty acid composition and the quality of dry cured Parma ham. Meat science 54: 119-126.

Cameron, N. et al. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Science 55: 187-195.

Folch, J., M. Lees, and G. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol chem 226: 497-509.

Jiménez-Colmenero, F., J. Carballo, and S. Cofrades. 2001. Healthier meat and meat products: their role as functional foods. Meat science 59: 5-13.

Uemoto Y, Nakano H, Kikuchi T, Sato S, Ishida M, Shibata T, Kadowaki H, Kobayashi E, Suzuki K. 2012. Fine mapping of porcine SSC14 QTL and SCD gene effects on fatty acid composition and melting point of fat in a Duroc purebred population. Animal genetics 43: 225-228.

Wilkinson S, Lu ZH, Megens H-J, Archibald AL, Haley C, Jackson IJ, Groenen MA, Crooijmans RP, Ogden R, Wiener P. 2013. Signatures of diversifying selection in European pig breeds. PLoS Genet 9(4):e1003453.

Wood, J. et al. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat science 78: 343-358.

Yang, B. et al. 2013. Genome-wide association analyses for fatty acid composition in porcine muscle and abdominal fat tissues. PloS one 8: e65554.

O-21-5

Estimation of Growth Curve Parameter in Japanese Quail

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Introduction

The Japanese quail (*Coturnix japonica*) is an excellent bird for commercial domestication because of its rapid growth, high egg-laying production, fecundity and environmental resistance, small body size, easily handled and large number of birds can be kept in a limited space as compared to chickens ((Baumgartner , 1994 Iwamoto et al., 2008 Zynudheen et al., 2008). In the last 20 years, it has become more popular as a source of meat and eggs in various parts of the world (Hemid et al., 2010), including Thailand (Suppadit et al., 2009). Growth traits are importance trait in the livestock industry. Growth is defined as an increase in tissues and organs of the animals per unit time and effected by genetic and environmental factors (Tariq et al., 2011). Growth of living cells, tissues, organs and organisms is a biological phenomenon and can be explained in terms of mathematical terms. Increase in number of cells and increase in size of cells results in overall increase in mass of living tissue (Ullah et al, 2013). Growth curve is to consider growth as being tripartite, with a self-accelerating phase, followed by a linear phase, and finally a decelerating phases which fads out as the animal reaches maturity (Lawrence and Fowler, 2002). Growth curve reveal time-dependent non-linear changes of the body weights in animal and the generated equations can be used to predict the expected weight of a group of animal at a specific age (Golian and Ahmadi, 2008).

The general shape of the growth curve is a sigmoid form and is explained reliably by non-linear growth models such as Brody, Logistic, two-phases Logistic, Gompertz, Von Bertalanfy, Asymptotic Exponential, Negative Exponential, Quadratic curvilinear, Cubic curvilinear and the four-parameter Richards functions (Fitzhugh, 1976 Tzeng and Becker, 1981 Goliomytis et al., 2003 Cooper, 2005 Golian and Ahmadi, 2008). Many studies reported that Logistic, Gompertz, Von Bertalanfy and Richard functions model is the best fit model to the growth data of Japanese quail (Sezer and Tarhan,2005 Gurcan et al., 2012) Therefore, the objective of this study was to study the growth curve parameter of Japanese quail in Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Thailand.

Objective

The objective of this study was to study the growth curve parameter of Japanese quail in Faculty of Science and Technology, Prince of Songkla University, Pattani campus, Thailand.

Methodology

The growth data of Japanese quail were obtained from an experiment at Faculty of Science and Technology, Prince of Songkla University, Pattani campus Thailand. The body weight data from hatching to 56 days of age on 2,745 observations of 305 quail were used in this study. The quails were fed ad libitum with 24 % crude protein and 2900 kcal ME/kg during 0 to 56 days of age. Body weights were measured at hatching and weekly up to 56 days of age using electronic scale. Wing band, sex were recorded. Sex was identified at 30 days of age. The data structure is presented in Table 1.

The models expressions are shown in Table 2. The Brody, Richard, Von Bertalanffy, Logistic and Gompertz functions were chosen to fit the data for body weights in Japanese quail. The growth curve parameters were estimated using non-liner procedure (NLIN) of SAS software (SAS, 1996). The mean square error (MSE) and coefficient of determination (R^2) were used to compare the models when fitted to the data.

Result and Discussion

The parameter for growth curve of Japanese quail was showed in Table 3. The parameter a (asymptotic weight) were found by Brody, Richard, Von Bertalanffy, Logistic and Gompertz as 311.700, 171.500, 172.400, 146.600 and 160.500, respective in the presented study. Similarly, the same parameters were detected 492.70, 222.00, 247.30, 201.90 and 222.10 for Brody, Richard, Von Bertalanffy, Logistic and Gompertz, respective (Narinc et al., 2010a). Raji et al. (2014) were also reported that these parameters were found 153.21, 151.22 and 153.11 for Richard, Logistic and Gompertz. Gurcan et al. (2012) reported that these parameters were detected minimum

174.20 and maximum 325.40 for Brody, Von Bertalanffy, Logistic and Gompertz, respective. Narinc et al (2010b) were reported a parameter was 227.57 for Gompertz model.

At the same time, the parameter c which rate of mature weight were detected 0.010, 0.039, 0.038, 0.084 and 0.050 for Brody, Richard, Von Bertalanffy, Logistic and Gompertz, respectively. Similarly, the same parameters were observed 0.013, 0.080, 0.054, 0.139 and 0.080 in the same model (Narinc et al, 2010a). Gurcan et al. (2012) were also reported that these parameters were found 0.018, 0.052, 0.090 and 0.066 for Brody, Von Bertalanffy, Logistic and Gompertz model, respective. Raji et al. (2014) were also reported that these parameters were found 0.387, 0.496 and 0.360 for Richard, Logistic and Gompertz model in quail.

According to the result, the coefficients of determination (R^2) were detected as 0.929, 0.981, 0.981, 0.980 and 0.981 for Brody, Richard, Von Bertalanffy, Logistic and Gompertz model, respective. The mean square errors (MSE) were detected as 178.200, 167.200, 167.100, 179.100 and 168.300 for Brody, Richard, Von Bertalanffy, Logistic and Gompertz, respectively. The smallest values of mean square errors in this study were obtained from Von Bertalanffy for these criteria. Von Bertalanffy has the best fitting to the data set and also Richard, and Gompertz models have shown higher accuracy. The result were disagree with previous study that Gompertz models was best fit for quail (Narinc et al ,2010a,b). Gurcan et al. (2012) and Beiki et al. 2013 were reported that Logistic model were best model for Japanese quail. Raji et al. (2014) were reported that Gompertz and Richard models were best model for Japanese quail. In addition, Sezer and Tarhan (2005) reported that Richard model was best fit model in three meat-type line of Japanese quail.

The overall calculation statistic values showed that the Von Bertalanffy model provides higher accuracy of goodness to the growth data, followed by Richard and Gompertz models. Von Bertalanffy model provides a better description of growth curve of quail summarizing age-weight data with the biologically meaningful parameters in this population.

Conclusion

The Von Bertalanffy model gave the best fit to the growth data of Japanese quail and this model was followed by Richard and Gompertz models in this present study. Therefore, these models might be used to determine of body weight-age relationship very well in Japanese quail studied.

KEYWORD : Japanese quail, growth curve, body weight, growth trait, non-linear

Table1. Data structure of body weight of Japanese quail.

Age (day)	No. of observation	Mean	Standard Deviation
1	302	7.90	0.76
7	302	17.72	2.89
14	297	36.25	6.84
21	296	63.80	10.92
28	295	90.20	13.14
35	293	110.99	13.64
42	292	127.81	12.46
49	291	132.62	15.70
56	290	141.86	22.29

Table 2. The growth curve model expression

Models	Expression[1]
Brody	$W_t = a\times(1-b\times\exp(-c\times t))$
Richard	$W_t = a\times(1-b\times\exp(-c\times t))^M$
Von Bertalanffy	$W_t = a\times(1-b\times\exp(-c\times t)^3)$
Logistic	$W_t = a/(1+b\times\exp(-c\times t)$
Gompertz	$W_t = a\times\exp(-b(\exp(-c\times t)))$

[1] W_t = weight (g) at time (day), a = asymptotic weight, b = integration constant, c = rate of mature weight, t = age (day), M = Shape parameter

Table 3 Parameters (±standard error) for growth curve of Japanese quail.

Models	a	b	c	m	MSE	R^2
Brody	311.700±	309.600±	0.010±	-	178.200	0.929
	15.363	15.003	0.001			
Richard	171.500±	0.644±	0.039±	3.193±	167.200	0.981
	4.058	0.094	0.003	0.720		
Von Bertalanffy	172.400±	0.670±	0.038±	-	167.100	0.981
	2.240	0.007	0.001			
Logistic	146.600±	-10.003±	0.084±	-	179.100	0.980
	0.986	0.262	0.001			
Gompertz	160.500±	2.939±	0.050±	-	168.300	0.981
	1.614	0.040	0.001			

a = asymptotic weight, b = integration constant, c = rate of mature weight, M = Shape parameter, MSE = mean square error, R^2 = coefficient of determination

REFERENCES

Baumgartner, J. 1994. Japanese quail production, breeding and genetics. World Poult. Sci. J. 50: 227-235,

Beiki, H., A. Pakdel, M. Moradi-shahrbabak and H. Mehrban. 2013. Evaluation of Growth Functions on Japanese Quail Lines. J. Poult. Sci. 50: 20-27.

Cooper, R.G. 2005. Growth in the Ostriches (Struthio camelus var.domsticus). J. Anim. Sci. 76: 1-4.

Fitzhugh, H. A. 1976. Analysis of growth curves and strategies for altering their shape. J. Anim. Sci. 42: 1036-1051.

Golian, A. and H. Ahmadi 2008. Non-linear hyperbolastic growth models for describing growth curve in classical strain of broiler chicken. Res. J. Biol. Sci. 3: 1300-1304.

Goliomytis, M., E. Panopoulou and, E. Rogdakis. 2003. Growth curves for body weight and major component parts, feed consumption, and mortality of male broiler chickens raised to maturity. Poult. Sci. 82, 1061-1068.

Gurcan, E.K., O. Cobanuglu and S. Genc. 2012. Determination of Body Weight-Age Relationship by Non-Linear Models in Japanese Quail. J. Anim. Vet. Adv. 11: 314-317.

Hemid A.E.A., A.H. Abd El-Gawad, I. El-Wardany, E.F. El-Daly and N.A. Abd ElAzeem. 2010. Alleviating effect of some environmental stress factors on productive performance in Japanese quail 2. Laying performance. World J. Agric. Sci. 6: 517-524.

Iwamoto S., S. Sato, K. Hosomichi, A. Taweetungtragoon, T. Shiina, H. Matsubayashi, H. Hara, Y. Yoshida and K. Hanzawa. 2008. Identification of heat shock protein 70 genes HSPA2, HSPA5 and HSPA8 from the Japanese quail, Coturnix japonica. Anim. Sci. J. 79: 171-181.

Lawrence, T.L.J. and V.R. Fowler. 2002. Growth of Farm Animal. 2nd ed. CABI international Cromwell Press, Trowbridge. New York.

Narinc, D., E. Karaman, M. Z. Firat and T. Aksoy. 2010a. Comparison of Non-linear growth models to describe the growth in Japanese quail. J. Anim Vet. Adv. 9(14): 1961-1966

Narinc, D., T. Aksoy and E. Karaman. 2010b. Genetic parameters of growth curve parameters and weekly body weights in Japanese quails (*Coturnix coturnix japonica*). J. Anim Vet. Adv. 9(3): 501-507.

Raji, A.O., S.T. Mbap and J. Aliyu. 2014. Comparison of different models to describe growth of the japanese quail (*Coturnix japonica*). Trakia J. Sci. 2: 182-188.

Sezer M. and S. Tarhan. 2005. Model parameters of growth curves of three meat-type lines of Japanese quail. Czech J. Anim. Sci. 50 (1): 22–30.

Suppadit, T., S. Jaturasitha, W. Selasat, R. Norkeaw, P. Poungsuk and N. Pripwai. 2009. Effect of dietary dried milky sludge on productive performance and egg quality in laying Japanese quails. Anim. Sci. J. 80: 310-315.

Tariq, M.M., M.A. Bajwa, A. Waheed, E. Eyduran, F.Abbas, F.A. Bokhari and A. Akbar. 2011. Growth curve in mengali sheep breed of Balochistan. J. Anim. Plant Sci. 21: 5-7.

Tzeng, R.Y. and W.A Becker. 1981. Growth patterns of body and abdominal fat weight in male broiler chickens. Poult. Sci. 60: 1101-1106.

Ullah M. A., M. Amin and M. A. Abbas. 2013. Non-Linear Regression Models to Predict the Lamb and Sheep Weight Growth. Pakistan Journal of Nutrition. 12 (9): 865-869.

Zynudheen, A.A., R. Anandan and K.G. Ramachandran Nair (2008). Effect of dietary supplementation of fermented fish silage on egg production in Japanese quail (Coturnix coromandelica). Afr. J. Agric. Res., 3: 379-383.

O-21-7 THE GENETIC DIVERSITY OF Mx|Hpy81 GENES IN NATIVE CHICKENS USING PCR-RFLP TECHNIQUE

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INTRODUCTION

Local chicken or Kampong Chicken is one of local germ plasma which existence close related to social and cultural values of rural communities in Indonesia. Beside that local chickens have economic value, because it have several advantages such as high adaptability and durability on environment, ability to utilize lower quality feed, easy maintenance and high economic value of its product, both meat and egg. Nevertheless local chicken development having some constrains, such as genetic quality and low productivity that resulting difficulty to produce good quality and availability breed. With some advantages of local chicken, its genetic quality and productivity can be optimized through breeding programs including sustainable targeted selection.

Local chickens in Indonesia are known have high genetic diversity, including identified has Mx gene. Mx gene is a gene that responsible on chicken immunity level due to viral diseases such as AI and ND. (Maeda, 2005). Tolaki chicken is one of the Indonesian local chickens originating from Southeast Sulawesi, known have antiviral genes with high allele A (Pagala, *et al*., 2013).

Targeted selection that can be programmed as approach was molecular selection. This selection optimized some key genes with responsible on economical expression such as growth rapidity, viral diseases resistance. The molecular selection advantages were spending shorter time with effective and low cost procedure. Selection by using marker gene, known as MAS method (Marker Assisted Selection) was effective to apply (Mauwissen, 2004). The MAS method application need initial information about genes as marker genes candidate. Based on description above, research was conducted to analyze the Mx gene genetic diversity of Southeast Sulawesi local chicken (Tolaki and native chicken)

MATERIALS AND METHODS

Research Samples

A total 71 samples of Tolaki and Kampong chicken DNA were used with 25 samples Kampong chicken taken from Konawe Regency, chicken 25 samples Kampong chicken taken from Southern Konawe Regency, and 21 other samples were Tolaki chicken from Kendari City.

DNA extraction and amplification by PCR

DNA extracted from the samples was performed using kit extraction *Phire Animal Tissue Direct PCR Kit* (Thermo Fisher Scientific Inc.). Extraction procedure was following manufacture instructions: ± 0.5 cm in the beginning (root/ kalamus) feather transferred into 1.5 ml tube, then cut into several tiny part. At the 1.5 ml tube was added 20µl *Dilution Buffer* and 0.5µl *DNA Release* ™ *Additive*. Tube of mixture processed with vortex and centrifuged, continued with incubation for 2-5 minutes at room temperature and continued for 2 minutes at 98℃ . The DNA sample is ready for use or stored at -20℃ . DNA samples were amplified by PCR machine (Polymerase Chain Reaction). Specific primers to amplify genes Mx according Sironi *et al*. (2010) were the forward primer (5'-GCA TCA CCT CTG CTT AAT AGA-3') and reverse (5'-GTA GTA GTT GTT GGC TTT GA-3'). Amplification of DNA carried out on a total volume of 25 µl consists of 2 µl (10-100 ng) of DNA, 15.75 mL sterile deionized water 2.5 mL of 10 × buffer without Mg 2+ 2 µl MgCl2 0.5 µl of 10 mM dNTP 0.25 µl Taqpolimerase 2 µl (25 pmol) of primer. First phase was conducted on 1 cycle, including initial denaturation process on 94°C for 4 minutes. Second phase was conducted on 30 x cycle, including denaturation at 94℃ for 10 seconds, primer annealing at 60℃ for 1 minute, DNA molecule elongation at temperature of 72℃ for 2 minutes. The third phase was done with 1 x cycle, including the end of DNA molecule elongation on 72℃ for 7 minutes. Sample incubation was at 4℃ until used for further analysis.

Genotyping Mx gene by PCR-RFLP

Identification of the Mx gene diversity was conducted using *Polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP). The used enzyme is Hpy8I which recognize the cutting site GTN | NAC. RFLP method

performed by adding 3 units enzimHpy8I (10 units/µL) and 0.7 mL of 10 X Buffer tango (Fermentas, Finland) in 5 µL of DNA produced by PCR. Continue by 16 hours incubation at 37℃ .
DNA fragments PCR-RFLP products electrophoresis using electrophoresis devices on 2% *agarose* gel (0.5 g/25ml 0.5XTBE). The device was run using 0.5 X TBE buffer, at 100 volts voltage for 30 minutes. Electrophoresis gel visualization was performed on *Alpha Imager* gel documentation device.

Data analysis

Based on genotyping results, allele frequency, genotype frequency, and Mx gene heterozygote was counted according to Nei (1987). Hardy-Weinberg balance value (Hartl and Clark 1997), Polymorphic Informative Content (PIC) value (Bostein *et al*. 1980).

RESULTS AND DISCUSSION

Figure 1 was the result of PCR-RFLP of Mx gene fragment (299pb), which was cut by restriction enzymes Hyp81, in *exon* 13, 2032nd sites (GTN | NAC). Cutting with Hpy81 produce two alleles (A and G) and three genotypes (AA, AG and GG). A alleles cannot be cut by Hpy81, produces a DNA fragment (299 bp), while the G allele can be cut by Hpy81 produces two DNA fragments (200 bp and 99 bp). Cutting results on cDNA 2032 of Mx gene were detected any transition base mutations (single mutation), there were mutations in base pairs from GC into AT.

Genetic frequency and allele frequency of Mx|Hpy81 Gene

The genetic diversity of Mx gene in Tolaki chickens was seen from the genotype frequencies and its allele frequency and presented in Table 1.
Based on genotypic analysis of 71 samples tested of local chicken feathers, the average values obtained genotype frequencies from high to low respectively AA genotype (0.42), genotype AG (0:32) and the GG genotype (0.26). A allele frequency average value (0.58) was slightly higher than the G allele (0.42). The genotyping results can be interpreted that the Mx gene in Hpy81 loci was polymorphic (various). This is consistent with the statement of Nei and Kumar (2000), if there is two or more alleles value relative frequency in the population more than 0.01 (1%), it is called polymorphic. It was indicated that both Kampong and chicken Tolaki have resistance against virus attacks (AI and ND). This resistance is due to the flow of A allele which causes serine amino acid (AGT) change to asparagines (AAT). The presence of asparagines amino acid (A) in *exon 13* indicates that chickens are resistant to viral infections, and contrast while occurs a bases mutation to be serine amino acids (G), its mean chicken is vulnerable to virus attacks, (Watanabe 2003 Ko *et al*. 2004).

Genetic Mx Gene Index in Population

The results of the analysis of genetic diversity Mx gene through genetic index value calculation were presented in Table 2.
Based on the results (Table 2), the average total value of Ho, He and PIC on the Mx gene *Hpy81* locus respectively 0:32, 0:48 and 0:37. This indicates the Mx gene genetic diversity in each population was high and was polymorphic with PIC values were moderate (Botstein *et al.* 1980). This is understandable in case of the chicken sample obtained from their natural habitat in the wild, which imply cause on naturally random mating with a low inbreeding chance.
The results of *chi-square test (x)2* in all populations were not significantly different values, which means that the Mx gene in the population were in Hardy-Weiberg balance, the allele and genotype frequencies were still heredities from generation to the next as result of the randomly gametes diffusion within population (Vasconcellos *et al*. 2003).

CONCLUSIONS

These results showed thatMx|*Hpy*81 gene was polymorphic in all types of chickens that was genotyped. The Allele A frequency of Mx|*Hpy81* gene in Tolaki chicken was relatively higher than Kampong chicken. The Mx|*Hpy*81 gene provide potential for used as genetic markers in resistance to*Avian Influenza*and*Newcastle Disease*infection in Indonesian native chickens.

KEYWORD : Native chicken, Mx gene, Antiviral, SNP, PCR-RFLP

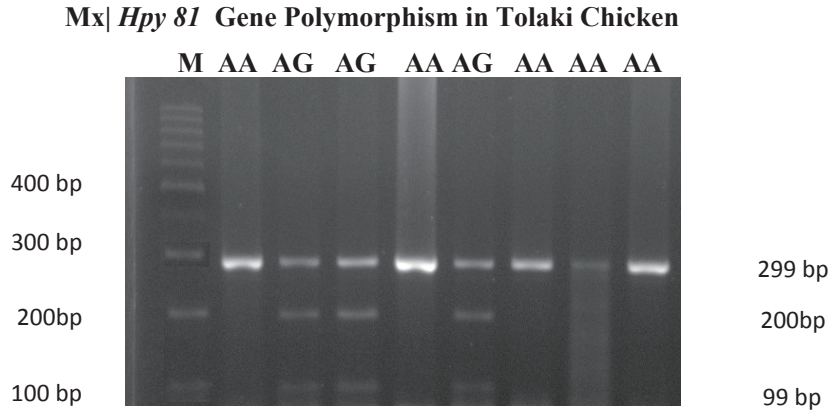

Figure 1. Products PCR-RFLP Mx gene amplification Mx on *exon13* (cut by Hpy81). M: marker 100 pb, N: Mx gene fragments (299 bp). AA, AG and GG: Mx Genotype gene in local chickens analyzed.

Table 1. Genetic and Allele Frequency of Mx|*Hpy81* Gene in Native Chicken

Sample Source	N	Genotype Frequency			Allele Frequency	
		AA	AG	GG	A	G
Tolaki Chicken (A)	25	0.40	0.44	0.16	0.62	0.38
Tolaki Chicken (B)	25	0.44	0.32	0.24	0.60	0.40
Kampong Chicken	21	0.43	0.19	0.38	0.52	0.48
Total	71	0.42	0.32	0.26	0.58	0.42

Table 2. Mx Gene Index Value (Value of Heterozygosity, Polymorphic Informative Content and Chi-Square) in Native Chicken

Generation	Ho	He	PIC	XHWE
Tolaki Chicken (A)	0.44	0.47	0.36	0.002^{tn}
Tolaki Chicken (B)	0.32	0.48	0.36	0.054^{tn}
Kampong Chicken	0.19	0.49	0.37	0.184^{tn}
Total	0.32	0.48	0.37	0.08^{tn}

tn: not significant, x^2hit $< x^2$ tabel (0.01,2)

REFERENCES

Botstein D, White RL, Skolnick, M, Davis RW. 1980. Construction of genetic linkage map in human using restriction frgamen lenght polymorphism. *Amer J Hum Genet*. 32:314-331

Hartl DL, Clark AG. 1997. *Priciple of Population Genetic*. Sinauer Associates, Sunderland, MA.

Ko JH, Takada A, Mitsuhashi T, Agui T, Watanabe T. 2004. Native antiviral specificity of chicken Mx protein depends on amino acid variation at position 631. *Anim Genet*. 35(2): 119-122.

Maeda. 2005. *Polymorphism of Mx Gene in Asian Indigenous chicken population. Seminar Nasional Tentang Unggas Lokal III* : 2005 Agustus 25; Semarang, Indonesia.Semarang (ID). Universitas Diponegoro.

Meuwissen T. 2003. Genomic selection: The future of marker assisted selection and animal breeding. Electronic forum on biotechnology in food and agriculture. *MAS a fast track to increase genetic gain in plant and animal breeding, session II, MAS in animal. FAO, Conference 10*. [Internet]. Torino 2004 Jul 24; [Diunduh 2013 Des 9].Tersedia pada :http://fao.org/Torino.

Nei, M, Kumar S. 2000 *Molecular Evolution and Phylogenetics*. Oxford University Press. Inc. New York

Nei, M. 1987. *Molecular Evolutionary Genetics*. New york (US) : Columbia University Pr.

Pagala, M.A., Muladno, C.Sumantri & S. Murtini. 2013.Association of Mx Gene Genotype with Antiviral andProduction Traits in Tolaki Chicken.J. Poult Sci. 12 (12): 735-739.

Vasconcellos, LPMK, Talhari DT, Pereira AP, Coutinho LL, Regitano LCA. 2003. Genetic characterization of aberden angus cattle using moleculer markers. *Genet Mol Biol*. 26:133-137.

Watanabe, T. 2003. Genomic analysis of antiviral resistant Mx gene in the chicken. Lab. Animal Breeding and Reproduction, Hokkaido University. Sapporo, Japan. *International workshop on Animal Genome Analysis*, KKR 2003 Nop 6; Sapporo (JP) : Hotel Tokyo.

O-22-1 DIETARY SUBSTITUTION OF SOYBEAN MEAL WITH SOY-MILK WASTE: EFFECTS ON GROWTH PERFORMANCE AND PHYSICAL MEAT QUALITY IN BROILER CHICKENS

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OBJECTIVE

In developing South-east Asia countries, mid-quality broiler chicken breeds, such as: New Lohmann, were normally harvested at body weight 1.5-2.0 kgs in 5-6 weeks. In European countries and the United States, hyline broiler chickens breeds, such as: Ross 308, Ross 708, or Cobb 500, can be harvested at weight 4.2-5.0 kgs in 9 weeks (Aviagen, 2007 Aviagen, 2014 Cobb-Vantress, 2015). Broiler chickens nowadays have very low feed conversion rate, high growth rate, and less costly nutrition. The fast growth of this meat-type chickens is supported by superior quality feed stuffs which contain high quality nutrients and energy that provided in proper amount.

In recent era, protein and amino acids which required were supplied by conventional protein source feed stuffs, such as: soybean meal (SBM). As a by-product in soybean oil industry, SBM contains not only high level of crude protein and digestible amino acids, but also is a good energy source for broiler chickens (Meng and Slominski, 2005). However, price of this commercial imported soybean meal becomes higher when the monetary crises is happened or when the national supply is low. Alternative locally available low-priced feed stuffs should be explored to change over the position of conventional high-priced poultry feedstuffs. One of the alternatives that might be investigated is soy-milk waste (SMW). SMW has also been shown as useful candidate as this by-product in soy-milk industry might contains high quality of nutrients (O'toole, 1999), which in turn should be beneficial in improving quality of meat yield. Aimon and Satrianto (2014) predicted a high trend in SMW availability in the next couple of years due to the increase of soybean consumption and import. A study must be done to explore the benefits of soybean meal dietary substitution with soy-milk waste using growth performance, protein-energy efficiency, and meat quality.

METHODOLOGY

Birds, Housing, and Experimental Design

The research was conducted in an opened-house poultry shed at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta. A total number of 60 day old New Lohmann male broiler chickens were allocated into 4 dietary treatments in a complete randomized fashion. Each treatment had 3 replicate pens with 5 birds per replicate pen. The four treatments were a yellow maize basal diet that was formulated to meet all nutrient-energy requirements recommended by the breeder (SMW-0 control). Soybean meal in the treatment diets was substituted with soy-milk waste (SMW) in different doses: 50 g/kg (SMW-1), 100 g/kg (SMW-2), and 150 g/kg (SMW-3). Each treatment was replicated 3 times, with 5 birds in each replicate pen. The diets were formulated to meet the recommendations of the National Research Council (1994) for broiler chickens. The ingredients and chemical compositions of the diets are presented in Table 1. All the diets for each period were prepared with the same batch of ingredients. Feed and water were provided for *ad libitum* intake.

Chicks were housed in floor pens (50 cm x 100 cm) equipped with a long feeder, bell drinkers, and brooder lamps. No coccidiostat, antibiotics, or enzymes were added to the experimental diets. The chicks were regular vaccinated at the hatchery against Infectious Bursal Disease, and no additional vaccinations were given during the study.

Sampling Procedures

Growth performance data were presented as feed consumption, slaughter weight, average daily gain, and feed conversion ratio (FCR). Feed consumption and slaughter weight data were taken on d 0 and 42 for calculation of body weight gain and FCR. Protein and energy efficiency data were presented as protein intake, energy intake, efficiency ratio (PER), and energy efficiency ratio (EER). PER (g/g) were calculated by dividing body weight gain (g) with protein intake (g) at the same duration of rearing period. EER (g/100 kcal) were calculated by multiplying body weight gain with 100, and followed by dividing the result with gross energy intake (kcal), according to calculation done by Dono (2012).

On day 42, two birds per replicate pen with body weight similar to the mean body weight of the pen were killed by humane slaughtering on anterior part of the neck using very sharp blade according to Islamic Law. Breast meat samples were removed and the meat quality traits were determined as meat pH, water holding capacity (Hamm, 1972), cooking loss (Bouton et al., 1971), and tenderness.

Statistical Analyses

Statistical analyses were conducted with the Statistical Package for Social Science (SPSS for Windows Version 15 SPSS GmbH, Munich, Germany) to determine if variables differed between groups. The data of growth performance, nutrient and energy utilization, as well as meat quality between groups were analyzed statistically by Oneway ANOVA. Duncan's new Multiple Range Test was used subsequently to separately significantly different means (Steel and Torrie, 1993). Significance was declared at probability values of less than 5% ($P<0.05$).

RESULTS

Results showed that SBM substitution with 50-150 g/kg SMW did not influence protein and energy consumption, energy efficiency ratio, as well as amount of feed consumed by the birds. However, results on Table 2 showed that 100 g/kg SMW substitution increased ($P<0.05$) slaughter weight and average daily weight gain by 1.79% and 3.53% improvement, respectively, resulting lower feed conversion ratio ($P<0.05$). The improvements of growth performance, as shown in the better average of daily gain (ADG) and slaughter weight could be attributed to the increase in protein efficiency ratio (Table 3). Protein efficiency ratio (PER) - efficiency in the use of protein that daily consumed - shows the contribution of dietary protein intake in improving ADG (Dono, 2012). Therefore, results clarify that the lower dietary protein intake in combination with the higher value of ADG, the higher value of PER will be achieved. As daily intake of protein is required and influential for growth and body enlargement, value of PER shows the effectiveness of protein in the diet for maximizing body development.

Although the SBM substitution with 100 g/kg SMW reduced crude protein content, but the lysine and methionine contents of the diet SMW-2 were increased (Table 1). This might be due to the higher lysine and methionine contents of SMW than those of SBM (Forster et al., 2002). Substitution of SBM with SMW in current study therefore increased the content and availability of essential amino acids in the experimental diets. Improvement of essential amino acids content in the experimental diets should increase the availability of micro nutrients which required by the fast growing of the broiler chickens. This improvement might be the answer on why replacement of SBM with SMW with the level of 100 g/kg resulted in lower FCR and higher average daily gain and slaughter weight.

Results in this study were in line with Hickling et al. (1990) where addition of diets with proper levels of methionine and lysine improved body weight gain and feed efficiency of 3-6 weeks old male Ross x Arbor Acres broiler chickens. Carefully worked with the same breed of broiler chickens in a corn-soybean meal basal diet, Han and Baker (1994) showed that increased of methionine and lysine levels in the diets had correlative effect with body weight gain and feed efficiency improvements. It has been shown in Labadan, Jr. et al. (1991) study that lysine requirement, as percentages of total amino acid in the diet, for maximum breast muscle growth were: 1.32 $\pm$ 0.01% (0 to 2 wk of age), 1.21 $\pm$ 0.06% (2 to 4 wk of age), 0.99 $\pm$ 0.02% (3 to 6 wk of age), and 0.81 $\pm$ 0.01% (5 to 8 wk of age), while lysine content of the experimental diets in current study was 1.13-1.21%.

Table 4 showed that no reductions were shown in meat pH, water holding capacity, cooking loss, as well as the meat tenderness. SBM substitution with 50-150 g/kg SMW in current study did not show any negative effect on meat physical quality. Feedstuffs containing high levels of fiber may be a good source of bio-active substances that may contribute to maximize growth performance and meat quality of broiler chickens. On the other hand, high fiber level in the diet can also have a minor effect on broiler performance. A study using high-fibre containing feedstuff (Maurão et al., 2008) showed that incorporating significant level of citrus pulp or dehydrated pasture in the diets reduced growth performance and meat characteristics of broiler chickens. However, Tabook et al. (2006) reported that dietary addition of date fibre had no significant effect on carcass or meat quality characteristics. In this study, substitution of SBM with 100% SMW increased crude fibre content of the diets but did not give any negative impact on physical meat quality responses. The absence of adverse effects on physical quality of meat might show that SMW can be used as alternative for SBM in the diets of broiler chickens.

CONCLUSION

Dietary substitution of soybean meal with 100 g/kg soy-milk waste might give positive effects in improving efficiency in protein utilization and growth performance, without any negative effects on meat quality of broiler chickens.

KEYWORD : Broiler chickens, Growth performance, Physical meat quality, Soybean meal substitution, Soy-milk waste

Table 1. Ingredient composition (g/kg, as-fed basis) and calculated nutrient and energy content of the diets used in the study

Item	Dietary treatments[1]			
	SMW-0	SMW-1	SMW-2	SMW-3
Ingredients composition, g/kg				
Yellow maize	487.5	485.0	488.5	488.5
Rice bran	171.7	171.6	165.8	160.4
Poultry meat meal	75.0	70.5	69.8	75.2
Fish meal	62.5	71.3	76.8	76.8
Soybean meal	150.0	100.0	50.0	0.0
Soy-milk waste	0.0	50.0	100.0	150.0
Palm kernel oil	21.0	21.0	19.4	19.5
Vitamin-mineral premix	25.0	25.0	25.0	25.0
Common salt	7.3	5.6	4.7	4.6
Total	1000.0	1000.0	1000.0	1000.0
Calculated Nutrients and Energy				
Metabolizable energy, kcal/kg	3057.4	3053.1	3034.7	3039.0
Crude protein, g/kg	212.1	211.8	211.2	210.9
Crude fibre, g/kg	30.5	32.2	33.0	34.1
Extract ether, g/kg	46.0	49.0	49.2	49.6
L-Lysine, g/kg	11.3	11.9	12.0	12.1
DL-Methionine, g/kg	3.8	4.0	4.5	4.8
Calcium, g/kg	7.7	7.9	8.3	8.6
Available Phosphorus, g/kg	5.0	5.2	5.3	5.4

Note: Soybean meal substitution with 0 g/kg (SMW-0), 50 g/kg (SMW-1), 100 g/kg (SMW-2), 150 g/kg (SMW-3) soy-milk waste.

Table 2. Growth performance responses of broiler chickens to soy-milk waste substitution[1]

Variable	Dietary treatments[2]				Significance level	
	SMW-0	SMW-1	SMW-2	SMW-3	SED	p-value
Feed intake, g/bird	2822.2	2827.1	2836.2	2841.0	7.977	0.288
Average daily gain, g/bird	1487.2^{b}	1495.4^{b}	1513.8^{a}	1524.6^{a}	16.588	0.016
Slaughter weight, g/bird	1604.6^{b}	1604.8^{b}	1661.2^{a}	1663.8^{a}	30.276	0.026
Feed conversion ratio	1.898^{a}	1.891^{a}	1.874^{b}	1.864^{b}	0.016	0.013

^{ab}Means within a row without a common superscript differ significantly ($P < 0.05$).
[1]Data represent means from 3 replicates pens of 5 birds per treatment.
[2]SMW-0=basal diet with 150 g/kg SBM (control; C), SMW-1=C with 50 g/kg SBM substitution, SMW-2= C with 100 g/kg SBM substitution, SMW-3=C with 150 g/kg SBM substitution.

Table 3. Energy and protein efficiency ratios of broiler chickens which receiving diets substituted with soy-milk waste[1]

Variable	Dietary treatments[2]				Significance level	
	SMW-0	SMW-1	SMW-2	SMW-3	SED	p-value
Energy Intake, kcal/bird	8629.86	8629.91	8630.91	8632.86	38.229	0.280
Protein Intake, g/bird	598.54	598.81	598.91	599.41	4.847	0.229
Energy Efficiency Ratio	17.233	17.329	17.540	17.660	0.201	0.111
Protein Efficiency Ratio	2.485[b]	2.497[b]	2.528[a]	2.543[a]	0.034	0.023

[1]Data represent means from 3 replicates pens of 5 birds per treatment.
[2]SMW-0=basal diet with 150 g/kg SBM (control; C), SMW-1=C with 50 g/kg SBM substitution, SMW-2= C with 100 g/kg SBM substitution, SMW-3=C with 150 g/kg SBM substitution.

Table 4. Meat physical quality responses of broiler chickens at 35 days of age in response to soy-milk waste substitution[1]

Variable	Dietary treatments[2]				Significance level	
	SMW-0	SMW-1	SMW-2	SMW-3	SED	p-value
Meat acidity (pH)	5.807	5.710	5.743	5.753	0.091	0.694
Water holding capacity	54.202	55.231	60.348	44.498	8.647	0.138
Cooking loss	27.389	29.775	29.737	33.319	4.344	0.474
Meat tenderness	1.893	1.593	2.267	2.243	0.717	0.697

[1]Data represent means from 3 replicates pens of 5 birds per treatment.
[2]SMW-0=basal diet with 150 g/kg SBM (control; C), SMW-1=C with 50 g/kg SBM substitution, SMW-2= C with 100 g/kg SBM substitution, SMW-3=C with 150 g/kg SBM substitution.

REFERENCES

Aimon, H. and A. Satrianto. 2014. Prospect of soybean consumption and import in Indonesia Year 2015 – 2020. Journal of Economic Studies. 3: 1-13.

Aviagen. 2007. Lohmann Meat Broiler Performance Objective. Huntsville, Alabama.

Aviagen. 2014. Ross 308 Broiler Performance Objective. Huntsville, Alabama.

Bouton, P.E., P.V. Harris, and W.R. Shorthose. 1971. Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. Journal of Food Science. 36: 435–439.

Cobb-Vantress. 2015. Cobb 500 Broiler Performance & Nutrition Supplement. Siloam Springs, Arkansas.

Dono, N.D. 2012. Nutritional strategies to improve enteric health and growth performance of poultry in the post antibiotic era. Ph.D. Thesis, University of Glasgow, Glasgow.

Forster, I.P., W. Dominy, and A.G. Tacon. 2002. The use of concentrates and other soy products in shrimp feeds. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., Simoes, N. (Eds.). Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Hamm, R. 1972. Kolloidchemie des Fleisches - das Wasserbindungsvermoegen des Muskeleiweisses. In: Theorie und Praxis. Paul Parey. Berlin.

Han, Y. and D.H. Baker. 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. Poultry Science. 73: 1739-1745.

Hickling. D., W. Guenteri, and M.E. Jackson. 1990. The effects of dietary methionine and lysine on broiler chicken performance and breast meat yield. Canadian Journal of Animal Science. 70: 673-678.

Labadan, Jr., M.C., K.-N. Hsu, and R.E. Austic. 2001. Lysine and arginine requirements of broiler chickens at two to three-week intervals to eight weeks of age. Poultry Science. 80:599–606.

Meng, X. and B.A. Slominski. 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poultry Science. 84: 1242–1251.

Mourão, J.L., V.M. Pinheiro, J.A.M. Prates, R.J.B. Bessa, L.M.A. Ferreira, C.M.G.A. Fontes, and P.I.P. Ponte. 2008. Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poultry Science. 87:733–743.

O'Toole, D.K. 1999. Characteristics and use of okara, the soybean residue from soy milk production A review. Journal of Agricultural and Food Chemistry. 47: 363–371.

Tabook, N.M., I.T. Kadim, O. Mahgoub, and W. Al-Marzooqi. 2006. The effect of date fibre supplemented with an exogenous enzyme on the performance and meat quality of broiler chickens. British Poultry Science. 2006. 47: 73-82.

O-22-5

The Effect of Lighting Regimens on Broiler Growth:2. On Weekly T3 and T4 Concentration

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INTRODUCTION

Light is one of the environmental factors that have an important role on the function of organs, especially the reproductive organs, behavior and social interaction poultry. Activities of eating the chicken depends on the presence or absence of light. Poultry meal at a time of light and almost no activity of eating in the dark. Thus the lighting functions to help optimize daily weight gain. Lighting is a strong exogenous factor in controlling many physiological processes and behavior, as well as the most critical factor of all the environmental factors for poultry. The integration of light with vision, including visual acuity and color difference (Olenrewaju et al., 2006). Scope of light that influence the physiological poultry there are four kinds, namely photoperiod, intensity, color, and light sources. Photoperiod is the length of time the light of natural lighting, which is ideal for activating hormones 11-12 hours.

Biologically light as a stimulus of hormonal activity of the hypothalamus and pituitary, which affect the growth. Stimulation of the physiological aspects of light affects the body's organs, starting with the mechanical stimulation through the visual nerve chemically followed, which ultimately leads to hormonal. It has been demonstrated that the use of blue light can increase performance of production in broiler chickens. Further reported weight gain during the 28-day maintenance of the normal lamp light color, blue, and green respectively in 1531, 1606, and 1537 gram/bird (Mauludin, 2014). Giving light for 24 hours would increase feed consumption and energy waste, otherwise broiler getting dark treatment (without lighting) longer than the light show better health levels. According Olenrewaju et al., (2006) broiler males were maintained during continuous illumination (23L: 1D) and Intermitteng Light (3L:1D) in 24 hour had better growth, the growth hormone is higher than the broiler given continuous illumination (24L: 0D). Light close relation to the chicken physiological development. Important influence of light is determined by three different aspects, namely the intensity of light, long exposure, and wavelength (Olenrewaju et al., 2012). Conventionally broiler strain Lohmann given lighting 23 hours of light and 1 hour dark on the first day later at the age of 4 days and so the lighting is required 20 hours of light and 3 hours dark (Aviagen, 2014). The green color is better than blue in spurring protein deposits. Colour green is the preferred color of chicken during the brooding off until harvested (Rozenboim et al., 2004). Blue lights helpful to provide a quiet atmosphere in poultry, while the red light will increase the activity of cannibalism and flapping wings (Rozenboim et al., 1999). Zhang et al. (2012) reported that the chicken senses sensitivity can be utilized to improve the performance of chicken, so the chicken can be given off brooding color blue or green light.

Thyroid tissue consisting of follicles of epithelial cells surrounding a lumen filled with colloid has been identifed in all vertebrates examined to date. In most vertebrates, these follicles are grouped together into a discrete gland, the thyroid, whilst in others they are diffusely distributed generally in the anterior region of the body (Etkin and Gona, 1974). This review will restrict itself to vertebrates. For two fascinating recent accounts of the function of thyroid hormones in invertebrates the reader is referred to Eales (1997) and Johnson (1997). The thyroid hormones are very hydrophobic and those that exhibit biological activity are 3',5',3,5-ltetraiodothyronine (T4), 3',5,3-l-triiodothyronine (T3), 3',5',3-l-triiodothyronine (rT3) and 3,5,- ldiiodothyronine (3,5-T2). At physiological pH, dissociation of the phenolic -OH group of these iodothyronines is an important determinant of their physical chemistry that impacts on their biological effects (Hullbert, 2000). When non-ionized these iodothyronines are strongly amphipathic. It is proposed that iodothyronines are normal constituents of biological membranes in vertebrates. In plasma of adult vertebrates, unbound T4 and T3 are regulated in the picomolar range whilst protein-bound T4 and T3 are maintained in the nanomolar range. The function of thyroid-hormone-binding plasma proteins is to ensure an even distribution throughout the body. Various iodothyronines are produced by three types of membrane bound cellular deiodinase enzyme systems in vertebrates. The distribution of deiodinases varies between tissues and each has a distinct developmental profile. Thyroid hormones have many effects in vertebrates. It is proposed that there are several modes of action of these hormones.

OBJECTIVE

Studying the performance and status of production of hormones triiodothyronine (T3) and thyroxine (T4) in broiler chickens 28 days in blue light treatment by means of continuous and intermittent blue lighting. Information about how blue lighting for 28 days were efficient on the broiler.

METHODOLOGY

Time and research location: The birds used in this study were 2700 day-old chicks (DOC). The research was conducted at a poultry farm in the village of Wonokerto, Turi, Sleman, Jogjakarta. Hormonal analyses were conducted at the Physiology Laboratory, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia. Blood examinations were performed at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine at the University of Gadjah Mada. A total of 2700 Lohmann DOCs were grouped randomly into 3 treatment groups: the control group, the group treated with intermittent monochromatic blue light (IBL) (12L:12D) and the group treated with continuous monochromatic blue light (CBL) (24L:0D). Each group consisted of 900 chickens. The body weights of the chickens were measured on days 1, 7, 14, 21 and 28.

Blood examination: At least 1 mL of blood was collected using a syringe and placed into a micro tube with ethylenediamine tetra acetic acid (EDTA). Blood samples were taken from decapitation of the chickens to measure T3 and T4 levels using a commercial kit DRG International, Inc., USA.

T3 and T4 assay: Plasma T3 and T4 concentrations were determined using a commercial enzyme ELISA immunoassay kit for T3 and T4 (DRG, International, Inc, USA).

Statistical analysis: The collected data were subjected to statistical analyses for the interpretation of the results using One way of ANOVA with a completely randomized design. Treatment means were compared with the Duncan Multiple Range Test (Steel *et al*., 1996).

RESULTS

Body weight gain: The body weight gain over 28 days of treatment are shown in Chart 1. There were significant differences among the control, IBL and CBL groups ($p<0.05$). The body weight gain was measured weekly on 1st week, 2nd week, 3rd week, and 4th week. The bird was measured randomly.

T3 levels: Data concentration of hormones triiodothyronine (T3) were obtained at ages 2, 7,14, 21, and 28 days are shown in Chart 2. Whole blood was taken by decapitation of five birds per treatment flock. Whole blood that has been in the centrifuge of 300 rpm for 10 minutes to obtain blood serum. To find out the status of hormone triiodothyronine (T3) using ELISA (enzyme linked immunosorbent assay) with the components in it to T3 is T3 Ab Coated Wells Standards, Liq Conjugate Diluent Antibody Reagent TMB Reagent Stop Solution and for components in T4 is Microtiterwells Standards, Liq Cone conjugate (11x) Conjugate Diluent TMB and Stop Solution. Production DRG International Inc., Mountain Ave, Spriengfield, USA.

The purpose of having the data hormone that is growing every week. In order to know at what age point or at concentrations of hormones triiodothyronine (T3) of the maximum . The concentration of the hormone triiodothyronine (T3) on lighting CBL has a high concentration in the age of 2 days ($P <0.05$), whereas the IBL has a concentration of hormones triiodothyronine (T3) is the highest at age 14 and 21 ($P <0.05$). In Control hormone concentrations triiodothyronine (T3) contained only the highest at 28 days.

T4 levels: The concentration of the hormone thyroxine (T4) were collected at ages 2, 7, 14, 21, and 28 are shown in Chart 3. Whole blood was taken by decapitation of five birds per treatment flock. Whole blood that has been in the centrifuge of 300 rpm for 10 minutes to obtain blood serum. Thyroxine (T4) analysis using ELISA (enzyme linked immunosorbent assay) Components in T4 is Microtiterwells Standards, Liq Cone conjugate (11x) Conjugate Diluent TMB and Stop Solution. Production DRG International Inc., Mountain Ave, Spriengfield, USA.
It has the same purpose, namely to describe the concentration of the hormone thyroxine (T4) which actually correspond to the desired age. The data obtained showed concentrations of the hormone thyroxine (T4) is highest in chickens aged 7 and 14 days, but no significant changes. Differences lighting has an influence on the

concentration of the hormone thyroxine (T4) is the highest CBL lighting at the age of 2, 14, and 21 days, the highest IBL lighting at the age of 7 and 28 days, while the lighting control always under CBL or IBL.

DISCUSSION

Continuous blue lighting and intermittent blue lighting treatment have a higher value of weight gain than control. Continuous and intermittent blue lighting given the result of weight gain that is optimal compared to the control, the influence of the blue color can give a quiet atmosphere in poultry so that the activity of chicken given lighting CBL and IBL make lower activity than the activity eat, so feed consumed many stored into muscle. Lighting blue can create a quiet atmosphere in poultry feed so that incoming energy efficient to be used (Rozenboim et al., 1999). Blue light is beneficial to provide a calm atmosphere so as to increase the body weight gain of chicken. Sensory sensitivity chicken utilized to improve the performance of chicken one of which increase the body weight gain. Color blue light can improve the performance of chickens. (Zhang et al., 2012). There are three color lights are optimal in improving production performance in broiler chickens, namely green, blue, and yellow (Rozenboim et al., 1999). Color blue lights give the best results (Mauludin, 2014).

The behavior of chickens in colors of blue light becomes quieter and can minimize behavior that can remove the chicken from the feed consumed energy (Rozenboim et al., 2006). Effect of light blue color on the hormonal status which can increase the concentration of the hormone Chart 2 on day 2, 14, and 21. Lighting color blue light through the retina of the eye will be passed through the eye nerve to the anterior hypothalamus, thus secreted tirotropik releasing hormone (TRH) and somatotropik hormone releasing factor (STH-RH). Releasing the lever will stimulate the anterior pituitary gland to secrete STH and TSH, TSH stimulates the thyroid gland to release thyroxin. STH and thyroxine will stimulate the body to increase the activity of growth (Kuhn et al., 1996) There is a difference concentrations of hormone triiodothyronine (T3) as it may result in differences are also body weight gain and feed convertion, note that the hormones triiodothyronine (T3) has a role in regulating the growth in broiler chickens.

There are two main hormones that regulate the expression of growth in broiler chickens, the GH and T3 (triiodothyronine). Growth hormone (GH) in poultry synthesized directly by somatotrof in the caudal lobe in the anterior pituitary (Darras et al., 1993). Phase starter in broiler chickens had T3 hormone production was higher in the treatment IBL and CBL. It makes the production performance significantly effect on body weight gain. T3 then GH produced will increase as well so will affect the performance of the production. Anterior pituitary gland to secrete growth hormone more quickly, in such a situation is T3 more potent than T4 (Colin, 2011). The fact about influence continuous and intermittent blue lighting on that birds, poultry included, can perceive light in different, non-pictorial ways. There are three key photosensitive areas: (1) Retina, which absorbed photons by photo-pigments rhodopsin (rods), iodopsin (cones) and recently melanopsin. (2) Pineal Gland and (3) Hypothalamus**.** Those 3 areas have a very important role in biological and physiological functions (Suwindra and Balnave, 1986 Apeldoorn *et al*., 1999 Hartwig and Vanveen, 1979 Hatori and Panda, 2010). Insulin like growth factors and the thyrotrophic [triiodothyronine (T3) and thyroxine (T4)] axis are considered to be prerequisite for normal growth and development (Decuypere and Buyse, 2005).

The production of T3 and T4 are activated by thyroid stimulating hormone (TSH) secreted by the pituitary through a negative feedback mechanism. When T3 and T4 decreased, TSH is secreted. The concentration of T3 is circulated governed by reducing deactivation of GH, this mechanism is done by T3-degrading type III deiodinase (Darras et al., 1993). There is a relationship between the growth hormone by the thyroid hormone status, the status of thyroid hormone (T3) is optimal GH secretion also can make optimal and may even increase the secretion of GH thereby increasing the rate of growth and division of tissue in it. There is a clear link between the status of thyroid hormone and GH secretion, if the T3 optimal GH secretion is also optimally even better, if the status is very high thyroid hormone can interfere with GH secretion that can inhibit tissue growth (Hullbert, 2000).

Thyroxine (T4) in the body of the chicken is first directly affect enzymes related to metabolic processes, spur activity increased oxygen consumption, accelerating the pulse, increases metabolic activities, backup nitrogen, energy provision and indirectly stimulate spending somatotropik hormone , At the cellular level, thyroid hormones increase the absorption and utilization of glucose. Hormones are also able to increase glycogenolysis, protein synthesis in all cells simultaneously upgraded followed by ribosomal activity RNA and a larger nucleus (Frandson, 1992). Calorigenic activity (generating heat) of the thyroid hormone is half of the Basal Metabolic Rate (BMR) from an animal that is normal, because this hormone increases oxygen consumption in all cell metabolism and

stimulate the cytoplasmic protein synthesis. Physical and emotional stress tends to inhibit the secretion of hormones. Thyroxine is essential for normal growth and differentiation of the network available. A reduction of this hormone in young animals causes dwarfism. Thyroxine hormone deficiency affects most of the systems in the body, as well as affect the metabolism of carbohydrates, fats, proteins, and electrolytes (Veerle et al., 2000).

CONCLUSIONS

Based this study, it can be concluded that continuous and intermittent blue lighting regimes can increase chicken body weight gains (P<0,05). And increase levels of T3 on 2,14 and 21 days of age chickens (P<0,05) but does not increase T4 levels, both of which indicators of Growth hormone secretion status. Thus, continuous and intermittent blue lighting usefully to increase the growth and more efficient in commercial broiler industry.

KEYWORD : blue light, chicken age, T3, T4

Chart 1. body weight gains of chicken over 28 days in the control, IBL, and CBL groups
Value (Mean ± SD) bearing different alphabets in a column is differ significantly (P<0.05).

	Weight gain over 28 days (g/chicken)		
	Control	IBL	CBL
1	1361,1	1510,2	1568,97
2	1365,43	1514,23	1573,63
3	1366,23	1514,6	1572,3
Average±SD	1364,922 ± 19,71[a]	1513,011 ± 49,84[b]	1571,644 ± 23,55[c]

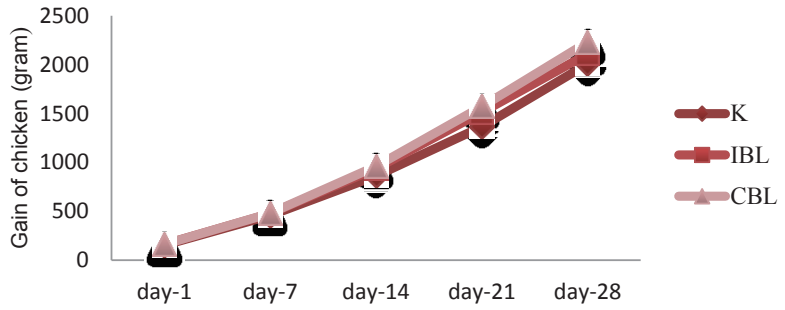
Chart 2. T3 levels (ng/mL) in Lohmann chickens on days 2, 7, 14, 21, 28
Value (Mean ± SD) bearing similar alphabets in a column is no differ significantly (P>0.05).
On the other hand, mean value different alphabets in a column is differ significantly.

	T3 levels (ng/mL) on experimental days				
Treatment	2	7	14	21	28
Control	5.23 ± 0.65[a]	5.51 ± 0.92[a]	4.33 ± 0.56[a]	2.80 ± 0.55[a]	3.07 ± 0.96[a]
IBL	5.67 ± 0.61[a]	5.35 ± 0.85[a]	6.06 ± 0.54[c]	4.50 ± 1.35[c]	2.80 ± 0.77[a]
CBL	6.65 ± 0.88[b]	5.83 ± 0.88[a]	5.57 ± 1.07[b]	4.09 ± 0.51[b]	2.07 ± 0.35[a]

Chart 3. T4 levels (ng/mL) in Lohmann chickens on days 2, 7, 14, 21, 28.

	T4 levels (ng/mL) on experimental days				
Treatment	2	7	14	21	28
Control	3.25 ± 1.05	7.73 ± 1.53	7.16 ± 1.17	4.76 ± 1.02	2.39 ± 0.58
IBL	2.53 ± 0.65	8.79 ± 3.76	7.01 ±1.22	3.17 ± 1.21	2.46 ± 0.28
CBL	3.56 ± 0.89	6.42 ± 1.91	7.64 ± 0.94	4.97 ± 1.62	2.21 ± 0.91

Chart 4. Body weight of chicken from day 1 untill day 28 of age (gram /chicken).



REFERENCES

Apeldoorn EJ, Schrama JW, Mashaly MM, Parmentier HK (1999) Effect of melatonin and lighting schedule on energy metabolism in broiler chickens. Poult Sci 78:223–229. doi: 10.1093/ps/78.2.223

Aviagen, 2014. Indian river meat broiler stock object. 2014. Cumming Research Park 5015, Bradford, Hunstville, Alabama, USA. Available at http://en.aviagen.com/assets/Tech_Center/LIR_Broiler/IRBroilerPerfObj2. Accession date Oct 23 rd 2015.

Colin G. S. 2011. Homones and Metabolisme in Poultry, University of Wisconsin Milwaukee, USA.

Darras, V.M., Rudas, P., Visser, T.J., Hall, T.R., Huybrechts, L.M., Vanderpooten, A., Berghman, L.R., Decuypere, E. dan Kühn, E.R. 1993. Endogenous growth hormone controls high plasma levels of 3,3',5-triiodothyronine (T3) in growing chickens by decreasing the T3-degrading type III deiodinase activity. J. Dom Anim Endocrinol 10 (1) : 55-65.

Decuypere, E., Buyse, J., 2005. Endocrine control of postnatal growth in poultry. J. Poult. Sci. 42:1-13.

Eales, J. G., Holmes, J. A., McLeese, J.M. & Youson, J.H. (1997). Thyroid hormone deiodination in various tissues of larval and upstream-migrant sea lampreys, *Petromyzon marinus. General & Comparative Endocrinology 106*, 202 ± 210.

Etkin, W.&Gona, A. G. (1974). Evolution of thyroid function in poikilothermic vertebrates. In *Handbook of Physiology. Section* 7, volume III, Thyroid (eds. M. A. Greer & D. H. Solomon), pp. 5 ± 20. American Physiological Society, Washington, DC.

Frandson. 1992. *Anatomy and Fisiologi of Animal*. Fourth Edition. Translated by B. Srigandono fan Koen Praseno Gadjahmada University Press. Yogyakarta.

Hartwig HG, Vanveen T (1979) Spectral Characteristics of Visible Radiation Penetrating into the Brain and Stimulating Extraretinal Photoreceptors - Transmission Recordings in Vertebrates. J Comp Physiol 130:277–282. doi: 10.1007/bf00614615

Hatori M, Panda S (2010) The emerging roles of melanopsin in behavioral adaptation to light. Trends Mol Med 16:435–446. doi: 10.1016/j.molmed.2010.07.005

Havenstain G. B, P. R. Feket, and M. A. Qureshi, 2003. Growth livability, and feed convertion of 1957 versus 2001 broiler when fed representative 1957 and 2001 broiler diet. J. Poult. Sci. 82: 1500-1508.

Hulbert, A. J, 2000. Thyroid hormones and their effects: a new Perspective. *University of Wollongong research online,* hulbert@uow.edu.au. Faculty of Science, Medicine, and Health. University of Wollongong. AU.

Johnson, L. G. (1997). Thyroxine's evolutionary roots. *Perspectives in Biology & Medicine 40*, 529 ± 535.

Mauludin M. 2014. The Effect of Monochromatic Blue and Green light on the Performance of Broiler. Script. Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Olanrewaju, H.A. et al. 2006. *A Review of Lighting Programs for Broiler Production. J. Poult*. Sci. 5 (4) : 301-308.

Rozenboim I, B. Robinzon, and A. Rosenstrauch, 1999. Effect of light source and regimen on growing broilers. *Br. Poult*. Sci. 40: 452-457.

Rozenboim I, I. Biran, Y. Chaiseha, S. Yahav, A. Rosenstrauch, D. Sklan, and O. Halevy, 2004. The effect of green and blue monochromatic light combination on broiler growth and development. J. Poult. Sci. 83:842-847.

Steel, R. G., J. H. Torry and D. A. Dickey, 1996. Principles and procedures of Statistics A Biometrical Approach. Multiple Comparisons Theory and Methods. Jason C. Hsu. Chapman Hall/CRC. Department of Statistics,

Ohio State University, Columbus, OH.
Suwindra N, Balnave D (1986) Intermittent Lighting and Feeding Schedules for Broilers Incorporating Long Periods of Darkness. Br Poult Sci 27:225–236.
Veerle M. Darras, Serge Van der Geyten, Eduard R. Kühn, 2000 Thyroid hormone metabolism in poultry. *Biotech. Agron. Soc. Environ*.(1), 13-20.
Zhang L, H. J. Zhang, X. Qiao, H. Y. Yue, S. G. Wu, J. H. Yao, and G. H. Qit, 2012. Effect of monochromatic light stimuli during embryogenesis onmuscular growth, chemical composition, and meat quality of breast musclein male broilers. *J. Poult. Sci*.,91 :1026–10.

O-22-9

BROILER FARMING IN TAIWAN: ON-FARM SURVEY IN HUALIEN COUNTY

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INTRODUCTION

In Taiwan, according to the agricultural statistical yearbook of 2014, the broilers consumed in a year were 430,000 mt totally (Statistic Office of C. O. A., 2014). Most of chickens were from the western region of Taiwan and where floor-rearing is common for commercial productions. Hualien County located in the eastern region of Taiwan where is sparsely populated and obstructed by Central Mountains Range. This natural and isolated environment is truly suitable for free-range farming. Therefore, free-range broilers farming was blooming around 2000 and free-range broilers became a famous specialty of Hualien County (Lin *et al.,* 2009). However, because the AI out broken and the price of feeds went up, the number of broilers reared significantly reduced in Hualien County during recent five years.

Although the broiler farming industry faced the critical challenges, the change of consumers' awareness showed a chance of revival of this industry. People realized that animal welfare and the food safety were becoming issues and concerns, only well-treated chickens can provide the high quality and safe chicken meat (Miao *et al.,* 2005). Therefore, the free-range broilers from Hualien County turned into a reliable choice for consumers undoubtedly. In this moment, it was an opportunity to figure out the superiorities and inferiors of free-range broiler farming industry in Hualien County. Consequently, we tried to investigate the current status of broiler farms in Hualien County and this study represented the problems of this industry needed to be solved urgently.

MATERIALS AND METHODS

Twenty one broiler farms were selected from a list of farms provided by Agriculture Bureau, Hualien County Government and phone interviewed during March, 2016. Totally, there are 342,050 broilers were covered in this survey and the breeds included black-feather native chicken, game hen, red-feather native chicken and silky. The items surveyed were general information, broiler breeds and chick sources, feeds source, farm facilities, products and their sale and management problems. In addition, these items were calculated as percentage of related farms out of the total number of farms surveyed (Kwak *et al.,* 1994).

RESULT AND CONCLUSION

Apparently, the broiler farming scale is very diverse in Hualien County and the numbers of broilers reared are from 150 to 120,000 per farm. All farms situated in the East Rift Valley and Shoufeng as well as Fuli were the two major districts which farms located. The results showed that approximately three quarters of farms (16 out of 21 farms) were managed by owners who considered broiler farming as a specialty and their careers averaged 18 years. The remainders were farming broilers as a sideline and their careers averaged 6 years. Overall, the livabilities of broilers from specialized farms were 87.0% and higher than sideline farms (83.5%). Furthermore, the daily labor requirement each farm were 2.07 full-time labors and 0.98 part-time labors in average. Usually, part-time workers helped the strenuous works like removing litters and grabbing the chickens before transportation. However, this investigation showed that the age of owners and employees were above 50 in average and youths rarely participated in this broiler farming industry.

This survey also demonstrated that the most popular breed was black-feather native chicken (64% of 342,050 broilers) and the next was game hen (28%) in Hualien County. Game hen is the crossbreed of fighting chicken and red-feather native chicken whose texture and flavor of meat are unique and preferred by gourmands. The minor breeds included red-feather native chicken and silky were around 8%. Generally speaking, one-day old chicks were purchased from the western region of Taiwan. 55% black-feather native chicken chicks came from Tainan City and 75% game hen chicks were mainly from Changhua County. On average, the marketed age of game hen (159 days) was longer than black-feather native chicken (116 days). Moreover, for the demands of consumers, farming multiple breeds of broiler showed the highest percentage (48%) in 21 broiler farms.

95% farms used commercial premix feeds and all of those commercial feeds were imported from the south-western region of Taiwan. In order to entertain the birds and to replenish the extra fibers out of the daily diet,

forage grass like pennisetum was supplied to chickens during the growing period. Moreover, the residue of vegetables and fruits from the local were given if possible. With regard to farm facilities, because Hualien County frequently suffered severe typhoons during summer, the automatic machinery could be destroyed by the gales. Farm owners preferred to maintain their own farm by labor rather than to establish the expensive automatic system. Therefore, merely 14% farms apply the automatic machinery. Even in 16 specialized farms, only 6 farms (38%) owned automatic or semi- automatic feeding system.

In the aspect of terminal products and their sale, although wholesalers could handle the slaughtering, selling and transportation of chickens, still about 62% farms marketed their own broilers by themselves to earn the higher profits. Applications of internet and home delivery made it possible and efficient to marketing and shipping the products by farm owners' self. Furthermore, 14% farms had developed the processed products like condensed chicken soup which is a popular nutrient supplement for Taiwanese people (Huang *et al.,* 2014). Compared to raw meats, processed products could increase the value as well as the sales of products.

At present, three major problems in the Hualien County free-range broiler farming industry were exposed from this survey. First of all, almost all of farm owners complained about the confused AI prevention policy. In order to prohibit the wild birds bringing the virus in, farms were enforced to surround their rangeland with nets. However, farmers criticized that the setting up of nets was time and money consuming and its consequent was doubtful. In addition, many owners pointed out that the shortage of chicks seriously harmed the survival of their business. Because AI was broken out in the western region of Taiwan and so far the produce of chicks reduced dramatically. Finally, the deficiency of slaughterhouses was another critical issue for local farms. The length of Hualien County is about 137.5 km, however, there were merely 3 registered slaughterhouses and located in the northern (Xincheng and Shoufeng) and southern (Yuli) terminals separately. Long distance transportation of chickens not only caused birds suffering but also increased the risk of pathogen spreading.

In conclusion, the broiler farming scale is very diverse in Hualien County and the most of farms are specialties. Due to the youths have no willing to take over this business, the population aging in the broiler farming industry is becoming an issue. Another critical problem is that the sources of chicks are dominated by the suppliers from the high-risk area of AI. Furthermore, the commercial premix feeds are popularly used, but automatic feeding machinery are not so prevalent because of the climate and topography in Hualien County. To gain higher profits, farmers have developed processed products like condensed chicken soup and other products are on planning. Undoubtedly, this study represents the current status of the broiler farming in Hualien County and it helps us to sustainably develop the industry of free-range broiler in Taiwan.

KEYWORD：Broilers, Free-Range, Hualien County, Taiwan

Table 1. General information of surveyed farms

Style of farms	Number of farms	Number of broilers reared (birds/farm)	Average career of farms (years)	Average age of farmers (years)
Specialty	16	250-120000	18	52
Sideline	5	50-1100	6	50
	Total 21			

Table 2. Daily labor requirement, level of automation and livabilities of broilers of surveyed farms

Style of farms	Daily labor requirement (workers/day)		Level of automation			Livabilities of broilers
	Full-time	Part-time	Automatic feeding system	Semi-automatic feeding system	None	
Specialty	2.30	1.20	15% (3/21)	15% (3/21)	48% (10/21)	87.0%
Sideline	1.20	0.20	0% (0/21)	0% (0/21)	24% (5/21)	83.5%
Average	2.07	0.98	15% (3/21)	15% (3/21)	72% (15/21)	85.9%

REFERENCE

Huang, W. C., C. I. Lin, C. C. Chiu, Y. T. Lin, W. K. Huang, H. Y. Huang and C. C. Huang. 2014. Chicken essence improves exercise performance and ameliorates physical fatigue. Nutrients. 6 : 2681-2696.

Kwak. W. S., K. H. Kim, C. W. Kim, B. T. Jeon and S. M. Lee. 1994. Deer farming in Korea : on-farm survey in Kyung-Kee and Chung-Buk provinces. Asian-Aust. J. Anim. Sci. 7(3) : 347-355.

Lin. C. Y., M. L. Lee, H. Y. Kuo, and M. S. Duh. 2009. A study on the optimal marketing age of Taiwan game hens. Taiwan Livestock Res. 42(1) : 35-45.

Miao. Z. H., P. C. Glatz and Y. J. Ru. 2005. Free-range poultry production - A review. Asian-Aust. J. Anim. Sci. 18(1) : 113-132.

Statistic Office of C. O. A. 2014. Livestock Production, in: Yearly Report of Taiwan's Agriculture, Council of Agriculture, Executive Yuan, Taipei, Taiwan.

O-23-4

Regulation of the cathelicidins expression in the vagina of laying hens in response to stimulation with microbial-associated molecular patterns

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Abstract

Objective The aim of this study is to examine the expression profile of Cathelicidins (CATHs) in the whole oviductal segments as well as the effects of viral and bacterial associated molecular patterns on their expression in the vagina.

Method The mRNA were extracted from all oviductal segments and examined for the profile of CATH1, CATH2, CATH3 and CATH1B by RT-PCR, whereas mucosal tissues of the vagina were cultured in TCM-199 medium and stimulated with or without viral associated molecular patterns namely, poly I:C (dsRNA virus, TLR3 ligand), R848 (ssRNA virus, TLR7 ligand) and CpG-ODN (DNA virus, TLR21 ligand) or bacterial associated molecular patterns such as Pam3CSk (TLR2 ligand) and flagellin (TLR5 ligand), followed by real-time PCR analysis.

Results The mRNA of CATH1, CATH2 and CATH3 were expressed in all segments of the oviduct except of CATH2 which was not expressed in the magnum, whereas CATH1B was not expressed at any segments of the oviduct. Pam3CSk4 and CpG-ODN did not affect the expression levels of CATH1, CATH2 and CATH3, Poly I:C down-regulated the expression of CATH1, CATH2 and CATH3, flagellin down-regulated the expression of CATH3, whereas R848 up-regulated the expression of CATH1 and CATH3 but down-regulated CATH2. These results suggested that three types of CATHs were expressed in the oviduct.

Conclusion These results suggest that mucosal tissues of the oviduct express CATHs to provide oviduct with defense mechanism against bacterial and viral infections, and the expression of CATH1 and CATH3 is up-regulated against ssRNA viruses, whereas, dsRNA virus may suppress the expression of CATH1, CATH2 and CATH3 and flagellin-containing bacteria may suppress the expression of CATH3.

Keywords:Cathelicidins,Hen oviduct,Toll-like receptor ligands,Microbial-associated molecular patterns

INTRODUCTION

The host innate immune defense plays an essential role in protecting the oviduct tissues as the first defense barrier against invading pathogenic microorganisms (Mageed et al., 2008 Sonoda et al., 2013). Recognition of specific molecular structures of the pathogens, which are known as pathogen-associated molecular patters (PAMPs), by specific receptors is the first step in the innate immune response.

PAMPs such as peptidoglycan and lipoproteins of the gram-positive bacteria and the synthetic analogue Pam3CSk4 are recognized by the heterodimer of TLR1 and TLR2 (Mintz et al., 2013) the analogue of dsRNA polyinosinic-polycytidylic acid (poly I:C) is recognized by TLR3 (Karpala et al., 2008) LPS of the Gram-negative bacteria is recognized by TLR4 (Karnati et al., 2014), bacterial flagellin is recognized by TLR5 (Keestra et al., 2008), whereas single-stranded RNA (ssRNA) of viruses is recognized by TLR7 (Diebold, 2008 Yue et al., 2014). Fungal and bacterial proteases are recognized by the chicken specific TLR15 (de Zoete et al., 2011 Keestra et al., 2013), whereas oligo-DNA with unmethylated CpG motifs is recognized by the TLR21, a homologous of mammalian TLR9 (Brownlie et al., 2009 Keestra et al., 2010). After recognition of different PAMPs, the innate immune response is triggered in the form of synthesis of cytokines and/or antimicrobial peptides such as defensins and cathelicidins.

Cathelicidins (CATHs) are a group of host defense peptides that have broad spectrum of antimicrobial effects against Gram-negative bacteria, Gram-positive bacteria, fungi and parasites (Lehrer and Ganz, 2002 Veldhuizen et al., 2013 Zaiou and Gallo, 2002 Zanetti, 2004). They are widely expressed in different tissues of different animals. In chicken, four Cathelicidins were discovered namely, CATH1, 2 and 3 which are expressed in various organs such as the lung, digestive tract, liver, bursa of Fabricius, testis, kidney, spleen and bone marrow (Xiao et al., 2006) and CathB1 which is expressed in the bursa of Fabricius only (Goitsuka et al., 2007(Goitsuka et al., 2007). However, no reports examined the presence of CATHs in the tissues of chicken oviduct. Thus, the aim of this study was to examine the mRNA expression profile of CATHs in the whole oviductal segments as well as the effects of

virus- and bacteria-associated molecular patterns (TLR ligands) on their expression in the vagina of laying hens. A part of the results that have been published was included here to state whole profiles of CATHs expression in the oviduct (Abdel-Mageed et al., 2014).

MATERIALS AND METHODS

Experimental birds

White Leghorn laying hens, approximately 250 days old were used in this experiment and handled in accordance with the regulations of Hiroshima University Animal Experiment Committee.

TLR ligands

TLR ligands (Pam3CSK4, Poly I:C, flagellin, R848 and CpG-ODN) used in this experiment were same as previously described in our report (Abdel-Mageed et al., 2014).

Tissue culture of the vaginal mucosa

The mucosal tissues of the vagina were collected, washed in saline containing 10 U/mL penicillin and 10 μg/mL streptomycin (Cosmo Bio Co., Ltd., Tokyo, Japan). The tissue specimens were placed in culture tubes containing 2 mL TCM-199 medium containing antibiotics. The different TLR ligands, Pam3CSK4 (0 to 100 ng/mL), poly I:C (0 to 100 μg/mL), flagellin (0 to 100 ng/mL), R848 (0 to 5 μg/mL), and CpG-ODN (0 to 10 μg/mL) were added to the culture tubes and incubated for 1.5 h or 3 h at 39 ℃ in 5% CO_2 and 95% air.

RNA extraction and reverse transcription (RT)

Total RNA was extracted from cultured tissues and from all oviductal segments and reverse transcribed into cDNA.

Semiquantitative PCR

The mRNA expression of *CATH*s in the mucosal tissues of all segments of the oviduct was examined by RT-PCR using specific primers for *CATH1, CATH2, CATH3* and *CATHB1* (Achanta et al., 2012) and the PCR products were separated by agarose gel electrophoresis.

Quantitative real-time PCR

Real-time PCR was performed using a Roche Light Cycler Nano System (Roche Applied Science, Indianapolis, IN, USA). The reaction mixture (20 μL) contained 1 μL of the cDNA, 1 × Thunderbird SYBR qPCR mix (Toyobo), 1 × ROX reference dye and 0.5 μM each primer. The amplification was performed with 3 steps cycles with 55 cycles for all *CATH*s primers.

RESULTS

The mRNA expression of *CATH1* and *CATH3* was expressed in the mucosal tissues of all segments of the oviduct from the infundibulum to vagina. The expression of *CATH2* was observed in the infundibulum, isthmus, uterus and vagina but not in the magnum whereas, the expression of *CATHB1* was not observed at any segments of the oviduct. Stimulation of the cultured vaginal tissues with Pam3CSK4 and CpG-ODN did not change the expression of *CATH1*, *CATH2* and *CATH3* either in time dependent manner nor dose dependent manner. Stimulation of the cultured vaginal tissues with Poly I:C downregulated the expression of *CATH1, CATH2* and *CATH3* in a dose dependent manner at a concentration of 10 μg/ml but not in a time-dependent manner. Flagellin stimulation of the cultured vaginal tissues did not change the expression of *CATH1* or *CATH2* in both time dependent and dose dependent manner. However, the expression of *CATH3* was downregulated in a dose dependency at 10 ng/ml but not in time dependency. R848 treatment of the cultured vaginal cells upregulated the expression of *CATH1* and *CATH3* in a dose-dependent manner at a concentration 10 μg/ml however the expression of *CATH2* was significantly downregulated in a dose-dependent manner at 10 μg/ml and 100 μg/ml. In examination of the time dependency, the expression of *CATH2* was downregulated at a concentration 100 μg/ml at 3 h but not 1.5 h post stimulation.

DISCUSSION

In the current study, we are reporting the expression profile of *CATH*s in the tissues of oviduct as well as the effect of some bacterial and viral components on their expression in the vagina. CATHs are widely distributed among

different mammalian and avian tissues such as human, goat horse and chicken (Kościuczuk et al., 2012 Scocchi et al., 1999 Srisaikham et al., 2016).

The expression of human cathelicidin (*LL37*) in the human neonatal foreskin keratinocytes was upredulated by Pam3Cys (Hau et al., 2013). Our study showed that no change in the expression of *CATH1, CATH2* and *CATH3* in the cultured vaginal tissues of chicken was obtained with Pam3CSK4 stimulation.

The expression of *LL37* in the human keratinocytes was upregulated by Poly I:C stimulation (Hau et al., 2013). Whereas the expression of *LL37* in the human primary foreskin KCS cells was not affected by Poly I:C stimulation (Abtin et al., 2008). Our current study reported that stimulating the cultured vaginal mucosal tissues with Poly I:C could downregulate the expression of *CATH1*, *CATH2* and *CATH3*.

The expression of *CRAMP* in the lung microphages and osteoblasts of SPF C57B mice was upregulated by flagellin stimulation (Horibe et al., 2013 Yu et al., 2010). However, the expression of *LL37* in the human primary foreskin KCS cells was not changed after stimulation with flagellin (Abtin et al., 2008) similar findings were notices in our study regarding to *CATH1* and *CATH2* which were not changed by flagellin stimulation of the cultured vaginal mucosal tissues. However, the expression of *CATH3* was significantly downregulated.

The TLR7 agonist and R848-homologue, imiquimod induced the expression of *CATH*s in the CD11c+ cells of mice (Sainathan et al., 2012). In the current study R848 upregulated the expression of *CATH1* and *CATH3* but suppressed the expression of *CATH2* in the cultured vaginal mucosal tissues. This difference in response between different types of *CATHs* may indicate a difference in the functions of those CATH molecules.

It was reported that CpG-ODN enhanced the expression of *LL37* in the human lung epithelial cells (Rivas-Santiago et al., 2008) compared to the current study, CpG-ODN did not affect the expression of *CATH*s in the cultured mucosal tissues of the vagina.

It was reported that the expression of proinflammatory cytokines *IL1B* and *IL6* was induced by stimulation of the vaginal cells and tissues by different TLR ligands it was suggested that IL1B may play role in stimulating the synthesis of avian beta-defensins (*AvBD*s) which are different group of antimicrobial peptides in chicken (Abdel-Mageed et al., 2014 Sonoda et al., 2013) however it was observed that the expression of almost all examined *CATH*s was downregulated by some of TLR ligands and others did not affect their expression. Hence, we assume that the intracellular signaling pathway downstream of TLRs responsible for *CATHs* synthesis may differ from that of *AvBDs* synthesis.

In conclusion, these results suggest that mucosal tissues of the oviduct express *CATH*s to provide oviduct with defense mechanism against bacterial and viral infections, and the expression of *CATH1* and *CATH3* is up-regulated against ssRNA viruses, whereas, dsRNA virus may suppress the expression of *CATH1, CATH2* and *CATH3,* and flagellin-containing bacteria may suppress the expression of *CATH3*.

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KEYWORD：Cathelicidins, Hen oviduct, Toll-like receptor ligands, Microbial-associated molecular patterns

REFERENCES

Abdel-Mageed, A.M., Isobe, N., Yoshimura, Y., 2014. Effects of different TLR ligands on the expression of proinflammatory cytokines and avian β -defensins in the uterine and vaginal tissues of laying hens. Vet. Immunol. Immunopathol. 162, 132-141.

Abtin, A., Eckhart, L., Mildner, M., Gruber, F., Schröder, J.-M., Tschachler, E., 2008. Flagellin is the principal inducer of the antimicrobial peptide S100A7c (psoriasin) in human epidermal keratinocytes exposed to Escherichia coli. The FASEB Journal 22, 2168-2176.

Achanta, M., Sunkara, L.T., Dai, G., Bommineni, Y.R., Jiang, W., Zhang, G., 2012. Tissue expression and developmental regulation of chicken cathelicidin antimicrobial peptides. Journal of animal science and biotechnology 3, 1.

Brownlie, R., Zhu, J., Allan, B., Mutwiri, G.K., Babiuk, L.A., Potter, A., Griebel, P., 2009. Chicken TLR21 acts as a functional homologue to mammalian TLR9 in the recognition of CpG oligodeoxynucleotides. Mol. Immunol. 46, 3163-3170.

de Zoete, M.R., Bouwman, L.I., Keestra, A.M., van Putten, J.P., 2011. Cleavage and activation of a Toll-like receptor

by microbial proteases. Proceedings of the National Academy of Sciences 108, 4968-4973.
Diebold, S.S., 2008. Recognition of viral single-stranded RNA by Toll-like receptors. Advanced drug delivery reviews 60, 813-823.
Goitsuka, R., Chen-lo, H.C., Benyon, L., Asano, Y., Kitamura, D., Cooper, M.D., 2007. Chicken cathelicidin-B1, an antimicrobial guardian at the mucosal M cell gateway. Proceedings of the National Academy of Sciences 104, 15063-15068.
Hau, C.S., Kanda, N., Noda, S., Tatsuta, A., Kamata, M., Shibata, S., Asano, Y., Sato, S., Watanabe, S., Tada, Y., 2013. Visfatin enhances the production of cathelicidin antimicrobial peptide, human β-defensin-2, human β-defensin-3, and S100A7 in human keratinocytes and their orthologs in murine imiquimod-induced psoriatic skin. The American journal of pathology 182, 1705-1717.
Horibe, K., Nakamichi, Y., Uehara, S., Nakamura, M., Koide, M., Kobayashi, Y., Takahashi, N., Udagawa, N., 2013. Roles of cathelicidin‐related antimicrobial peptide in murine osteoclastogenesis. Immunology 140, 344-351.
Karnati, H.K., Pasupuleti, S.R., Kandi, R., Undi, R.B., Sahu, I., Kannaki, T., Subbiah, M., Gutti, R.K., 2014. TLR-4 signalling pathway: MyD88 independent pathway up-regulation in chicken breeds upon LPS treatment. Vet. Res. Commun., 1-6.
Karpala, A.J., Lowenthal, J.W., Bean, A.G., 2008. Activation of the TLR3 pathway regulates IFN< i> β production in chickens. Dev. Comp. Immunol. 32, 435-444.
Keestra, A.M., de Zoete, M.R., Bouwman, L.I., Vaezirad, M.M., van Putten, J.P., 2013. Unique features of chicken Toll-like receptors. Dev. Comp. Immunol. 41, 316-323.
Keestra, A.M., de Zoete, M.R., Bouwman, L.I., van Putten, J.P., 2010. Chicken TLR21 is an innate CpG DNA receptor distinct from mammalian TLR9. The Journal of Immunology 185, 460-467.
Keestra, A.M., de Zoete, M.R., van Aubel, R.A., van Putten, J.P., 2008. Functional characterization of chicken TLR5 reveals species-specific recognition of flagellin. Mol. Immunol. 45, 1298-1307.
Kościuczuk, E.M., Lisowski, P., Jarczak, J., Strzałkowska, N., Jóźwik, A., Horbańczuk, J., Krzyżewski, J., Zwierzchowski, L., Bagnicka, E., 2012. Cathelicidins: family of antimicrobial peptides. A review. Molecular biology reports 39, 10957-10970.
Lehrer, R.I., Ganz, T., 2002. Cathelicidins: a family of endogenous antimicrobial peptides. Current opinion in hematology 9, 18-22.
Mageed, A.A., Isobe, N., Yoshimura, Y., 2008. Expression of avian β-defensins in the oviduct and effects of lipopolysaccharide on their expression in the vagina of hens. Poult. Sci. 87, 979-984.
Mintz, M., Mintz, D., Ilia-Ezra, R., Shpigel, N.Y., 2013. Pam 3CSK 4/TLR2 signaling elicits neutrophil recruitment and restricts invasion of Escherichia coli P4 into mammary gland epithelial cells in a murine mastitis model. Vet. Immunol. Immunopathol. 152, 168-175.
Rivas-Santiago, B., Hernandez-Pando, R., Carranza, C., Juarez, E., Contreras, J.L., Aguilar-Leon, D., Torres, M., Sada, E., 2008. Expression of cathelicidin LL-37 during Mycobacterium tuberculosis infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infection and immunity 76, 935-941.
Sainathan, S.K., Bishnupuri, K.S., Aden, K., Luo, Q., Houchen, C.W., Anant, S., Dieckgraefe, B.K., 2012. The TLR7 ligand Imiquimod induces type I IFN and antimicrobial peptides to ameliorate DSS-induced acute colitis. Inflammatory bowel diseases 18.
Scocchi, M., Bontempo, D., Boscolo, S., Tomasinsig, L., Giulotto, E., Zanetti, M., 1999. Novel cathelicidins in horse leukocytes. FEBS Lett. 457, 459-464.
Sonoda, Y., Mageed, A.M.A., Isobe, N., Yoshimura, Y., 2013. Induction of avian β-defensins by CpG oligodeoxynucleotides and proinflammatory cytokines in hen vaginal cells in vitro. Reproduction 145, 621-631.
Srisaikham, S., Suksombat, W., Yoshimura, Y., Isobe, N., 2016. Goat cathelicidin‐2 is secreted by blood leukocytes regardless of lipopolysaccharide stimulation. Animal Science Journal 87, 423-427.
Veldhuizen, E.J., Brouwer, E.C., Schneider, V.A., Fluit, A.C., 2013. Chicken cathelicidins display antimicrobial activity against multiresistant bacteria without inducing strong resistance. PloS one 8, e61964.
Xiao, Y., Cai, Y., Bommineni, Y.R., Fernando, S.C., Prakash, O., Gilliland, S.E., Zhang, G., 2006. Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. Journal of Biological Chemistry 281, 2858-2867.
Yu, F.-s., Cornicelli, M.D., Kovach, M.A., Newstead, M.W., Zeng, X., Kumar, A., Gao, N., Yoon, S.G., Gallo, R.L.,

Standiford, T.J., 2010. Flagellin stimulates protective lung mucosal immunity: role of cathelicidin-related antimicrobial peptide. The Journal of Immunology 185, 1142-1149.

Yue, M., Gao, C.-f., Wang, J.-j., Wang, C.-j., Feng, L., Wang, J., Yu, R.-b., Peng, Z.-h., Xue, X.-x., Cai, L., 2014. Toll-like receptor 7 variations are associated with the susceptibility to HCV infection among chinese females. Infect. Genet. Evol.

Zaiou, M., Gallo, R.L., 2002. Cathelicidins, essential gene-encoded mammalian antibiotics. Journal of molecular medicine 80, 549-561.

Zanetti, M., 2004. Cathelicidins, multifunctional peptides of the innate immunity. Journal of leukocyte biology 75, 39-48.

O-23-5

A Comparison of Growth Performance, Carcass Quality of Male and Female Meat Type Ducks

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INTRODUCTION

Ducks account for 5% of world poultry production, and around 87% of this being produced in regions of Asian (Michael, 2004). Thailand is a major duck meat producer, total production was around 24,176 tons per month in 2008 and total export market value was 2,100 million Baht (Department of Livestock Development, 2008). Commercially, due to the cost of sexing ducklings at hatching and the lack of a pronounced sexual dimorphism in Pekin ducks, mixed sexes are usually kept in the same houses (Normand, 1997 Farhat and Chavez, 2000). However, several investigators reported that Pekin male ducks have a faster growth rate, better feed efficiency and lower carcass fat than the female (Farrell, 1990 Leeson and Summers, 1997 Normand, 1997). In term of carcass development, the percentages of muscle and skin with fat are increased, whereas the percentage of bones decrease as ducks grow older (Bochno *et al.,* 2006).

Since reports of physiological or growth development of commercial strain of ducks are less, this study was focused on a comparison of growth development and carcass quality of male and female commercial meat-type ducks.

MATERIALS AND METHODS

Animals and Management

Total of ninety-six Grimaud ducks (48 male and 48 female) were divided into 2 groups, and each group consisted with 6 replicates of 8 chicks each. During eight weeks experimental period, an evaporative cooling system was used to control air ventilation and temperature. Feed and water were offered *ad libitum*. The diets were formulated according to minimum requirement of Grimaud strain's recommendation.

Measurements of Growth Performance and Carcass Quality

The body weight and pool feed intake of the ducks were measured weekly. Subsequently, average daily gain, and feed conversion ratio (FCR) were calculated from these data at 2, 4, 6 and 8 weeks of age. FCR was calculated as feed intake (g) divided by body weight gain (g).

At 2, 4, 6 and 8 weeks of age, after overnight feed deprivation, all birds were weighted. Six ducks of each sex were randomly selected, and killed for determination of carcass composition. The carcass yield was defined as the carcass without blood, feathers and giblets. Body skin with fat was removed from the carcass (with the forearms and wing tips) (Ziolecki and Dorchowski, 1989). Breast (without skin), wing, legs meat, bones, abdominal fat and skin with fat were individual weighted and expressed as a percentage of live weight.

Statistical analysis

Significant difference among the mean of groups was separated by Student's t-test significant difference test at a 5% probability level.

RESULTS

Growth performance of male and female ducks shown in Table 1. There was no significant difference between male and female ducks on the body weight during 1-5 weeks of age, while the body weight of male ducks was heavier than that of the female at 6, 7 and 8 weeks of age ($P<0.01$).

Carcass compositions of male and female ducks are presented in Table 2. There were no significant differences on percentage of dressing carcass and bone between male and female throughout the experimental period. At 2, 4 weeks of age, wing and abdominal fat of the female was significantly bigger than that of the male, respectively ($P<0.05$). At 6 weeks of age, legs meat of male was significantly bigger than that of the female ($P<0.01$), while breast meat and skin with fat of female were bigger than the male ($P<0.05$).

DISCUSSION

Growth rate and body weight varies with breeds, ages and sexes (Janiszewska, 1993 Onbasilar *et al.,* 2011). Wilkiewicz-Wawro *et al.* (2005) showed that from 2 weeks of rearing, the differences in body weight of duck between males and females were becoming more visible, but this study shows that body weight was significantly affected by sex from 6 weeks of age. In agreement with Galal *et al*. (2011) who found that the body weight of Pekin and Muscovy ducks were heavier for the males from 5 weeks of age. Unlike broiler chickens, it is suggested that mixed sexes of meat type ducks can be kept in the same house up to 5 weeks of age.

Carcasses of duck are characterized by a relatively low lean content and high percentage of skin with fat, in compassion with other poultry species (Bochno *et al.,* 2005). For example, carcass of ducks at 7 weeks of age contained on average 39.8% of lean and 35.7% of skin with fat (Bochno *et al.,* 2005), while carcass of broiler chickens at 6 weeks of age contained 58% of lean and 18% of skin with fat (Bochno and Brzozowski, 1998). Generally, carcass yields are varied with the sexes (Solomon *et al.,* 2006). In this study, at 6 weeks of age, the breast and skin with fat of females were heavier than that of the males. Similarly, Farhat and Chavez (2000) reported that the female had a smaller body size, but have higher percentage of breast meat than the males. The reason is that females mature earlier and are expected to have a higher relative breast yield (Hay and Scott, 2007), although Baeza *et al.* (1999) found that from 6 to 12 weeks of age, the male had higher breast meat yield than the female. Interestingly, at 6 week of age, legs meat of male was bigger than that of the female. Bochno *et al.* (2005) and Bochno *et al.* (2007) also found that male Pekin ducks had thigh and shank bigger than that of the female. This indicates that sexes closely influence to carcass composition of meat type ducks.

The abdominal fat and skin with fat of female were heavier than those of male at 4 and 6 weeks of age. This is similar to the finding of Kleczek *et al.* (2006) who conducted with Muscovy ducks (10 weeks of age). However, Witak (2008) reported that males were characterized by larger contents of skin with fat and abdominal fat than females in the 9 weeks of age.

In conclusion, from 6 weeks of age, the male of Pekin ducks has faster growth rate than the female. There are difference effects of sexes on carcass composition, particular edible meat (breast and leg meat) and fat accumulation (abdominal and subcutaneous fat).

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KEYWORD : Growth performance, Carcass quality, Meat-type ducks

Table 1 Growth performance of male and female meat-type ducks.

Trait	Age (weeks)							
	1	2	3	4	5	6	7	8
BW[1] (g)								
Male	232.67 ± 9.02	701.16 ± 22.74	1301.07 ± 57.54	1953.60 ± 71.53	2778.63 ± 70.04	3459.41 ± 80.33**	3830.58 ± 110.30**	4131.53 ± 168.52**
Female	232.25 ± 5.68	700.82 ± 16.15	1303.91 ± 14.80	1957.27 ± 37.87	2684.44 ± 60.92	3227.70 ± 59.24	3489.03 ± 65.17	3726.11 ± 91.54
ADG[2] (g/d)								
Male	6.77 ± 1.29	66.96 ± 2.91	85.59 ± 4.85	93.23 ± 2.93	110.26 ± 5.48	97.10 ± 7.44**	52.50 ± 9.06*	40.09 ± 11.39
Female	6.70 ± 0.76	66.92 ± 2.10	86.36 ± 3.16	93.33 ± 4.14	103.80 ± 6.74	78.17 ± 5.84	40.15 ± 3.28	32.50 ± 4.64
FI[3]	28.86 ± 1.14	77.76 ± 2.95	124.96 ± 5.13	162.17 ± 8.79	208.82 ± 10.17	242.53 ± 8.76	201.01 ± 27.24	237.86 ± 48.63
FCR[4]	0.87 ± 0.07	1.06 ± 0.03	1.24 ± 0.02	1.41 ± 0.02	1.54 ± 0.02	1.77 ± 0.03	2.00 ± 0.09	2.28 ± 0.06

* Significant differences for traits between males and females (P<0.05)

** Significant differences for traits between males and females (P<0.01)

Values reported represent the mean ± SD.

[1]BW = Body weight

[2]ADG = Average daily gain

[3]FI = Feed intake (mixed-sex)

[4]FCR = Feed conversion ratio (mixed-sex)

Table 2 Carcass quality (% body weight) of male and female meat-type ducks.

Trait	Sex	Age (weeks)			
		2	4	6	8
Carcass (%)	Male	80.17 ± 7.15	79.78 ± 1.40	81.18 ± 1.88	82.17 ± 1.49
	Female	79.63 ± 6.79	79.79 ± 0.73	81.42 ± 1.21	84.33 ± 1.99
Breasts (%)	Male	1.93 ± 0.23	5.18 ± 0.56	11.07 ± 1.71	15.59 ± 0.81
	Female	2.11 ± 0.25	5.51 ± 0.51	13.53 ± 1.18*	16.54 ± 1.61
Wings (%)	Male	2.41 ± 0.15	6.03 ± 0.89	7.65 ± 0.38	7.33 ± 0.36
	Female	2.73 ± 0.26*	6.35 ± 0.21	7.40 ± 0.30	7.77 ± 0.36
Legs meat (%)	Male	17.63 ± 0.87	16.25 ± 0.47	13.23 ± 0.74**	10.92 ± 0.37
	Female	17.54 ± 0.72	15.49 ± 0.74	11.66 ± 0.61	11.34 ± 0.61
Skin with fat (%)	Male	17.29 ± 0.60	16.59 ± 0.80	16.88 ± 1.63	19.69 ± 2.16
	Female	17.40 ± 1.15	16.48 ± 2.28	19.53 ± 1.59*	19.76 ± 1.06
Abdominal fat (%)	Male	0	0.87 ± 0.13	1.17 ± 0.18	1.65 ± 0.63
	Female	0	1.13 ± 0.20*	1.40 ± 0.29	1.48 ± 0.39
Bones (%)	Male	32.40 ± 0.67	31.30 ± 1.55	27.31 ± 1.72	25.93 ± 1.33
	Female	32.76 ± 1.99	30.57 ± 1.10	25.99 ± 2.06	26.35 ± 0.73

* Significant differences for traits between males and females ($P<0.05$)

** Significant differences for traits between males and females ($P<0.01$)

Values reported represent the mean ± S

REFERENCES

Baeza, E., G. Marche and N. Wacrenier. 1999. Effect of sex on muscular development of Muscovy ducks. Reproduction Nutrition Development. 39: 675-682.

Bochno, R. and W. Brzozowski. 1998. The effect of age and sex of broiler chickens on slaughter value, feed conversion and production costs. J. Anim. Feed Sci. 7: 63-72.

Bochno, R., D. Murawska and U. Brzostowska. 2006. Age-related changes in the distribution of fat with skin and bones in goose carcasses. Poult. Sci. 85: 1987-1991.

Bochno, R., W. Brzozowski and D. Murawska. 2005. Age-related changes in the distribution of lean, fat with skin and bones in duck carcass. Br. Poult. Sci. 46: 199-203.

Bochno, R., W. Brzozowski and D. Murawska. 2007. Prediction of meatness and fatness in ducks by using a skin slice with subcutaneous fat and carcass weight without skin. Poult. Sci. 86: 136-141.

Department of Livestock Development. 2008. Thailand Livestock report. Available http://www.ice.gov.it/paesi/asia/thailandia/upload/177/Thailand%20Livestock%20Report.pdf , May 14, 2016.

Farrell, D. J. 1990. Nutrition and carcass quality in ducks. In: Recent Advances in Animal Nutrition. Butterworths, London, UK. 177-197.

Farhat, A. and E. R. Chavez. 2000. Comparative Performance, Blood Chemistry, and Carcass Composition of Two Lines of Pekin Ducks Reared Mixed or Separated by Sex. Poult. Sci. 79: 460-465.

Grimaud Freres. 2010. Rearing guide roasting pekin ducks. Groupe Grimaud LTD., France.

Galal, A., W.A.H. Ali, A.M.H. Ahmed and K. A. A. Ali. 2011. Performance and carcass characteristics of Dumyati, Muscovy, Penkin and Sudani Duck Breeds. Egyptian J. Anim. Prod. 48: 191-202.

Hay, G.C. and T.A.Scott. 2007. Growth performance and its prediction in two commercial strains of meat ducks. Australian Poult. Sci. Symp. 19: 45-48.

Janiszewska, M. 1993. Changes in body weight and tissue components of white Italian geese during the rearing period. Acta Acad. Agricult. Techn. Olst. Zoot. 37: 1-39 (Suppl).

Kleczek, K., K. Wawro, E. Wilkiewicz-Wawro and Makowski. 2006. Multiple regression equations to estimate the content of breast muscles, meat and fat in Muscovy ducks. Poult. Sci. 85: 1318-1326.

Leeson, S., and J. D. Summers. 1997. Commercial Poultry Nutrition. 2nd ed. University Books. Guelph. Canada. pp. 248-259.

Michael, D. 2004. Adding Value to New Animal Product Supply Chains. Rural Industries Research and

Development Corporation. ACT. Australia.
Normand, L. 1997. Recycling of agro-industrial food wastes into feed for Pekin duck meat production towards a sustainable agriculture in the province of Que ´ bec. M.Sc. Thesis. McGill University, Montreal, QC, Canada.
Onbasilar, E., E. Erdem, S. Gurcan and O. Poyraz. 2011. Body weight and body measurements of male and female Pekin ducks obtained from breeder flocks of different age. Archiv Fur Geelugelkunde. 75(4): 268-272.
Solomon, J. K.. Q., R. Austin, R. N. Cumberbatch, J. Gonsalves and E. Seaforth. 2006. A comparison of live weight and carcass gain of Pekin, Kunshan and Muscovy ducks on a commercial ration. Livestock Research for Rural Development.
Witak, B. 2008. Tissue composition of carcass, meat quality and fatty acid content of ducks of a commercial breeding line at different age. Archiv Fur Tierzucht. 51: 266-275.
Wilkiewicz-Wawro, E., K. Szypulewska and K. Wawro. 2005. Age-related changes in tissue component distribution in Muscovy duck carcasses. Archiv fur Geflugelkunde. 69(3): 128-134.
Ziolecki, J. and W. Doruchowski. 1989. Metody oceny wartosci rzeznej drobiu. COBRD, Poznan, 1-22 (in Polish).

O-23-6

Analysis of growth curves of male and female Pekin ducks

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Introduction

Poultry production needs to make decisions in the production cycle that affects the profitability of operation for example, nutrient supply to birds, cost and type of feed, bird health, and environmental issues (Darmani et al., 2010). The mathematical models of growth curves are of great importance for animal production because it provides results for visualizing growth patterns over time, and the generated equations can be used to predict the expected weight of a group of animals at a specific age (Tzeng & Becker, 1981 Yakupoglu & Atil, 2001 Sengul & Kiraz, 2005). Brody's, logistic, Gompertz, and the four-parameter Richards function are available to fit the growth curve of poultry (Goliomytis et. al., 2003). Two nonlinear growth models, Gompertz and Exponential, were used to estimate growth curve of duck (Faridi et al., 2014). Gompertz, Logistic and Richards were fitted by Norris et al. (2007) to estimate and compare the growth curve parameters for body weight of indigenous Venda and Naked Neck chickens. Anthony et al. (1991) compared the Gompertz, Logistic and Bertalanffy models in their study on quails.

The objective of the current study was comparison of average growth curves with the mean of age-weight data of male and female Pekin ducks using Gompertz, Logistic and Von Bertalanffy models. Results generated by these models were compared and the best model showing high performance for describing the growth curves of Pekin ducks was revealed.

Materials and Methods

Data were collected from male and female commercial Pekin ducks and a total of 144 (72 male and 72 female) Pekin ducks were used. Ducks were raised from day-old to 8 weeks of age (56 days) on a litter floor in evaporative cooling house system. Standard starter and grower diets were provided *ad libitum* and the birds had free access to water. Throughout the experiment, all ducks were weighed individually at 7 days intervals.

In this study, 3 different growth functions were fitted to estimate the mean age-live weight relationship. The mathematical models are as follows:

Gompertz : $W=A*Exp(-Exp(-b(t-c)))$

Logistic : $W=A/(1+Exp(-b(t-c)))$

Von Bertalanffy : $W=A(1-1/3Exp(-b(t-c)))^3$

Where W is the corresponding weight at time t. A is the adult value or asymptote, b, c, are the model parameters. Parameters were estimated using the non-linear regression and the Levenberg-Marquardt methods of SAS package (2016). Models were compared using Coefficients of determination (R^2). Parameter values, the Durbin-Watson Statistic (DW) test for autocorrelation and residual variances were calculated to evaluate the goodness of fit. The first criterion was Coefficient of determination measuring the proportion of total variation in the mean, explained by the model. The Durbin-Watson Statistic ranges from 0 to 4 and a value close to 2 indicates non-autocorrelation. If result has a value close to 0 indicating positive autocorrelation and a value close to 4 indicating negative autocorrelation. Residual variances were also estimated for goodness of fit.

Results and Discussions

Predicted Gompertz, Logistic and Bertalanffy growth curve models for male and female Pekin ducks are shown in figure 1, 2 and 3. Results generated using all models showed that male and female had same growing rate up to 3 weeks of age. Sexual dimorphism found between 3 and 4 weeks of age in all models. The values of parameter A (and the standard errors) from different models are presented in Table 1. In this study, the parameter A of the body weights using the Gompertz and Logistic model were closer to the observed values. Whereas, the largest value of the parameter A was observed using the Bertalanffy model and may possibly indicate overestimation of mature weight. The standard errors of parameter A estimated using the Bertalanffy model were also large. These results corresponded to the work of Yakupoglu & Atil (2001) claiming that of the values of parameter A generated using Bertalanffy model were higher than Gompertz model in broiler chickens.

According to the results, the values of R^2 were higher than 0.997 in all models for male and female Pekin ducks (Table 1). The highest value of R^2 was obtained from the Gompertz model in both sexes. Yakupoglu & Atil (2001) also found high R^2 values in a study of growth in broilers using Gompertz and Bertalanffy growth models. Norris et. al. (2007) also found high R^2 for Gompertz, Logistic and Richards in a study of growth curves of indigenous male Venda and Naked Neck chickens. Based on R^2 alone, all models seemed to be appropriate to use for describing the relationship between age and live weight.

Autocorrelation derived from the Durbin-Watson procedure for both sexes are given in Table 2. Durbin Watson statistic showed a good fit (no autocorrelation) for Gompertz model in both sexes. On the other hand, Durbin Watson statistic showed a trend of positive autocorrelation for female Pekin duck in Logistic model ($P<0.06$). Fitting the growth functions led to the lowest residual variance 65.67 and 31.42 values of male and female Pekin ducks respectively for Gompertz model (Table 2). Yakupoglu & Atil, 2001 stated that the residual variance may vary according to increasing and decreasing asymptotic body weight and small values would be desirable for the decision of best-fit model. Results of this study were consistent with the work of Anthony et. al. (1991) showing that growths of tukeys, quail and broiler were describing well using the Gompertz model.

In conclusion, the Gompertz model was the most appropriate for describing the age-live weight relationship in the male and female of Pekin duck. However, the estimated values of the mature weights using the Logistic model were closer to the observed values. It is possible to follow the change of the growth as taking advantage of both the growth models in Pekin duck. Thus, it is concluded that we need further study to examine the most appropriate model, in which the growth model parameters and growth characteristics used in order to develop more accurate results for Pekin ducks.

Acknowledgement

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KEYWORD : Pekin ducks, growth models, body weight

Figure 1 Predicted (Gompertz model) values for male and female body weight (BW) of Pekin ducks.

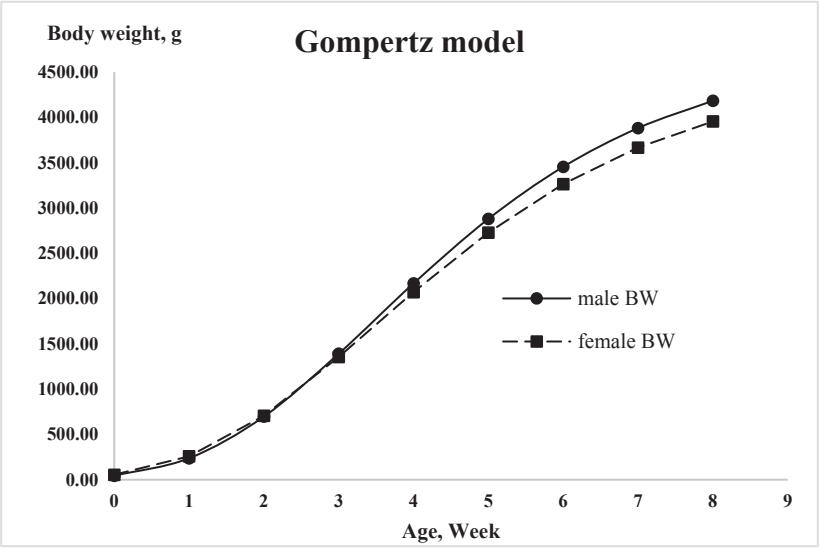

Figure 2 Predicted (Logistic model) values for male and female body weight (BW) of Pekin ducks.

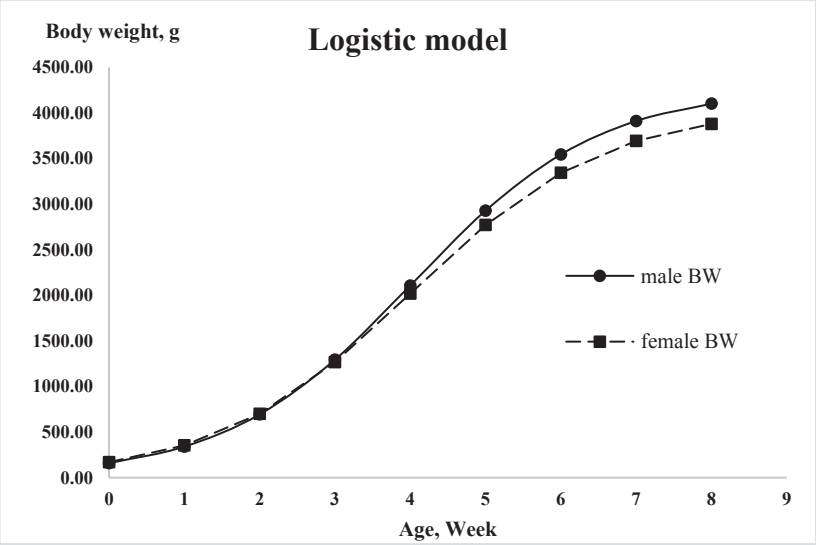

Figure 3 Predicted (Bertalanffy model) values for male and female body weight (BW) of Pekin ducks.

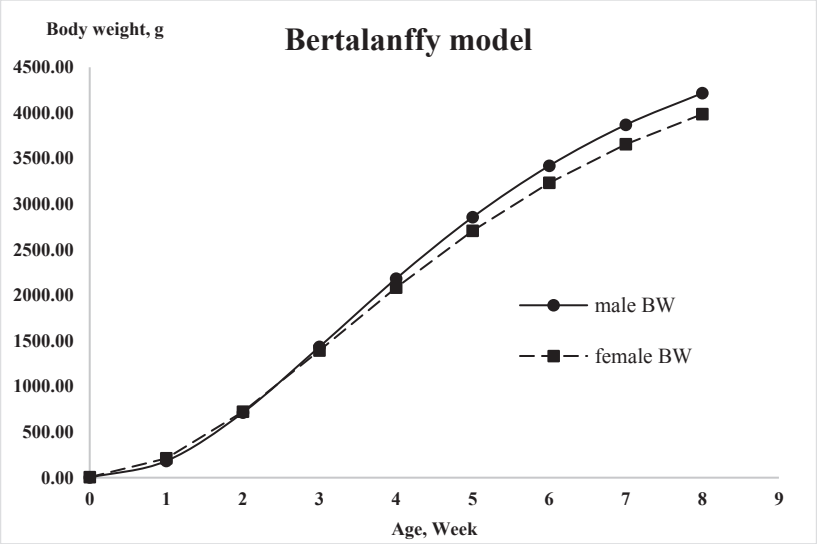

Table 1 Coefficients of determination (R^2) and Parameter A values of non-linear models

Model	Sex	R^2	A	s.e.
Gompertz	Male	0.9982	4781.9	168.8
	Female	0.9995	4552.9	83.4
Logistic	Male	0.9977	4267.9	100.7
	Female	0.9975	4052.8	103.5
Bertalanffy	Male	0.9970	5206.4	326.6
	Female	0.9989	4971.8	194.5

s.e. = standard errors

Table 2 Durbin-Watson statistic (DW) values and residual variances for non-linear models relating growth by age for male and female Pekin ducks.

Model	Sex	DW	Residual variances
Gompertz	Male	2.14	65.67
	Female	2.28	31.42
Logistic	Male	1.75	73.13
	Female	1.08	72.17
Bertalanffy	Male	1.62	85.32
	Female	1.42	48.60

REFERENCES

Anthony, N. B., D. A. Emmerson, K. E. Nestor and W. L. Bacon. 1991. Comparison of growth curves of weight of selected populations of turkeys, quail and chickens. Poult. Sci. 70, 13-19.

Darmani K., H., T. Porter, S. López, E. Kebreab, A.B. Strathe, A. Dumas, J. Dijkstra, and France J. 2010. A review of mathematical functions for the analysis of growth in poultry. Wold's Poultry Sci. J., 66: 227–240

Faridi, A., D. Murawska, A. Golian, M. Mottaghitalab, A. Gitoee, S. Lopez and France, J. 2014. Alternative growth functions for predicting body, carcass, and breast weight in ducks: Lomolino equation and extreme value function. Poultry Science. 93, 1031–1042.

Goliomytis, M., E. Panopoulou, and Rogdakis, E. 2003. Growth curves for body weight and major component parts, feed consumption, and mortality of male broiler chickens raised to maturity. Poult. Sci. 82, 1061-1068.

Norris, D., J. W. Ngambi, K. Benyi, M. L. Makgahlela, H. A. Shimelis and Nesamvuni, E. A. 2007. Analysis of growth curves of indigenous male Venda and Naked Neck chickens. S. Afr. J. Anim. Sci. 37, 21–26

SAS. Base SAS university edition installation guide for Windows. SAS Institute, Cary, NC, USA. 2016.

Sengul, T. and Kiraz, S. 2005. Non-linear models of growth curves in large white turkeys. J. Vet. Anim. Sci. 29, 331-337.

Tzeng, R. and Becker, W.A. 1981. Growth parameters of body and abdominal fat weights in male broiler chickens. Poult. Sci. 60, 1101-1105.

Yakupoglu, C. and Atil, H. 2001. Comparison of growth curve models on Broilers. II. Comparison of models. Online J. Biol. Sci. 1 (7), 682-684.

O-24-1

PRODUCTIVITY OF TWO VARIETIES Brachiaria sp ON DIFFERENT LEVEL OF FERTILIZER IN YOGYAKARTA INDONESIA

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INTRODUCTION

Cattle production in Yogyakarta Indonesia has been increasing rapidly over the last decade. Therefore, the increase should be balanced by the increase productivity especially the availability of forage feed that is an important factor in supporting the productivity of ruminants. *Brachiaria decumbens cv Basilisk* and *Brachiaria ruziziensis cv Kennedy* grass is a source of forage that grows well in Indonesia throughout the year. Lascano and Euclides (2009) stated, some plant varieties Brachiaria such as *Brachiaria decumbens cv Basilisk* and *Brachiaria ruziziensis cv Kennedy* had a different response to fertilizer absorption. Marassing (2013) stated, the large amount of fertilizer applied depending on the response of several varieties of grass plants Brachiaria sp. The more complete and appropriate a given nutrient amounts, the better and the maximum result obtained. Miles et al (1996) suggested that *Brachiaria decumbens cv Basilisk* responds strongly to fertilizer N and P, while Brachiaria ruziziensis cv Kennedy respond well to N fertilizer.

Brachiaria grass production would be better if it is conducted with proper and appropriate fertilization dose. Therefore we need to conduct a study on the influence of NPK fertilizer on the growth, production and quality of *Brachiaria sp* in Yogyakarta, Indonesia.

MATERIAL AND METHODS

This research was conducted over one year at the Forage and Pasture Science Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, Indonesia. The soil in this location is classified as regosol soil. Based on the soil test taken in June 2014, the result showed that it was netral (pH 7.12), with organic matter of 3,23%, N (0.2%) and P (0.22%) and K (0.10%).

Fertilization was conducted in accordance with the level of the corresponding dosage of NPK fertilizer, (P1 =0 kg , P2=150 kg, P3= 300 kg) sprinkled around the plant then covered by the soil. Planting was arranged with 1 x 1 m spacing. Furthermore, the plant development were observed and recorded.

The variables measured were growth (plant height, leaf numbers, plants length) productivity (dry matter production, organic matter production) and chemical composition. Observations on number of leaves was conducted by calculating the amount of green leaves on each plants. The rate of the number of leaves per week was calculated by the number of leaves day 60 was reduced with the number of leaves day 0 divided by time (weeks). The length was observed after the plant was moved to the land until day 60. The measurement started from the ground up to the longest length. The rate of leaf plants per week was calculated by the length on day 60 was reduced with the length on day 0 divided by time (weeks). Plant weight (canopy) at harvest was converted in tons/ha, then was multiplied with the percentage of dry matter (DM). Dry matter production (tons/ha) was multiplied with percentage of organic matter (OM).

The data collected was analyzed and if interaction exists, it was analyzed by using Duncan Multiple Range Test (DMRT)

Results and Discussion

The length of plants (cm) and the length rate of plant per week (cm/week) of several varieties of *Brachiaria sp* until the age of 60 days with provision of NPK fertilizer at certain levels with doses of 0 kg/ha, 150 kg/ha and 300 kg/ha, are *B. decumbens cv. Basilisk* has long accretion average per week was 16.71 cm, while *B. ruziziensis cv. Kennedy* had a mean of length per week was 12.86 cm. *B. decumbens cv. Basilisk* had a length of 155.23 cm whereas plants *B. ruziziensis cv. Kennedy* has a length of 128.64 cm. Sulistya and Mariyono (2013) stated, the length of *B. decumbens cv. Basilisk* grass was 98 cm, while B. ruziziensis cv. Kennedy was 85.5 cm.

Plant height (cm) and the rate of increase per week (cm/week) of *B. decumbens cv. Basilisk* and *B. ruziziensis cv. Kennedy* grass until the age of 60 days with the provision of NPK fertilizer with a dose level of 0 kg/ha, 150 kg/ha

and 300 kg/ha, are had no significant effect towards plant height (cm) and plant growth (cm/week). *B. decumbens cv. Basilisk* plant has lower average height (cm) and growth (cm/week) compared to *B. ruziziensis cv. Kennedy* per week. B. *decumbens cv. Basilisk* had a mean of growth of 5.65 cm/week, while *B. ruziziensis cv. Kennedy* was 6.24 cm/week. *B. decumbens cv. Basilisk* was 63.20 cm height, while *B. ruziziensis cv. Kennedy* was 67.67 cm. This may be due to *B. decumbens cv. Basilisk* has a type of high creeping growth and plant height increment per week lower than the *B. ruziziensis cv. Kennedy*. *B. decumbens cv. Basilisk* has a characteristic of spread growth and comes from tropical Africa. Carrilho *et al.* (2012) stated that *B. decumbens cv. Basilisk* grown in direct sunlight was 58 cm height, whereas in 50% radiation, it was higher, that was 63 cm.

Plant with fertilization level of 150 kg/ha has the highest height (cm) and growth (cm/week) compared with fertilization level of 0 kg/ha and 300 kg/ha. At the level 300 kg/ha, plant height and growth decreased, but not significantly. Sulistya and Mariyono (2013) stated, the height of *Pennisetum purpureum cv. Mott* at NPK fertilization level of 100 kg/ha and 200 kg/ha were respectively 78.8 cm and 74.8 cm. *Pennisetum purpureum cv. Hawaii* had the highest growth at the NPK fertilization level of 200 kg/ha, it was 130.8 cm. Meanwhile, at the fertilization level of 100 kg/ha, it was only 118.0 cm height. Plant height was influenced by genetics of each grass variety and the environment.

The number and the rate of increase in the number of leaves per week for several varieties of *Brachiaria sp* until the age of 60 days with the provision of NPK fertilizer with a dose level of 0 kg/ha, 150 kg/ha and 300 kg/ha, are had no effect on the growing number of leaves per week. It was because the number ploidization only affected the leaf surface area only. This is consistent with the statement Anggraito (2004) which stated that polyploid is a state of an individual who had more than two sets of chromosomes. Polyploid plants generally have the physical characteristics of the increase in cell size, slower cell growth rate, thicker leaves, less and larger flowers, larger fruit and declining fertility at various levels compared with diploid plants.

Dry matter content of some *Brachiaria sp* varieties with the provision of NPK fertilizer with dose level of 0 kg/ha, 150 kg/ha and 300 kg/ha, are listed in Table 1.

The results showed that the different varieties of *Brachiaria sp* had a significant effect ($P < 0.05$) towards dry matter content. *B. decumbens cv. Basilisk* had a higher dry matter content of 14.80% rather than *B. ruziziensis cv. Kennedy* of 12.60%. Carrilho et al. (2012) stated that *B. decumbens cv. Basilisk* grown under the sun had a higher dry matter content of 25%, whereas those with exposure to sunlight of 30% and 50% has a dry matter content of 22% and 23% respectively.

Data Table 1 shows that the levels of fertilization had a significant effect ($P < 0.05$) towards dry matter content. *B. decumbenscv. Basilisk* and *B. ruziziensis cv. Kennedy* has the highest dry matter content at NPK fertilization level of 150 kg/ha. This shows that the fertilization level of 150 kg/ha responds best in increasing dry matter content compared with NPK fertilization level of 0 kg/ha and 300 kg/ha. Seseray et al. (2013) stated that, elephant grass (*Pennisetum purpureum*) with the fertilization of 100 kg urea/ha 50 kg TSP/ha 50 kg KCl/ha had a dry matter content of 22.61%, while the fertilization of 200 kg urea/ha TSP 100 kg/ha 100 kg KCl/ha had a dry matter content of 24.56%, and those without fertilization had a dry matter content of 23.91%.

The content of organic matter of several *Brachiaria sp* varieties until the age of 60 days with NPK fertilizer doses of 0 kg/ha, 150 kg/ha and 300 kg/ha, are listed in Table 2 below.

Mean of organic matter content of *B. decumbens cv. Basilisk* was higher ($P < 0.5$) compared to *B. ruziziensis cv. Kennedy*. This is because the dry level in *B. decumbens cv. Basilisk* was high and it was proportional to the concentration of organic matter in it.

Several varieties of dry matter production of *Brachiaria sp* with the provision of NPK fertilizer with a dose level of 0 kg/ha, 150 kg/ha and 300 kg/ha, are listed in Table 3.

Table 3 shows the different varieties of *Brachiaria sp* had no real influence on the production of dry matter. Dry matter production of *B. ruziziensis cv. Kennedy* was higher (1.28 tons/ha) compared with *B. decumbens cv. Basilisk* (0.97 tons/ha). Gobius et al. (2001) stated that the production of *B. decumbens cv. Basilisk* dry material was 9.57 tons/ha, while *B. ruziziensis cv Kennedy*, according to Tekletsadik (2004), was 18.44 tons/ha.

Novizan (2007) stated that the availability of phosphorus in the soil was determined by many factors, but the most important was pH of the soil. In the land with low pH (acidic), phosphorus reacts with ions of iron and aluminum. This reaction formed iron phosphate or aluminum phosphate which were not soluble in water and so it cannot be absorbed by plants. In the land with a high pH (acid), phosphorus reacts with calcium ions. This reaction formed calcium phosphate that were not soluble and cannot be used by plants. Thus, regardless to the soil pH, phosphorus fertilization will not take effect for plant to grow.

Conclusion

Based on the research results, the addition of NPK fertilizers of 150 and 300 kg/ha can increase dry matter content, crude protein and crude fat. Differences in Brachiaria sp varieties influenced the fresh production, dry matter and organic matter, *B. decumbens cv. Basilisk* had a dry matter content and organic matter higher rather than *B. ruziziensis cv. Kennedy*. The interaction between Brachiaria sp varieties and the best interaction of *B. decumbens cv. Basilisk* with fertilization level of 150 kg/ha.

KEYWORD : Brachiaria, Productivity, Fertilizer

Table 1. Mean of dry matter level of two *Brachiaria sp* varieties with different level NPK fertilization

Varietas *Brachiaria sp*	Level of Fertilizer (kg/ha) 0	150	300	Average
B.decumbens cv. Basilisk	15.12±2.53	16.36±1.53	12.92±1.83	14.80±2.37
B. ruziziensis cv. Kennedy	12.00±1.05	13.51±2.15	12.29±1.87	12.60±1.76
Average	13.56±2.46^{ab}	14.93±2.31^{b}	12.61±1.78^{a}	

^{a,b}: different superscript on the same row shows the significant difference (P<0.05)

Table 2. Mean of organic matter of two *Brachiaria sp* varieties with different levels of NPK fertilization

Brachiaria sp varieties	Fertilizer level (kg/ha) 0	150	300	Mean
B.decumbens cv. Basilisk	84.46±0.95	84.70±0.51	83.65±1.41	84.27±1.06
B. ruziziensis cv. Kennedy	82.34±1.07	83.03±1.68	82.73±1.94	82.70±1.52
Mean^{ns}	83.40±1.47	83.87±1.47	83.19±1.67	

^{ns}: *non significant*

Table 3. Mean of dry matter production (tons/ha) of two varieties *Brachiaria sp* with different levels of NPK fertilization.

Brachiaria sp Varieties	Fertilizer Level ^{ns} 0	150	300
B.decumbens cv. Basilisk	8.95±1.35	9.65±1.59	9.03±1.98
B. ruziziensis cv. Kennedy	8.93±1.42	9.99±1.01	9.88±1.05
Mean^{ns}	8.94±1.38	9.82±1.30	9.45±1.51

^{ns} : *non significant*

REFERENCES

Anggraito YU, 2004 Identifikasi berat, diameter dan tebal kultival Action 434 tetraploid akibat perlakuan Colchicin. Berkala Penelitian Hayati 10(1):37-42

Carrilho, P. H. M., J. Alonso, L.D.T. Santos dan R. A. Sampaio. 2012. Vegetative and reproductive behavior of *Brachiaria decumbens* cv. Basilisk under different shade levels. J. Agric. Sci. 46 : 85-90.

Gobius, N. R., C. Phaikaew, P. Pholsen, O. Rodchompoo dan W. Susena. 2001. Seed yield and its components of Brachiaria decumbens cv. Basilisk, *Digitaria milanjiana* cv. Jarra and *Andropogon gayanus* cv. Kent in north-est Thailand under different rates of nitrogen application. 35: 26-33.

Lascano, O. E. dan V. P. B. Euclides. 2009. Nutritional quality and animal production of *Brachiaria* pastures. (16) : 106-122.

Marassing, J., Kaunang, F. Dompas dan N. Bawole. 2013. Produksi dan kualitas rumput gajah dwarf (*Pennisetum purpureum*) cv. Mott yang diberi pupuk organik hasil fermentasi EM4. 32:158-171.

Miles, J. W., Maass dan Valle. 1996. *Brachiaria* : Biology, agronomy and Improvement. Joint Publication. Brazil.

Miller, W.J. 2012. Dairy Cattle Feeding and Nutrition. Academic Press. United States of America.

Novizan. 2007. Petunjuk Pemupukan yang Efektif. Agromedia Pustaka. Jakarta.

Tekletsadik, T., S. Tudsri, S. Juntakool dan S. Prasanpanich. 2004. Effect of dry season cutting management on subsequent forage yield and quality of ruzi (*Brachiaria ruziziensis*) and Dwarf Napier (*Pennisetum purpureum* L.) in Thailand. Press. Yogyakarta.

Seseray, D. Y., B. Santoso dan M. N. Lekitoo. 2013. Produksi rumput gajah (*Pennisetum purpureum*) yang diberi pupuk N, P dan K pada devoliasi hari ke-45. 11: 49-55.

Sulistya, T. A., Mariyono 2013. Produktivitas Rumput Unggul di Area Tambang. Pasuruan. Jawa Timur.

O-24-3

Performance of tropical dairy cows fed on cassava top silage in rice straw based diet

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Abstract

The objective of this study was to investigate the effect of feeding cassava top silage (CTS) levels on feed intake and milk production fed rice straw. Four lactating crossbred dairy cows (75% Holstein-Friesian, HF and 25% Thai cows) in early lactation with average age at 4 years old were randomly assigned to four dietary treatments in a 4 × 4 Latin square design. The treatments were different supplementation levels of CTS at 0, 0.75, 1.50, and 2.25 kg/day of DM, respectively. All cows were fed concentrate at 2% of BW while rice straw was offered *ad libitum*. The present findings revealed that all cows had consumed CTS well as offered. Interestingly, milk production was dramatically increased with increasing level of CTS while milk composition was similar among treatments. Highest milk yield was obtained at high level of CTS feeding ($P<0.05$). This study concluded that feeding CTS could increase milk yield without adverse effect on feed intake and milk composition of dairy cows fed rice straw based diet. This study additionally suggested that CTS could be used as a high quality roughage source for improvement of milk and meat production in the tropics.

Introduction

Cassava is a highly productive tropical crop that is traditionally cultivated to produce roots or tubers for human food and for industrial extraction of starch. Apart from that, cassava also produces major amounts of leaves and the yields of cassava leaves at root harvesting may be as much as 4.6tonnes DM/ha. Fresh cassava forage including the tender stems, can be utilized directly for ruminants feeding, while sun-dried leaves can be utilized in non-ruminants as pig and poultry diets (Ravindran, 1991). Wanapat et al.(1997),reported cassava foliage in Thailand made into hay has been used efficiently as a source of rumen undegradable protein with a high content of digestible nutrients.

The HCN content is normally between 200 and 800mg/kg DM in fresh cassava leaves (Ravindran,1991). Sun-drying the leaves to produce cassava hay will result in a reduction in HCN (Wanapat et al., 2000). The concentration of HCN in the diets, between 18.3 and 49mg/kg DM, was below the recommended safe level of HCN in the diet of 50mg/kg DM (Bolhuis, 1954). Much research has been conducted to find out the effective methods of utilization cassava tops to reduce HCN content such as chemical treatment, heat treatment and fermentation. Cassava tops silage (CTS) can improve feed intake and nutrients digestibilities in ruminants and cyanogenic glucosides content is reduced significantly by 70 to 75% (Louembe et al., 1997 Kobawila et al., 2003) after 14-21 days of silage. Moreover, HCN can be rapidly detoxified by rhodanese and β-mercapto pyruvate sulfur transferasein ruminants by rumen microbes.

However, CTS has abundant and highest biomass productivity in the rainy season so, it should be harvested and stored as important source of protein for animals feeding in dry season and its feeding effects on ruminants animal are still limited. Therefore, this study was conducted to determine the effect of feeding CTS levels on feed intake and milk production fed rice straw base.

Materials and method

Preparing of ensilage

Cassava top were harvested at local farms in Khon Kaen province, Northeast of Thailand, at 9-12 months (containing young stem, leaf and petiole) from the top down in the length of 50 cm. It was then chopped to the length of 5 cm. Solution mixture was prepared by using ration of water: molasses: urea at 10: 2: 1, mixed well, and then prayed on to 100 kg chopped cassava top and kept into the barrel pressing well (about 60 kg/barrel), and covered tightly, store for at least 14 days before feeding to animal. After 21days of ensilage, temperature and pH of the silage were measured, and 500g of CTS were sampled for later chemical analysis.

Animals, experimental design and dietary treatments

Four lactating crossbred dairy cows (75% Holstein-Friesian, HF and 25% Thai cows) in early lactation with average age at 4 years old were randomly assigned to four dietary treatments in a 4 × 4 Latin square design. The treatments were different supplementation levels of CTS at 0, 0.75, 1.50, and 2.25 kg/day of DM, respectively. All cows were fed concentrate at 2% of BW while rice straw was offered *ad libitum*. Feed ingredient and chemical compositions of dietary treatment are shown in Table 1. The experiment was conducted for four periods and each was run for 21 days. First 14 days were for feed adaptation and intake measurement while the last 7 days were for sample collection. Animals were housed in individual pens and individually fed with dietary treatments twice daily at 06.00 and 16.00 after each milking time. Clean fresh water and mineral blocks were available at all times. Body weights of each cows was weighed at the first and last day of each period for feed offered calculation. Milk yield was recorded during the 21 day-period and samples were collected during the last 7 days of each period.

Sampling procedure, data collection and analysis

Feed offered and refusals were recorded daily throughout the experimental period for dry matter (DM) intake measurement. Samples of concentrate, rice straw and CTS were collected daily during the collection period and composited by period, stored at 4℃ for later chemical analysis. The samples were divided into two parts first part was analyzed for DM while second part was for analyses of ash, crude protein (CP) according to AOAC (1995). Acid detergent fiber (ADF) was determined according to an AOAC method (1995) and was expressed inclusive of residual ash. Neutral detergent fiber (aNDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite and results are expressed with residual ash. In addition, CTS was rinsed with distill water and collected for volatile fatty acid analysis by using high-pressure liquid chromatography (Intrusments by water and Novapak model 600E water mode 1484UV detector column Novapak C18 column size 3.9 × 300 mm mobile phase 10 mM H_2PO_4 [pH 2.5] according to Samuel et al. (1997)
Milk yield were recorded daily and milk samples were composited daily, according to yield, for both the morning and afternoon milking time, preserved with 2-bromo-2 nitropropane-1, 3- dial, and stored at 4 ℃ until analysis for fat, protein, lactose, totals solids, and solids-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO).

Statistical analysis

All data were subjected to ANOVA according to a 4× 4 Latin square design using the General Linear Models (GLM) procedures (SAS, 1998). The results were presented as mean values with the standard error of the means. Difference among means with P

Results and discussion

The chemical compositions of dietary feed are shown in Table 1. Rice straw nutritive value was similar to the report by Wanapat (1985). The DM content at 32.9 and 36.3 % DM was obtained by CTS ensilaged with molasses at 6% which also reported by Man and Wiktorsson (2002). It is reported that DM contents (25.0 to 35.0 %) and pH (<4.5) of silage can be considered to be a high quality silage (Petterson, 1988). The CP content in CTS 23.0% the fresh material reduced slightly after ensiling and the reduction was more evident with the addition of molasses. Values obtained are similar to values reported by previous (Man and Wiktorsson, 2002 Khang et al., 2005).
Rice straw an total feed intake were not affected by level of CST feeding (Table 2). All cows consumed CTS well as offered. However, Aminah et al. (1999) reported supplementation of CTS mixed with grass based diet tended to increase feed intake which could have been a result of a stimulatory effect of silage on intake or the effect of the protein in the cassava leaves when added to a low protein roughage diet (Merkel et al. 1999).
On the other hand, milk yield were increased with the increasing level of CTS feeding (Table 3). However, there was no effect of feeding CTS on milk composition in the present study.

Conclusions and recommendations

Based on this study, it could be concluded that feeding CTS could increase milk yield without adverse effect on feed intake and milk composition of dairy cows fed rice straw based diet. This study additionally suggested that CTS could be used as a high quality roughage source for improvement of milk and meat production in the tropics.

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KEYWORD : Cassava top silage, Rice straw, Lactating cow

Table 1. Feed ingredients and chemical composition of concentrate, rice straw and cassava top silage

Items	Concentrate	Rice straw	Cassava top silage (CTS)
Ingredients, kg			
Cassava chip	47		
Corn meal	7		
Soybean meal	20		
Rice bran	5		
Palm kernel meal	9.5		
Bean hull	4		
Urea	2.5		
Molasses	3		
Di-calcium	1		
Mix vitamin	0.5		
salt	0.5		
Chemical composition, %			
Dry matter		90.0	24.8
Crude protein		2.2	23.0
		……. % dry matter……..	
Organic matter		96.4	92.9
Neutral detergent fiber		71.7	44.3
Acid detergent fiber		47.7	32.4
pH	-	-	4.2
Lactic acid, g/L	-	-	2.3
Acetic acid, g/L	-	-	0.6
Hydrocyanic acid, mg/kg	-	-	71.9

Table 2. Effect of cassava top silage on voluntary feed intake in lactating dairy cows

Items	Cassava top silage (kg/day of DM)				SEM	P-value
	0	0.75	1.50	2.25		
Rice straw DM intake						
kg/day	4.2	3.8	3.7	3.2	0.54	0.74
%BW daily	0.8	0.9	0.9	0.9	0.56	0.55
Concentrate DM intake						
kg/d	10.6	9.6	9.3	10.3	1.05	0.88
Cassava top silage DM intake						
kg/day	0.0^{a}	0.75^{b}	1.50^{c}	2.25^{d}	1.67	0.03
%BW daily	0.0	0.07	0.13	0.19	0.23	0.47
Total DM intake						
kg/day	14.8	14.1	14.5	15.7	1.06	0.28
%BW daily	2.8	3.0	3.1	3.1	0.28	0.08

^{a,b,c} Means in the same row with different superscripts differ ($P<0.05$)

Table 3. Effect of cassava top silage supplementation on milk yield and composition in lactating dairy cows

Items	Cassava top silage (kg/day of DM)				SEM	P-value
	0	0.75	1.50	2.25		
Production						
Milk yield, kg/day	12.7[a]	13.2[a]	13.3[a]	14.0[b]	0.93	0.04
3.5% FCM, kg/day[1]	14.6[a]	14.9[a]	16.1[b]	17.2[c]	1.01	0.03
Milk composition, %						
Fat	4.4	4.3	4.8	5.0	0.35	0.58
Protein	3.2	3.5	3.6	3.8	0.39	0.34
Lactose	4.4	4.4	4.3	4.5	0.14	0.75
Solids-not-fat	9.3	9.3	9.2	9.0	0.55	0.97
Total solids	14.1	14.0	14.2	13.9	0.58	0.45
Milk urea N, mg/dL	13.7	14.1	14.4	14.5	0.88	0.75

[a,b,c] Means in the same row with different superscripts differ ($P<0.05$). [1]3.5% FCM (fat collected milk)=0.432 (kg of milk/d)+16.23 (kg of fat).

REFERENCES

Aminah, A., Abu Bakar, C. and Izham, A. 1999. Silages from Tropical Forages: Nutritional Quality and Milk Production. FAO Electronic Conference on Tropical Silage 1999. Rome.http://www.fao.org.

AOAC. 1995. Official method of analysis. In: Animal Feeds, 16th ed. Association of Official Analytical Chemists, VA, USA.

Bolhuis, G. G. 1954. The toxicity of cassava root. J. Agric. Sci. 2: 176–185.

Khang D. N., Wiltorsson, H. and Preston, T. R. 2005. Yield and chemical composition of cassava foliage and tuber yield as influenced by harvesting height and cutting interval. Asian-Aust. J. Anim. Sci. 18: 1029–1035.

Kobawila, S. C., Louembe, D., Keleke, S. and Traore, A. S. 2003. Aspects physico-chimiques et biochimiques de l fermentation des feuilles de manioc en ntoba mbodi. Procédés Biologique et Alimentaire 1: 106–119.

Louembe, D., Malonga, M., Kobawila, S. C. and Mavoungou, O. 1997. Evolution de la teneur en composes cyanés des tubercules de manioc au cours du rouissage- Activité linamarasique de bactéries lactiques. Microbiol. Alim. Nutr. 15: 53–60.

Man, N.V. and Wiktorsson, H. 2002. Effect of molasses on nutritional quality of cassava and gliricidia tops silage. Asian-Aust J. Anim Sci. 15: 1294–1299.

Merkel, R. C., Pond, K. R., Burns, J. C. and Fisher, D. S. 1999. Intake, digestibility and nitrogen utilization of three tropical tree legumes. II As protein supplement. Anim. Feed Sci. Technol. 82:107–120.

Petterson, K. 1988. Ensiling of forages. Factorsaffecting silage fermentation and quality.Dissertation, Swedish Univ. of Agric. Sci. Report179. Uppsala, Sweden.

Ravindran, V. 1991. Preparation of cassava leaf products and their use as animal feed. In: Roots, Tubers, Plantains and Bananas in Animal Feeding. Anim. Prod. Health Paper 95. FAO, Rome, pp. 81–98.

Samuel, M., Sagatheman, S., Thomas, J., Mathen, G., 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian. J. Anim. Sci. 67, 805.

SAS. 1998. User's Guide: Statistic, Version 6, 12th ed. SAS Inst. Inc., Cary, NC.

Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Book Company, New York, NY.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597.

Wanapat, M., Petlum, A. and Pimpa, O. 2000. Supplementation of cassava hay to replace concentrate use in lactating Holstein Friesian crossbreds. Asian–Aust. J. Anim. Sci. 13: 600–604.

Wanapat, M., Sundstol, F. and Garmo, T. H. 1985. A comparison of alkali treatment methods to improve the nutritive value of straw. I. Digestibility and metabolizability. Anim. Feed Sci. Technol. 12: 295–309.

Wanapat, M., Pimpa, O. Petlum, A. and Boontao, U. 1997. Cassava hay: a new strategic feed for ruminants during the dry season. Livest. Res. Rur. Dev. 9: 1–6.

O-24-4

Fatty Acid Composition in M. longissimus dorsi muscle of Thai Swamp Buffalo Feeding by Soybean Oil Supplement within Concentration Rations

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INTRODUCTION

Buffalo is the domestic animal that it plays the important role as a source of meat, milk, leather, on-farm power, and transportation. Thai swamp buffalos (Bubalus Bubalis) are widely distributed in the remote area of Thailand. Buffalo is well adapted to convert the poor quality feed to be the meat and the milk. They are traditionally kept as draft animals. Buffalo meat in Thailand is normally from the retired draft animal (Nanda and Nakao, 2003).

Fatty acid composition data are important for solving the issue of health in the view of the high incidence of disease in recent year. The relevant affords were towards decreasing the percentage of saturated fatty acid (SFA) and increasing the percentage of unsaturated fatty acid (USFA), in particular the polyunsaturated fatty acid (PUFA). The beneficial effects of PUFA depend on the ratio of the fatty acid omega 6 (n-6) to omega 3 (n-3). The fatty acid component of meat might be influenced by a variety of factors, including animal breeding, feeding, and rearing condition. Feeding dietary PUFA supplements soybean oil which is rich in linoleic acid (18:2n-6) has been studied. There is no documented result on the fatty acid composition of Thai swamp buffalo because it's lack of buffalo meat production in Thailand. Therefore, the objective of this study was to investigate the fatty acid composition in M. longissimus dorsi muscle of Thai swamp buffalos feeding by soybean oil supplement within concentrate rations.

MATERIALS AND METHODS

The research was carried out at Lam Phraya Klang Animal Husbandry and Research Center, Lopburi province, Thailand. This place is located at 15° 16'35.5"N and 101° 20'36.0"E. The mean rainfall is approximately 1,521 mm annually with the mean minimum and maximum temperature of 17.3℃ to 37.3 ℃ , respectively.

Eight male, one year old with the initial body weight 300 kg, Thai swamp buffalo (Bubalus bubalis) were randomly allocated to 3 groups. One group fed on 0% soybean oil supplement within concentrate (S0), and the others 2 groups on 3 and 6 % soybean oil supplement within concentrate (S3) and (S6), respectively. Bufflaos were raised and fed in the conventional opened housing system. Concentrate was fed 2% of the body weight per day with free choice Pagola (Digitaria erianhta) and hay. Forage was harvested daily by hand and was cut into 50 cm for feeding. Buffalos were weight every month till the body weight reaching to 450 kg. From each carcass meat, test samples from M. longissimus dorsi were taken for laboratory analyses of the component as the following: crude protein- by the Kjeldahl method crude fat- by a Soxhlet extractor moisture- by oven drying at 105℃ and lipid composition- by gas chromatographers. The experiment consisted of a completely randomized design with 3 groups. The statistic used for determine the different among groups was one-way ANOVA. Significant treatment effects were subjected to multiple comparison by Duncan-test.

RESULTS AND DISCUSSION

The nutritional analysis indicates that the nutritional value of buffalo meat moisture, crude protein, crude fat, and ash are described in Table1. There was no different ($P>0.05$) of all nutritional value traits among those groups. Moisture was higher in S0 than in S3 and S6 (70.21 vs 69.89 and 69.34% respectively). Meat protein was slightly higher in S3 (25.14) than those S6 (24.49) and S0 (23.53). Meanwhile, crude fat of S3 was lower than those S0 and S6 (1.05 vs 3.55 and 3.46 respectively). While, ash was similarly among all those groups (0.01), which was in keeping with another comparative study (Dimov et al., 2012 and de Almeida et al., 2006)

The fatty acid values obtained in the experiment are described in Table 2. There was no different ($P>0.05$) of saturated fatty acid (SFA) profiles among those groups. The experimental data indicate that Thai swamp buffalos presented higher proportions of SFA and lower proportions of PUFA for all those groups of soybean supplement within concentrate rations, as shown in Figure1. Fatty acid content has become very important as it implicates the health issue of human, the high ration of PUFA:SFA is desired (Demirel et al.,2006). For monounsaturated fatty acid (MUFA), Cis-10-heptadecanoic acid (C17:1) of S0 and S3 are significant difference ($P<0.05$) from S6 (86.46

and 79.77 vs 124.65 respectively). This was particularly evident in polyunsaturated fatty acid (PUFA) that tran-9-elaidic acid (C18:1n9t) of S3 and S6 were significant lower than S0 (26.24 and 22.73 vs 530.31). Moreover, Linolelaidic acid (C18:2n6t) and Linolenic acid (C18:3n3) of S0 and S3 were significant difference from S6 (6.14 and 17.52 vs 39.94) and (22.81 and 32.28 vs 46.89) respectively. Feeding the dietary PUFA supplements such as soybean oil which is rich in linoleic acid (LA, 18:2n6) increase the proportions of PUFA in meat (Scollan et al., 2014). It shows that soybean/soybean oil is altered to be healthful fatty acid profile. Both linoleic acid (C18:2n6c) and linolenic acid (C18:3n3) are essential fatty acid which cannot synthesized in animal body, therefore must be get from the diet of animal (McNivena et al., 2004). A lower n − 6/n − 3 ratio, or a higher intake of n − 3 fatty acid has been recommended (Simopoulos, 1991).

CONCLUSIONS

Different soybean oil supplement within concentrate rations make the different effect on the fatty acid composition of Thai swamp buffalos resulting on meat quality. Soybean oil which is rich in PUFA enhance the amount of PUFA in meat, in particular the essential fatty acid Linolenic acid (C18:3n3).

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KEYWORD : buffalo, meat quality, fatty acid composition, soybean

Table 1 The Nutritional value of buffalo meat fed soybean oil supplement within Concentrate

Traits	Soybean oil supplement within concentrate		
	S0	S3	S6
moisture, %	70.21	69.89	69.34
Crude protein, %	23.53	25.14	24.49
Crude fat, %	3.55	1.05	3.46
Ash, %	0.01	0.01	0.01

Table 2 The fatty acid profiles of *M. longissimus dorsi* muscle of Thai swamp buffalo fed on 0, 3, and 6 % soybean oil supplement within concentrate

Fatty acid	Soybean oil supplement within concentrate		
	S0	S3	S6
Saturated Fatty Acid (SFA)			
Lauric acid (C12:0)	34.47	66.42	92.36
Myristic acid (C14:0)	493.70	702.30	924.60
Pentadecanoic acid (C15:0)	61.39	105.36	137.42
Palmitic acid (C16:0)	3914.00	5357.00	6920.00
Heptadecanoic acid (C17:0)	238.48	341.11	424.93
Stearic acid (C18:0)	4430.80	2996.60	3944.70
Arachidic acid (C20:0)	37.99	65.40	94.89
Heneicosanoic acid (C21:0)	4.49	8.57	11.77
Monounsaturated Fatty Acid (MUFA)			
Myristoleic acid (C14:1)	18.05	17.25	29.33
Palmitoleic acid (C16:1)	276.78	286.67	428.22
Cis-10-heptadecanoic acid (C17:1)	86.46[a]	79.77[a]	124.65[b]
Eicosanoic acid (C20:1)	18.85	21.51	37.50
Polyunsaturated Fatty Acid (PUFA)			
Tran-9-elaidic acid (C18:1n9t)	530.31[a]	26.24[b]	22.73[b]
Cis-9-oleic acid (C18:1n9c)	58.41	50.05	77.60
Linolelaidic acid (C18:2n6t)	6.14[a]	17.52[a]	39.94[b]
Linoleic acid (C18:2n6c)	263.88	379.83	451.31
Linolenic acid (C18:3n3)	22.81[a]	32.93[a]	46.89[b]
Cis-8,11,14-eicosatrienoic acid (C20:3n6)	13.44	13.28	15.68
Eicosadienoic acid (C20:3n3)	20.83	15.89	17.58

[a, b] Within rows, mean with different superscripts differ ($p<0.05$, dancan-test)

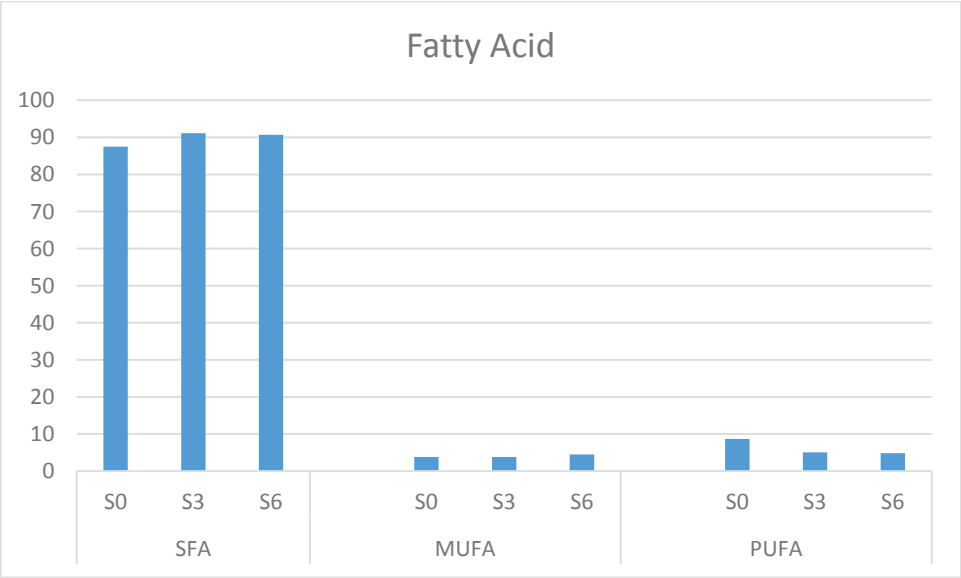

Figure 1 Proportion of fatty acid (%) in different groups of soybean oil supplement within concentrate

REFERRENCES

de Almeida, J.C., M.S. Perassolo, J. L. Camargo, N. Bragagnolo, J. L. Gross. 2006. Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. Brazilian Journal of Pharmaceutical Sci. 24, 1.

Demirel, G., H. Ozpinar, B. Nazli, O. Keser. 2006. Fatty acids of lamb meat from two breeds fed different forage: concentrate ratio. Meat Sci. 72:229-235.

Dimov, K., R. Kalev, M, Tzankova, P. Penchev. 2012. Fatty-acid composition of the lippids in M. longissimus dorsi of bovine and buffalo calves and buffalo cows. Bulgarian Journal of Africultural Science. 18(5), 778-783.

McNivena, M.A., J. Duynisveldb, E. Charmleyb, A. Mitchella. 2004. Processing of soybean affects meat fatty acid composition and lipid peroxidation in beef cattle. Animal Feed Science and Technology, 116(2004), 175-184.

Nanda, A.S. and T. Nakao. 2003. Role of buffalo in the socioeconomic development of rural Asia: current status and future prospectus. Amim.Sci.J. 74:443-455.

Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J. -F., et al. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science, 97(3), 384–394

Simopoulos, A.P., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54, 438–463.

O-24-5

Potential of Mixed Feed Palm Kernel Meal and Oil Palm Decanter Cake was Fermented by Rhizopus oligosporus for Bali Cattle

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Abstract

The aim of this study was to determine potential of mixed feed PKM and OPDC for Bali cattle performance. Palm kernel meal (PKM) and oil palm decanter cake (OPDC) are potential feed ingredients because of their abundance and low price. They have high protein contain but also high fat contain. High fat containing in PKM and OPDC might have negative effect on rumen fermentation. Solid state fermentation is an alternative bioconversion process for enhance nutrient quality of PKM and OPDC. Mixed feed PKM and OPDC each 50% (PKM/OPDC 50/50) were fermented by 0.2 and 0.4% of *Rhizopus oligosporus* from tempe inoculums. They were tested by in vitro true digestibility test and in vivo Bali cattle performance experiments. Control feed (PKM/OPDC 50/50 with *Rhizopus oligosporus* 0%), feed A (PKM/OPDC 50/50 with *Rhizopus oligosporus* 0.2 %) and feed B (PKM/OPDC 50/50 with *Rhizopus oligosporus* 0.4%) were assigned and analyzed in completely randomized designed experiment. This study resulted that solid fermentation of mixed feed PKC/OPDC 50/50 by *Rhizopus oligoporus* was significant ($P<0.05$) increasing Bali cattle performance with average daily gain (284.09, 386.36 and 518.18 g/day, for 0%, 0.2% and 0.4% of *Rhizopus oligosporus* , respectively) due to enhancement some nutrient of the mixed feed especially protein contain, hence support to increasing in vitro true dry matter digestibility and energy efficiency. It was conluded that fermented mixed feed PKM/OPDC 50/50 by 0.4% *Rhizopus oligosporus* from Tempe inoculum was optimum.

INTRODUCTION

Palm oil industriy produces hugh amount of waste product for feed ingredient such as palm kernel meal (PKM) and oil palm decanter cake (OPDC). Solid state fermentation (SSF) could be an alternative processing biotechnology for optimizing PKM and OPDC as potential feed ingredient for ruminant animals. SSF is defined as the growth of microorganisms on a solid substrate with several advantages which they are are produced with low water content, large quantities, not too much energy required, using fermentation media simple, reducing bacterial contamination and uses a few of water (Pandey et al., 2001). Solid state fermentation biotechnology use fungi, yeast and bacteria as microorganisms fermenting agent in the raw material feed ingredients byproduct for the purpose of improvement of nutritional protein and the production of additional feed (feed aditive) in the form of enzymes, vitamins, natural dyes and antibiotics (Ajila et al., 2012).

For all the reasons, the aim of this study was to determine potential value of mixed feed PKM and OPDC for increasing Bali cattle performance.

MATERIAL AND METHODS

Animal and Experimental DietsTwelve male growing Bali Cattle were used for studies and the studies were carried out in Farm of Farmer Group "Kelompok Tani Makmur" in Pelalawan Regency, Riau. During 2 weeks pre-experiment adaptation period they were fed ad libitum with mixed feed 50% palm kernel meal (PKM) and 50% Oil palm decanter cake (PKM/OPDC 50/50) and 2% dry matter of grass of cattle body weight, and had free access to water at all time. The treatments using mixed feed PKM/OPDC 50/50 are :

Control feed : Mixed Feed PKM/OPDC 50/50 without fermentation (0 % of Rhizopus oligosporus)Feed-A : Fermented Mixed feed PKM/OPDC 50/50 Rhizopous oligosporus 0.2%Feed-B : Fermented Mixed Feed PKM/OPDC 50/50 by Rhizopous oligosporus 0.4%

Solid State FermentationThe fermentation was carried out in plastic bag bioreactors, with dimension 30 x 40 cm. The PKC and OPDC substratum (each 1 Kg) bag was mixed well with Rhizpous oilgosporus from tempe inoculums and mineral vitamine. It was placed in plastic bioreactor in the holes to provide enough air during fermentation and in the from of fine layer of 2 cm. The mixed feed was fermented was fermented from 5 days on the wood rack. After 5 days fermentation the fermented feed was fed by cattle directly in the same day.

Chemical analysisMixed feed PKC/OPDC 50/50 before and after fermentation which were used for this experiment were analyzed for proximate fraction, fiber fraction , ADICP and NDICP. Method of the analysis is suggested by AOAC (1998), modified Van soest (1998) and ANKOM method protocol (ANKOM Tech., 2005) on dry matter basis. Fiber fraction was determined by the method of van soest modification using fiber analyzer A200 (Ankom techology). NDF and ADF contents in feed was analysed using a heat stable α -amylase at 0,2 ml/g DM (NDFa). Neutral detergent fiber (NDFa), Detergent fiber ash (DIA), NDFom (NDFa - DIA), Acid detergent fiber (ADF) and Lignin were evaluated. Other fiber fraction, Hemicelluloce and Celluloce were calculated from ADF, NDF and lignin values. All measurement were made in triplicate

In Vitro true digestibility test and Estimation of Energy valuesIn vitro true digestibility was tested using ANKOM DAISY^{II} Incubator as ANKOM Methodology protocle (Ankom, 2005). Estimation energy of feed (TDN, DE, ME nad NE) was approached by UC Davis factorial[8]. In vitro true DM disappearance (IVTDMD) and in vitro NDF disappearance (IVNDFD) were calculated with equation from Robinson, 2004.

Performance studyAfter pre-exprimental period, cattle received ad libitum of mixed feed and 5 days fermented mixed feed every day. Feed intake was measured daily during 6 weeks experiment period.dry matter intake, protein intake and metabolisable intake was calculated from feed intake. Daily gain was calculated from different between cattle final weight and initial weight during 6 weeks. Feed conversion was ratio value of daily dry matter consumption to daily gain.

Statistical AnalysesThe data from in vitro test was analyzed by using GLM procedure of SPSS 16 for windows (SPSS Inc., 2007) statistical package. All treatments were assigned in three treatments and three replicate arrangement in completely randomized designed. The model used was as follow :

$Y_{ijk} = \mu + \alpha_i + _{ij} + e_{ijk}$

Where Y_{ij} = observed value

μ = general mean

α i = Level of doses of *Rhizopus oligosporus* effect (i = 1,2,3)

e_{ij} = standart error

RESULTS

Mixed feed PKM/OPDC 50/50 Nutrient QualityThe chemical composition and feed evaluation content of mixed feed PKM/OPDC for this experiment are given in table 1.

Table 1. Chemical composition of Mixed Feed Palm Kernel cake (PKC) and Oil palm decanter cake (OPDC) was fermented by Rhizopus oligosporus (RO) 0%, 0.2% and 0.4%

Item	Treatments			SEM
	Control	Feed-A	Feed-B	
Proximate fraction				
Dry Matter/DM (%DM)	52.65[a]	61.19[b]	54.61[b]	0.64
Organic Matter (%DM)	92.19[c]	91.29[b]	90.24[a]	0.24
Crude Fiber (%DM)	21.53[a]	20.35[a]	23.85[b]	0.45
Crude Protein (%DM)	18.35[a]	20.33[b]	20.58[b]	0.36
Extract Eter (%DM)	2.69	2.86	2.81	0.06
Ash (%DM)	7.81[a]	8.71[b]	9.76[c]	0.24
Nitrogen free Extract (%DM)	49.62[b]	47.74[b]	42.99[a]	0.84
Fiber Fraction (Modified van Soest)-%DM				
NDFa	69.19[b]	67.42[a]	69.10[b]	0.42
NDFom	66.16[b]	64.24[a]	67.64[b]	0.45
DIA	3.04[b]	3.18[b]	1.46[a]	0.17
Hemicelluloce	22.73[b]	17.58[a]	27.00[c]	0.61
ADF	46.47[b]	49.83[c]	42.10[a]	0.54
Celluloce	40.71[b]	43.54[c]	36.72[a]	0.70
Lignin	5.75[b]	6.30[ab]	5.38[a]	0.18
NDIN (%DM)	0.85	0.64	0.62	0.09
NDIN (%CP)	4.49	3.11	3.05	0.52
NDICP (%DM)	5.31	3.96	3.89	0.57
NDICP(%CP)	28.10	19.46	19.08	3.25
ADIN (%DM)	0.40[a]	0.78[c]	0.52[b]	0.02
ADIN (%CP)	2.20[a]	3.83[c]	2.51[b]	0.06
ADICP (%DM)	2.52[a]	4.87[c]	3.23[b]	0.11
ADICP (%CP)	13.71[a]	23.95[c]	15.69[b]	0.36
ACP	17.86[b]	16.89[a]	18.79[c]	0.16

NDFa = Netural detergent fiber alpha amylase, NDFom = organic matter in NDF, DIA = Detergent Insoluble ash, NDIN = Nitrogen NDF, NDICP = crude protein in NDF, ADIN = nitrogen in ADF, ADICP = crude protein in ADF, ACP = adjust crude protein. [a,b,c]Different superscript indicate significance in the same row ($P<0.05$)

Some nutrient of mixed feed PKM/OPDC 50/50 were enhanced after *Rhizopus oligoporus* fermentation when others were decreasing. Dry matter, crude fiber, crude protein, ash, and adjust crude protein (ACP) were increasing after fermentation. Nitrogen free extract (NFE) and most of fiber fraction (NDF, ADF) are decreasing at 0.2% doses of *Rhizopus oligosporus*. However, extract ether of all treatments were same.

In Vitro true digestibility and Estimation of Energy Values of Mixed Feed PKM/OPDC 50/50The in vitro true digestibility resulted significantly increasing of NDF digestibility and true dry matter digestibility at 0.4% Rhizopus oligosporus fermentation ($P<0.05$). Their values were same between 0.2% and 0.4% Rhizopus oligosporus fermentation.

Table 2. Effect of different doses of Rhizopus oligosporus (RO) for Solid state fermentation Mixed feed PKC/OPDC on In Vitro True digestibility parameters

Invitro True Digestibility	Treatments			
Parameter	Control	Feed-A	Feed-B	SEM
NDFD (%NDF)	39.29a	40.63a	48.34b	4.07
dNDF (g/Kg DM)	271.90a	273.78a	333.89b	28.01
IVTDMD (% DM)	58.07a	60.04a	64.31b	2.79
IVTDMD (g/Kg DM)	580.73a	600.37a	643.09b	27.94
TDN (%DM)	48.41	49.03	52.54	2.91
DE (%DM)	2.14	2.16	2.32	0.13
ME (%DM)	1.71	1.74	1.89	0.13
NEm (%DM)	0.91	0.93	1.05	0.10
NEg (%DM)	0.36	0.39	0.50	0.10

NDFD = Neutral detergent fiber digestibility in % NDF, dNDF = digestible value of NDF
IVTDMD = in vitro true dry matter digestibility, TDN = total digestible nutrient
DE = digestible energy level maintenance, ME = metabolizable energy level maintenance
NEm = Net energy for maintenance NEg = Net energy for gain at production level,
[a,b,c]Different superscript indicate significance in the same row (P<0.05)

In vitro true digestibility values effect to same way affect in energy values estimation. The estimation which used UCD30 hour equation (Robinson, 2004) using NDF digestibility 30 hours data for calculation of Digestible energy (DE) values. However, estimastion energy values, DE, ME, NEm and NEg are not significantly different between treatmens (P >0.05). Total digestible nutrient (TDN) value was also not significant different between treatment (P >0.05).

Bali Cattle Performance Fed Mixed feed PKM/OPDC 50/50Performance study resulted an increasing some parameters of Bali cattle production as in table 3. Production parameters including nutrient and energy consumption, daily gain and feed conversion were significantly increasing (P<0.05) at 0.4% of doses of *Rhizopus oligosporus* during 6 weeks experiments period.

Table 3. Effect of different doses of Rhizopus oligosporus (RO) for Solid state fermentation Mixed feed PKC/OPDC on Bali cattle performance

Production	Treatments			
Parameter	Control	Feed-A	Feed-B	SEM
Initial weight (Kg)	203.50	168.67	164.37	
DMI (g/d)	2775.09[a]	3277.81[c]	3042.63[b]	49.57
OMI (g/d)	2553.25[a]	2997.23[c]	2765.01[b]	45.42
Protein Intake (g/d)	379.04[a]	500.22[c]	453.25[b]	8.09
MEI (Mcal/d)	4.84[a]	5.77[b]	5.64[b]	0.09
MEI (MJ/d)	20.17[a]	24.04[b]	23.50[b]	0.09
MEI (MJ/Kg $LW^{0.75}$)	0.37[a]	0.51[b]	0.51[b]	0.09
LWG (g/d)	284.09[a]	386.36[b]	518.18[c]	21.50
FC (DMI/ADG)	9.80[b]	8.59[b]	5.88[a]	0.46

Control = mixed feed without fermentation, Feed-A = Mixed Feed PKC/OPDC 50/50 RO 0.2%
Feed-B = Mixed Feed PKC/OPDC 50/50 RO 0.4%, DMI = dry matter intake, OMI = organic matter intake.
$LW^{0.75}$ =metabolism live weight
MEI = metabolizable energy intake, LWG = average live weight gain, FC = feed convetion
[a,b,c]Different superscript indicate significance in the same row (P<0.05)

Dry matter, organic matter,crude protein and metabolizable energy intake of fermented mixed feed PKM/OPDC 50/50 were higher than mixed feed without fermentation (controlled feed). Highest value of protein intake is fermented mixed feed PKM/OPDC 50/50 by 0.2% *Rhizopus oligosporus* while metabolizable energy intake was same between fermented mixed feed PKM/OPDC 50/50 (0.2% and 0.4% of *Rhizopus oligosporus*).

DISCUSSION

Nutrient Quality of Mixed Feed PKM/OPDC 50/50Solid fermentation of mixed feed PKM/OPDC 50/50 by Rhizopus oligosporus was significantly increasing dry matter, crude fiber, crude protein, ash, and adjust crude protein (ACP) in 0.2% and 0.4% dose of *Rhizopus oligosporus*. Highest percentage values of the increasing was protein containing in fermented mixed feed PKM/OPDC 50/50 with 0.4% *Rhizopus oligosporus* (12.15%). The result support other experiment using Rhizopus oligospporus for fermented agriculture by-product as animal feed which mostly increasing protein contain after fermentation because of biomass cell production if microorganism during fermentation (Oluseyi et al., 2008). Increasing of crude fiber after fermentation at 0.4% doses of Rhizopus oligosporus because of the value of hemicelluloce was also increasing at same doses due to fiber fraction breakdown by fermentation process. However, nitrogen free extract (NFE) of mixed feed which is refer to simply carbohydrate containing was decreasing 13.36% at 0.4% doses of *Rhizopus oligosporus*. It is in agreement with another research that the fermentation process increased reducing sugar content in rice brand using *Rhizopus oligosporus* (Oliveira et al., 2010). Most of fiber fractions are decreasing after fermentation (NDFa, NDFom, ADF, Hemicelluloce, cellulose and lignin) due to degradation process of fiber fraction.

Decreasing of this fiber fraction of fermented mixed feed PKM/OPDC 50/50 generally because microb ability which produced cellulose enzyme for ligno-cellulose degradation (Belewu et al., 2003).

In Vitro True digestibility and Estimated Energy Value of Mixed feed PKM/OPDC Mixed feed PKM/OPDC 50/50 had had highest value of in vitro true dry matter digestibility (IVTDMD) 64.31 %DM at fermented mixed feed 0.4% *Rhizopus oligosporus* which its value increased 10.75% from 58.07% DM at Mixed feed PKM/OPDC without fermentation. This resulted could be explained as high value of some nutrients degraded during fermentation. It is also could be explained by opposite effect result that IVTDMD was decrease due to decreasing of rumen microorganism by essential oils which help degraded nutrient during fermentation (calsamiglia et al., 2007 Rofiq, 2013).

Total digestible Nutrient (TDN) of all treatments ranged 48.41 to 52.54 %. The values were not significantly different ($P > 0.05$) which was opposite with IVTDMD values. However, the result was tend to highest value at fermented mixed feed PKM/OPDC 50/50 with 0.4% Rhizopus oligosporus. Fermentation process activity on energy values og Mixed feed PKM/OPDC 50/50 which was estimated by in vitro digestibility NDF (dNDF) completed others support nutrient effect such as ADICP. Less value of ADICP could be increased the estimation of energy values when it was calculated by UCD 30 hour equation (Robinson, 2004).

The Performace of Bali Cattle Fed Mixed feed PKM/OPDC 50/50Bali cattle is Indonesia native cattle which had low performance characteristic but high adaptation with tropical condition. There for, optimize feed which resulted high performance of Bali cattle was important for the strategy of Bali cattle fattening. Bali cattle had wide live weight daily gain 0.02 Kg/bull.day to 0.82 kg/bull.day depend on feeding strategy in a year with average daily gain 0.4 Kg/bull.day (Dahlanuddin et al., 2014). The average of daily gain was resulted from bali cattle which had feed containing more protein intake from peanut adn soybean residues (Dahlanuddin et al., 2014). Performance study using fermented mixed feed PKM/OPDC 50/50 had range of live weight daily gain (DG) from 0.284 kg/bull. day to 0.518 kg/bull.day. Highest values of ADG was 0.518 Kg/bull.day when Bali cattle fed fermented mixed feed PKM/OPDC 50/50 with 0.4% *Rhizopus oligosporus,* although organic matter and protein intake was not highest. The highest value of organic matter and protein intake was Bali cattle fed fermented mixed feed PKM/OPDC 50/50 with 0.2% *Rhizopus oligosporus*. The result was caused by same metabolizable energy intake between fermented mixed feed and more efficient feed conversion when Bali cattle fed fermented mixed feed PKM/OPDC 50/50 with 0.4% *Rhizopus oligosporu.* Bali cattle fed fermented mixed feed PKM/OPDC 50/50 with 0.4% *Rhizopus oligosporus.* Metabolisable energy intake (MEI) for this study for Bali cattle fed fermented mixed feed PKM/OPDC (0.51 MJ/Kg $LW^{0.75}$) was more for MEI maintenance according to Quigley et al (2014) that Bali Cattle has metabolisable energy requirements for maintenance 0.47 MJ ME/Kg $LW^{0.75}$.day. However MEI values were not efficient according to Quigley et al (2014) that Bali cattle need 29 g LWG/MJ ME. It was could be explained that energy source from this mixed feed is high fat contain

CONCLUSSIONThe results revealed that fermented mixed feed palm kernel meal and oilpalm decanter cake (PKM/OPDC 50/50) was optimize with 0.4% *Rhizopus oligosporus* from Tempe inoculums. It was significantly ($P < 0.05$) increased daily gain of male growing Bali cattle due to nutrient efficiency, nutrient value, in vitro true digestibility improvement. The improvement might happened from fermentation product of *Rhizopus oligosporus* and useful

microorganism amount in PKC/OPDC 50/50 substrate.

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KEYWORD : Palm Kernel Meal (PKM), Oil Palm Decanter cake (OPDC), Mixed Feed, Solid Fermentation, *Rhizopus oligosporus*, Bali Cattle

REFERRENCES

Abo-state MAM, Hammad Al, Swelim M, Gannam RB., 2010. Enhanced production of cellulose (S) by Aspergillus spp.isolated from agriculture wastes by solid state fermentation. American-Eurasian J Agric Environ Sci. 8:402-410

Adam M.A., A. Silaiman, C. M. S. Said. 2014. Preliminary study of oil palm decanter cake natural polymer composite (OPDC-NPC). Advamced Material research. 911:40-44

Afdal, M. A. Kasim, A.R. Alimon and N. Abdullah. 2012. Some chemical properties of oil palm decanter meal. African J. of Biotech. 11 (27) : 7128-7134

Ajila, C.M., S.K. Brar, M. Verma, R.D. Tyagi, S. Godbout and J.R. Valero. 2012. Bio-processing of agro-byproducts to animal feed. Critical rev in Biotechno. 32(4) : 382-400.

AOAC, 1998.Official Methods of analyses.12th ed. Washington, DC.

AOAC, Association of official analytical chemist. 2012. Official methods of analysis of AOAC International. 19th ed. Association of Official Analytical Chemist. Washington DC. USA.

ANKOM. 2005. In vitro true digestibility using th DAISY incubator. Accessed Sep. 13, 2010. http://www.ankom.com/media/documents/ IVDMD_0805D200.pdf.

Belewu MA., 2003. Nutritional qualities of corncob and waste paper incubated with edible mushroom. Nigerian J. Anim. Prod., 30 1-6.

Camassola M., Dillon AJ., 2010. Cellulases and Xylanases production by penicillium echinulatum grown on sugar cane bagasse in solid state fermentation. Appl. Biochem Biotechnol. 162 : 1889-1900

Corley R.H.V., P.B.Tinker. 2003. The oil palm : chapter 13-The products of the oil palm and their extraction. 4 ed. Blackwell sci. Ltd.

Dahlanuddin, B. T.Yuliana, T. Panjaitan, M. J.Halliday, E. V. Fliert and H. M. Shelton. 2014. Survey of Bali Bull fattening practices in central Lombok, estern Indonesia, based on feeding of Sesbanina grandiflora. Animal Prod. Sci. Csiro Pub. 54 : 1273-1277

Diraj A., A. vattem and K. Shetty. 2002. Solid state production of phenolic antioxidants from cranberry pomace by Rhzopus oligosporus. Food Biotechnol J. 16(3) : 189-210

Faithfull, N. T., 2002. Methods in Agricultural Chemical Analysis. CABI pub. NY.USA.

ANKOM. 2005. In vitro true digestibility using th DAISY incubator. Accessed Sep. 13, 2010. http://www.ankom.com/media/documents/ IVDMD_0805D200.pdf.

Feng X. M., Anders R.B. Eriksson and J. Schnurer. 2005. Growth of lactic acid bacteria and Rhizopus oligosporus during barley tempeh fermentation. Int.J. of Fooc Microbiol. 104:249-256.

Feng X.M., T.O. Larsen and J. schnurer. 2007. Production of volatile compounds by rhizopus oligosporus during soybean and barley tempeh fermentation. Int. J. of food Microbiol. 113:133-141s

Han b., Yong Ma, F.M. Rombouts, and M.J. Robert Nout. 2003. Effects of temperatur and relative humidity on growth and enzyme production by actinomucor elegans and Rhizopus oligosporus during sufu pehtze preparation. Food Chem. 81:27-34

Jalil Azizah Ab., N. Abdullah, A.R. Alimon and S. Abd-Aziz. 2015. Nutrient enhancement of ground sago (Metrxylon sagu rottboll) pith by solid state fermentation with rhizopus oligosporus for poultry feed. J. of Food Res. 4(2) : 1-15

Mabjeesh S. J., M. Cohen, and A. Arieli. 2000. In Vitro Methods for Measuring the Dry Matter Digestibility of Ruminant Feedstuffs: Comparison of Methods and Inoculum Source. J. of Dairy Sci. Vol. 83, No. 10.

Oliveira M.dos S., V. Feddern, L. Kupski, E.P. Cipolatti, E Badiale-Furlong and L.A. de Souza-soares. 2010. Physico-chemical characterization of fermented rice bran biomass. CyTA J. of Food. 8(3) : 229-236

Oluseyi O. Oduguma, Mojisola O. Edema, Ayodeji O. Ayeni. 2008. Physico-chemical and microbiological analyses

of fermented corn cob, rice bran and cowpea husk for use in rabbit feed. Biores. Techno. 99 : 1816-1820.
Pandey A., Szakacs G., Soccol C.R., Rodriquea A and Soccol V.T. 2001. Bioresource technol. 77 : 203-214.
Quigley, S. P., Dahlanuddin, Marsetyo, D. Pamungkas, A. Priyanti, T. Saili, S.R. McLennan and D.P. Poppi. 2014. Metabolisable energy requirements for maintenance and gain of liveweight of Bali cattle (Bos javanicus). Animal Prod. Sci. Csiro Pub. 54 : 1311-1316
Robinson T, Nigam P. 2003. Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. Biochem Eng J., 13:197-203.
Robinson, P.H., Mathews, M.C., Fadel, J.G. 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fiber as 48h and comparison to 48 h in sacco neutral detergent fiber digestion. Anim. Food. Sci. Technol. 80, 257 – 266.
Robinson, P. H., D. D. Givens, G. Getachew. 2004. "Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equation utilizing chemical assays and in vitro determinations," J. Anim.Feed.Sci. & Tech. vol. 114, pp 75-90, 2004.
Rofiq M. N. 2013. The Effects Of Some Essential Oils On Milk Yield, Milk Composition and Methane Production in Goat. PhD.Dissertation. University of Cukurova.
Rofiq, M.N., S. Martono, M. Gorgulu and M. Boga, "Combination effect of Clove and Cinnamon Oil on in Vitro Rumen Gas and Methane Production,' in Proc.2nd International Seminar on Animal Industry, Jakarta, 2014, pp431-437.
Sabu A., S. Sarita, A. Pandey, B. Bogar, G. Szakacs and C.R. Soccol. 2002. Solid-state fermentation for production of phytase by Rhizopus oligosporus.
SPSS inc. 2007. SPSS ver 16.0 Statitical package. IBM. USA
Van Soest, P.J. 1963. Use of detergent in the analysis of fibrous feed, II.A rapid method for the determination of fiber and lignin.Journal. Of the association of official agriculture chemists 46, 829-835
Van Soest P.J., Robertson J.B., Lewis B.A., 1991.Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal Dairy Science, 74: 3583 — 3597
Vig A.P. and A, Walia. 2001. Beneficial effects of Rhizopus oligosporus feremention on reduction of glucosinolates, fibre and phytic acid in rapesedd (Brassica napus) meal. Biores. Tech. 78:309-312
Yahya A., C.P. Sye, T.A.Ishola, H. Suryanto. 2010. Effct of adding Palm oil mill decanter cake slurry with regular turning operation on the composting process and quality of compost from oil palm empty fruit bunches. Biorer. Tech. 101:8736-8741

O-24-7

Supplementation of Sesbania grandiflora to pregnant and lactating cows increased birth weight, milk production and preweaning growth of Bali cattle

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Introduction

Cattle performance under traditional production system rely on native grass where the availability and quality fluctuate following season. Native grass is low in protein content and digestibility (Sutaryono et. al., 2009) As a result gowth rate of Bali cattle are generally low at 0.2 kg/day (Wirdahayati, 1994).

One of the efforts to overcome this problem is to match cattle reproduction cycle with feed availability and quality in order to have calves born when high quality feeds are available. Dahlanuddin et. al. (2016) reported that implementation of an integrated village management system that includes early weaning, controlled mating using a selected bull and improve supply of high quality feed during late pregnancy and during lactation increase calving rate from less than 60% to more than 85%.

Availability of high protein feeds such as sesbania (*Sesbania grandiflora*) in Lombok is limited due to limited land availability (Dahlanuddin et. al., 2014) so it should be used effectively to meet the critical point of the cow reproduction cycle. The objective of this experiment was to evaluate the effect of sesbania supplementation during the last two month of pregnancy on cow feed intake, calf birth weight, milk production and preweaning growth

Materials and methods

Twenty four pregnant Bali cattle (*Bos javanicus*) at their last two month of pregnancy were divided into two groups, one fed 100% native grasses (mainly *Brachiaria sp*, *Cynodon sp* and *Heterppogon contortus*) and the other fed 70% native grasses and 30% sesbania. All cows were housed in individual pens equipped with feed through and water bucket. Feeds were provided at 3% dry matter of cow body weight. Feeds were given twice a day at around 07:00 AM in the morning and at around 17:00 PM in the afternoon. Clean drinking water was provided *ad libitum*.

Newborn calves remained with their cows in the individual pens. Feed throughs and water buckets for the calves were placed separately within the pens. Variables measured were feed intake, calf birthweight (measured within 24 hours after birth), cow body condition score (BCS) using 1-5 scale according to Teleni et. al. (1993), calf vigour score according to Wiley et. al. (1999), calf mortality and preweaning growth.

Data analysis

Data were analysed by analysis of variance for T test (Gomez and Gomez, 1995) using SAS for Windows software (SAS, 2001).

Results and discussions

Feed intake

Sesbania supplementation increased total dry matter (DM), crude protein (CP) and total digestible nutrient (TDN) intakes ($P<0.01$) as shown in Table 1. The increased in CP intake and TDN intake was associated with an increased in CP content of the diets from 6.65% to 11.85%, and TDN from 65.03% to 66.63% due to sesbania supplementation.

Sesbania supplemetation increased CP content of the diet within the range required to support rumen function (Church and Pond, 1988).

Table 1. DM intake, CP intake birth weight, BCS during lactation, calf mortality, calf vigour score and preweaning growth.

Variables	Control	Supplemented with sesbania
Cows		
- DM intake (kg/day)	6.92 [p] ± 0.22	7.17 [q] ± 0.51
- CP intake (kg/day)	0.46[p] ± 0.01	0.85 ± 0.02
- TDN intake (kg/day)	4.50 [p]	4.78[q]
Diet TDN (%)	65.03 ± 0.10	66.67 ± 0.11
CP content of diet (g/Kg)	66. 5	118.5
Birth weight (kg)	14.82 [p] ± 2.63	18.21 [q] ± 1.37
Milk production (kg)	0.97 ± 0.33	1.47 ± 0.26
Cow BCS at lactation	3.09 [p] ± 0.60	3.28 [q] ± 0.50
Calf vigour score	1	1
Milk production (kg/day	0.97 ± 0.33	1.47 ± 0.26
Calf preweaning ADG (kg/day)	0.46 [p] ± 0.05	0.55 [q] ± 0.04

Calf birth weight and vigour score

Supplementation of sesbania increased birth weight of calve to 18.2 kg than that of calve from cow fed 100% grass of 14.1 kg. Calf birth weigh recorded in this experiment was within the range of birthweight reported by Muzani et. al. (2006) who recorded birth weight of 15.39 ± 2.5 female and 17.88 ± 1.5 kg for male and by Dahlanuddin et al. (2016) who reported birthweight 16 kg regradless of the sex. Vigour score for calves was 1.0 (meaning all calves were born normal) and the effect of sesbania supplementation was not significant on calf vigour.

Milk production

Supplementation with sesbania increased milk production by 52%. Similar result was also reported by Oka (2005) that milk production of Bali cattle fed elephant grass was 1.1 kg/day and it increased to 1.6 kg/day when the cow was fed 40% grass and 60% concentrate. Supriyadi (2015) also reported that milk production of Bali cow fed grass only was 0.90 kg/day and increased to 2.06 Low milk production in Bali cattle may be related to cattle type as Bali cattle are dual purpose cattle to produce beef and power (Wirdahayati and Bamualim, 1990).

Pre weaning ADG

Supplementation with sesbania increase calf ADG from 0.46 kg to 0.55 kg/day which is higher than commonly reported elsewhere. Putra et. al. (2009) reported that birth weight of male and female Bali calves in the Bali cattle breeding centre in Bali were 0.30 ± 0,08 kg/day (male) and 0.31 ± 0.07 kg/day (female). Supriyadi (2015) also reported that the preweaning ADG of calves born to cows fed tree legumes during pregnancy and lactation was 0.38 kg/d, almost twice the ADG of calves born to cows fed Kinggrass throughout pregnancy and lactation. Results of this experiment support previous finding that ADG of Bali calves driven by cow milk production (Parakkasi, 1999).

Conclusion

Supplementation of native grasses with sesbania during late pregnancy and during lactation improve cow milk production, birth weight and preweaning growth of Bali cattle.

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KEYWORD : Bali cattle, Calf growth, Milk production, Sesbania

REFERRENCES

Church, D.C. and W.B. Pond. 1988. Basic Animal Nutrition and Feeding. 3th ed. John Wiley and Sons. New York.

Dahlanuddin, B T Yuliana, T Panjaitan, M J Halliday, E van de Fliert and H M Shelton (2014) Survey of Bali bull fattening practices in central Lombok, eastern Indonesia, based on feeding of Sesbania grandiflora. Animal Production Science, 2014, 54, 1273–1277.

Dahlanuddin, Zaenuri L A, Sutaryono Y A, Hermansyah, Puspadi K, McDonald C, Williams L J, Corfield J P and van Wensveen M 2016. Scaling out integrated village management systems to improve Bali cattle productivity under small scale production systems in Lombok, Indonesia. *Livestock Research for Rural Development. Volume 28, Article #79*. Retrieved June 29, 2016, from http://www.lrrd.org/lrrd28/5/dahl28079.htm

Gomez, K. A. dan A. A. Gomez, 1995. Prosedur statistik untuk penelitian pertanian. Terjemahan E. Sjamsudin, J. S. Baharsjah dan A. H. Nasution. Penerbit Universitas Indonesia

Muzani A. ,W. R. Sasongko , dan T. S. Panjaitan. 2006. Dampak penerapan paket manajemen terpadu terhadap bobot lahir dan pertambahan bobot badan harian pra sapih ternak sapi Bali, Laporan Penelitian. Balai Pengkajian Teknologi Pertanian Nusa Tenggara Barat.

Oka, I. G. L. 2005. Performance of Bali Heifers and calves prior to weaning in a feedlot system. http:// ejournal,unud,ac,id/abstrak/ ,pdf akses: 10-5-2012.

Parakkasi, A. 1999. Ilmu Nutrisi dan Makanan Ternak Ruminan. Penerbit Universitas Indonesia Press, Jakarta.

Putra, B. A. W., E. Baliarti, dan Sumadi. 2009. Kinerja induk dan pedet sapi Bali di Balai pembibitan Ternak Budidaya Sapi Bali. Proceeding Seminar Nasional Pengembangan Ternak Potong untuk Mewujudkan Program Kecukupan Swasembada Daging. Fakultas Peternakan Universitas Gadjah Mada. 99 - 112.

SAS (1999) 'SAS/STAT user's guide. Version 8.'(SAS Institute: Cary, NC)

Supriyadi M 2015. Pengaruh skor kondisi tubuh fase akhir kebuntingan hingga laktasi terhadap performa pertumbuhan pedet sapi Bali prasapih. Masters Thesis University of Mataram Indonesia.

Sutaryono, Y.A., Imran, dan J. Corfield. 2009. Penyediaan pakan ternak pada pemeliharaan sapi Bali sistem potong angkut (*Cut and Carry System*) di Kabupaten Lombok Tengah. Proceeding Seminar Nasional Pengembangan sapi Bli berkelanjutan Dalam Sistem Peternakan Rakyat. : 100 - 111.

Teleni, E., R. S. F. Champoel, and D. Hoffman. 1993. Draught Animal system and Management an Indonesian study. ACIAR Monograph. No 19.

Wiley, J. S., M. K. Petersen, R. P. Ansotegui, and R. A. Bellows. 1999. Production from first-calf beef heifers fed a maintenance or low level of prepartum nutrition and ruminally undegradable or degradable protein postpartum. J. Anim. Sci. 69: 4279 - 4293.

Wirdahayati, R.B. 1994. Reproductive characteristics and productivity of Bali and Ongole cattle in Nusa Tenggara, Indonesia. PhD Thesis, University of Queensland, Brisbane Australia

Wirdahayati, R. B. dan A. Bamualim, 1990. Penampilan produksi dan struktur populasi ternak sapi di pulau Timor. Nusa Tenggara Timur. Proceeding Seminar Nasional Sapi Bali. 20 - 22 September 1990. Fakultas Peternakan Universitas Udayana. Denpasar. Bali.

O-24-8 Ensiling fruits byproduct for feed use aiming at long term preservation and methane mitigation in the rumen

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Due to emerging worldwide demands for animal products (meat and milk), shortage of feed materials has become a global issue. As the use of food wastes or food processing residues for animal feed is regarded as a reasonable and economical solution, feed utilization of food-derived materials containing inedible parts have been considered. Fruit byproducts (FB), especially apple pomace (AP) and grape pomace (GP), have already been widely evaluated as food-derived feed materials. In addition, persimmon skin (PS), which is generated during preparation of dried persimmon (hoshigaki) and is rich in soluble crude fiber, such as pectin, as well as soluble carbohydrates, seems attractive as an energy source for animal feed. As these FB are usually watery materials, preventive measures such as drying or lactic fermentation (ensiling), will enable long-term preservation, which is important because the generation of FB is generally limited to the harvest season each year. In humid Southeast Asia and East Asian countries where efficient conditions for sun-drying are not always available, lactic acid fermentation of BP may be a more practical method to achieve storage stability. Here, we conducted fermentation experiments of a variety of FBs (apple pomace [AP], grape pomace [GP], and persimmon skin [PS]) to compare lactic acid bacteria (LAB) species with regard to both fermentation quality and aerobic stability. The potential benefits of feeding such FB that contain certain amount of plant secondary metabolites include manipulation of the rumen community capable of decreasing methane eructation and improving feeding efficiency. Methane production in ruminants has attracted a great deal of attention in relation to its contribution to the greenhouse gas effect and global warming. Therefore, we also aimed at evaluating ensiled FB in view of whether these materials are effective on mitigating methane emission from ruminants by means of *in vitro* assessment.

Three types of FB (AP, GP, and PS) were obtained from different food processing factories in Nagano prefecture, Japan. Apple pomace was generated by mechanical squeezing of apples, which had been washed three times with tap water after harvesting. Grape pomace was collected from a brewery after juicing of *Vitis labrusca* (Niagara). Persimmon skin, which was peeled off from fresh persimmon during the making of dried persimmon, was collected from a processing plant. All FB samples were frozen immediately after collection until use. Nutritional values of the FB were as follows: AP, 17.6% ± 2.0% dry matter (DM) GP, 33.5% ± 0.5% DM, 9.5 ± 0.9 DM% crude protein (CP), 25.0 ± 3.6 DM% crude fiber (CF), and 53.7 ± 5.7 DM% nitrogen-free extract (NFE) PS, 25.1% ± 1.1% DM, 4.5 ± 0.2 DM% CP, 12.2 ± 0.5 DM% CF, and 79.0 ± 0.6 DM% NFE. Before ensiling experiments, the water content of samples were adjusted for AP and PS with addition of corn cobs, resulting in 27.5% of DM for AP and 36.1% of DM for PS. No adjustment of water content for GP was required. With reference to our previous report, we chose three LAB strains (*Lactobacillus plantarum* NBRC15891, *Lactobacillus buchneri* NBRC107764, *Leuconostoc mesenteroides* NBRC100496, and *Lactococcus lactis* NBRC100933), which performed well in our previous test of apple pomace fermentation (Hiramori et al., 2015). These strains were restored and maintained according to the instructions of the distributor. Materials were mixed with 1% (v/w) inoculant or MRS medium (Oxoid, Basingstoke, UK) as a control. The inoculated material was mixed well by hand and 20 g of each was packed in a three-layer film bag. These bags were vacuum packed and tightly heat sealed (SQ-205S Asahikasei Packs Co. Ltd., Tokyo, Japan), and then incubated at 25℃. Ensiled content was diluted by addition of 180 ml of saline to the bag and kept for 2 hours at 5℃. The dilution was used to determine pH, LAB counts (using MRS agar), yeast counts (by chloramphenicol-added potato dextrose agar), and organic acids (by HPLC as described previously [Hiramori et al., 2015]).

We periodically analyzed the contents of the sealed bags for time course data acquisition, and found that the nature of fermentation characteristics could be expressed as data obtained at 28 days of fermentation (Fig. 1). In all materials tested, the addition of LAB did not further decrease the pH compared to the control. However, the LAB counts were marginally higher in silage including LAB than in the control. *Lb. buchneri* NBRC 107764 showed higher counts in one or more logarithm degrees than the control, and yeast counts were significantly decreased in AP and PS samples fermented with this strain. With regard to organic acid composition, in all

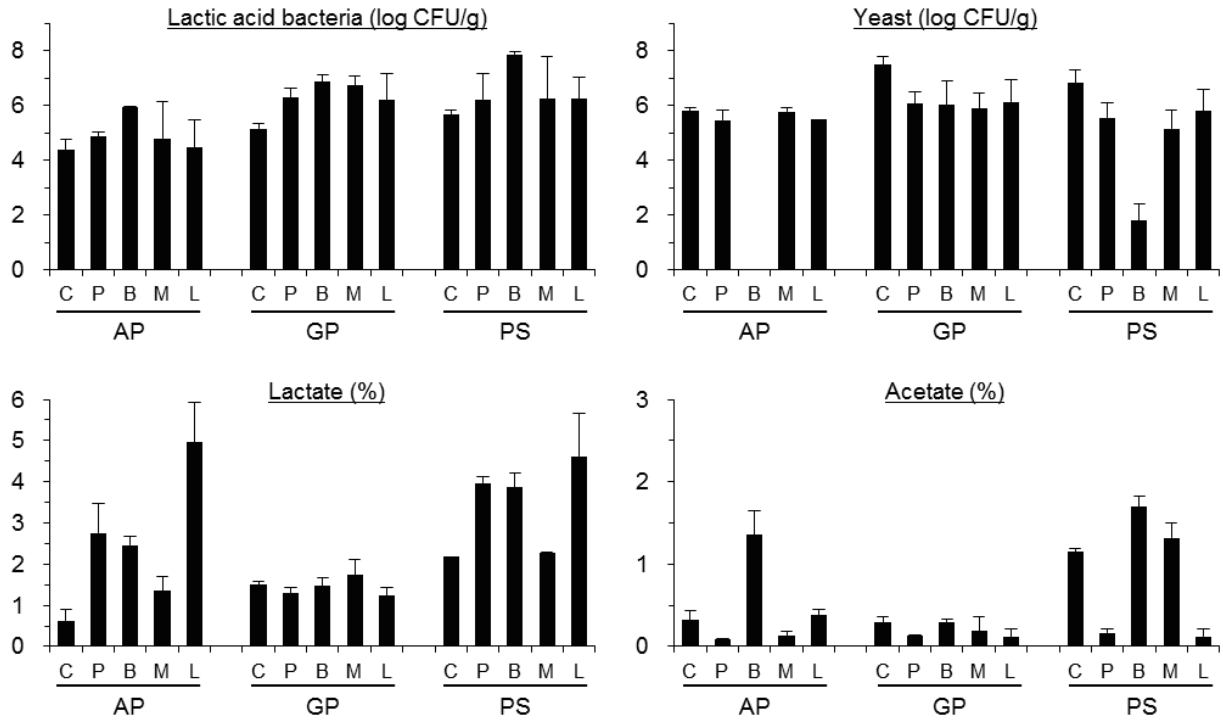
samples only lactate and acetate were detected, while neither propionate nor butyrate was detected. These data suggested that the addition of LAB may contribute to increased lactic acid fermentation in AP and PS silage, but not in GP silage. One reason for this may be differences in pH at the starting points of the materials, where inoculated LAB could not well work at the low pH of GP. Otherwise, it is possible that GP may contain substances that hamper the activity of LAB, such as polyphenols. As increased lactate production was detected in silage using *Lb. plantarum* NBRC 15891 and *Lc. lactis* NBRC100933, these strains are also considered to contribute to stable lactic fermentation when ensiling AP and PS.

We further determined the effect of the ensiled FB with LAB on the prevention of aerobic deterioration after opening the bag. Samples of 500 g of AP, GP, and PS incorporating 5 ml of *Lb. plantarum* (NBRC 15891) culture, *Lb. buchneri* (NBRC 107764) culture, or MRS medium (as a control) were packed anaerobically and incubated at 25℃ . Six weeks later, they were opened, added to a vacuum mug (300 ml), and then covered with plastic wrap. They were kept in an incubator at 25 ℃ to monitor the temperature inside the samples. A weak positive (i.e., preservative) effect was observed in the GP silage. On the other hand, the addition of *Lb. plantarum* or *Lb. buchneri* succeeded in avoiding the temperature increase in the PS silage, suggesting that aerobic fermentation by yeast was prevented. These data suggest that inoculated LAB may have a positive effect on storage stability of opened silage even when yeast would survive within it. *Lb. buchneri* is known to be effective in preventing aerobic deterioration when applied as an inoculant for ensiling plant feeds (Nishino et al., 2004 Tabacco et al., 2011). Based on our findings, ensiling AP, GP, and PS with appropriate LAB inoculant will provide high-quality silage with long-term preservation and open the way for year-round feed use of these FBs, which have been expected as one of the most feasible resources to counteract feedstuff shortages.

Among the various strategies reported to date, systematic intervention in the rumen microbial populations with feeding natural PSM, such as tannin, is the most feasible means of mitigating methane emission. These phytochemicals have potential to significantly impact animal health, animal production, and gut microbial community. Inclusion of an optimum level of tannin in the ruminant feed has been shown to favorably modulate rumen fermentation, such as reducing protein degradation in the rumen, prevention of bloating, and inhibition of methanogenesis thereby improve animal performance. Since PS and GP have been recognized as PSM-rich materials, we subsequently screened ensiled PS and GP for their methane suppression properties using *in vitro* culture method. Method of the incubation was according to described in our previous paper (Uyeno et al., 2016). Rumen fluid samples were collected from Holstein dry cows via cannula just prior to morning feeding. Collected rumen fluid was filtered through four layers of cheesecloth. Subsequently strained rumen fluid was diluted (1:2) with pre-warmed McDougall buffer which had been flushed with CO^2 gas. 50 mL of the diluted rumen fluid was dispensed into a 120 mL serum bottle with substrate (1.0 g) then was flushed with CO_2 gas. Inoculated bottles (n=3 per group) were sealed with a butyl rubber stopper and aluminum cap, then incubated anaerobically for 24 h at 39℃ with shaking at 180 rpm in a water bath. After 24 h of incubation, fermentation parameters (headspace gas composition, short-chain fatty acids, methane generation, and a gene encoding alfa-subunit of methyl co-enzyme M reductase [*mcrA*]) were analyzed according to methods in previous studies (Denman et al., 2007 Abrar et al., 2016). Results of the *in vitro* cultivation experiment indicated that ensiling or not in the FBs affect *in vitro* rumen cultivation characteristics and demonstrated negative impact of these silages on methanogenic groups compared to the non-fermented materials, Bhatta et al. (2009) evaluated the effects of 6 commercially available natural sources of tannins on total archaea using mixed cultures. They found that condensed tannin (CT) reduced methane production by 5.5% and suppressed the population of methanogenic archaea by 12.0%. The total archaeal population was lower with the combination of hydrolyzed tannin (HT) and CT than with HT alone. The different modes of action of two kinds of tannins may explain why the effects of HT+CT on total gas and methane production were greater than those of HT alone. Our results imply that suppressive effect of ensiled FBs on metanogen population is probably due to changing form of tannins during silage fermentation.

Ensiled FBs possibly affect in vitro rumen fermentation patterns and consecutive methane generation. In addition, our data implied that ensiling of PSM-rich FB such as GP and PS may also affect rumen fermentation. Detail monitoring of the digestion kinetics of nutrients, as well as of microbial interactions within the ecosystem may be warranted to clarify the mechanism and to open ways of practical use of ensiled FB as a feed which optimizes rumen fermentation.

KEYWORD : Aerobic stability, Ensiling, Fruit byproducts, Lactic acid bacteria, Methane emission



REFERENCES

Abrar, A., Kondo, M., Kitamura, T., Ban-Tokuda, T., Matsui, H., 2016. Effect of supplementation of rice bran and fumarate alone or in combination on in vitro rumen fermentation, methanogenesis and methanogens. Anim. Sci. J. 87, 398-404.

Bhatta, R., Uyeno, Y., Tajima, K., Takenaka, A., Yabumoto, Y., Nonaka, I., Enishi, O., Kurihara, M., 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J. Dairy Sci. 92, 5512-5522.

Denman, S.E., Tomkins, N.W., McSweeney, C.S., 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. FEMS Microbiol. Ecol. 62, 313-322.

Hiramori, C., Uyeno, Y., Koh, K., 2015. Effects of lactic acid bacteria on fermented apple pomace (FAP) production and the inclusion of an FAP into a medium for bunashimeji (*Hypsizygus marmoreus*) cultivation. Mushroom Sci. Biotechnol. 23, 120-124.

Nishino, N., Wada, H., Yoshida, M., Shiota, H., 2004. Microbial counts, fermentation products, and aerobic stability of whole crop corn and a total mixed rRation ensiled with and without inoculation of *Lactobacillus casei* or *Lactobacillus buchneri*. J. Dairy Sci. 87, 2563-2570.

Tabacco, E., Righi, F., Quarantelli, A., Borreani, G., 2011. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. J. Dairy Sci. 94, 1409-1419.

Uyeno, Y., Wang, C., Jayanegara, A., Kondo, M., Ban-Tokuda, T., Matsui, H., 2016. Increase in rumen fibrolytic bacteria and the improvement of fiber degradability of ensiling total mixed ration assessed by *in vitro* rumen culture. Adv. Anim. Vet. Sci. 4, 183-186.

Figure legends

Fig. 1. Fermentation characteristics of fruit byproduct silages with or without inoculum after 28 days fermentation. CFU, colony forming units AP, apple pomace GP, grape pomace PS, persimmon skin. C, Control P, *Lb. plantarum* B, *Lb. buchneri* M, *Leuc. mecenteroides* L, *Lc. lactis*. Values are indicated as means ± SD of three samples.

O-24-9

Supplementation of suckling Bali calves with maize grain or sesbania increases growth rates prior to weaning

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Introduction

Bali cattle (*Bosjavanicusd'alton*) are an indigenous cattle species in Indonesia that are believed to have been domesticated around 3500 BC (Darmadja, 1980). Bali cattle are well adapted to tropical environments where seasonal feed quality and quantity vary significantly. Under these extreme environmental and nutritional conditions they are able to maintain relatively high BCS and reproduction rates (Moran 1973 Moran 1978 Martojo 2012) with calving rates above 80% (Toelihere 2003 Panjaitan *et al.*, 2008 and Gunadi *et al.*, 2013). Females have a small mature size (300 kg), low feed requirements and are the preferred cattle species of smallholder farmers across eastern Indonesia.

Low preweaning calf growth and high preweaning calf mortality negate many of the positive traits of Bali cattle. Low calf growth and high mortality are likely to be directly related to the inherently low milk production of Bali cows. Previous studies reported milk production of Bali cows from 0.8 to 1.5 kg/day in East Nusa Tenggara (Jelantik *et. al*. 2009) and 1.6 kg/day from days 30 to 142 of lactation in West Nusa Tenggara (Gunadi *et. al.*2015). Milk production of up to 3.0 kg/day was reported by Wirdahayati *et al*. (1997) but this was dependent on feed availability. The issue of low milk production is influenced by a strong tendency for Bali cattle to give birth in the early to mid-dry season (Jelantik *et al*. 2009) when feed supply and feed quality is insufficient to support high milk production. Milk production of first calf Bali cows has been increased through improved nutritional management (Supriyadi 2015, 0.9 to 2.1 kg/day) resulting in increased calf growth rates prior to weaning. However increases in milk production have required the inclusion of large quantities of concentrates in the diet of the pregnant and/or lactating cow which are largely unavailable to smallholder cattle farmers. Even at the maximum rates of milk production reported for Bali cowsit is unlikely sufficient nutrients will be available solely in the milk to allow the calf to reach its potential liveweight gain during the pre-weaning period. This lack of milk results in small calves that become dehydrated, malnourished and progressively weaker leading to mortality. Those calves that do survive are likely to take a longer time reach slaughter or mating liveweight (Jelanti *et. al*., 2008) thereby having a longer term impact on total herd productivity.

Creep feeding is one strategy that has been used to provide additional nutrients to suckling calves to overcome the nutrient deficit faced by Bali calves, thereby increasing preweaning growth rates and decreasing mortality rates (Jelantik *et al*. 2009). Creep feeding is considered a more efficient nutritional management strategy than supplementing the cow as smaller quantities of high quality feeds are required and the feeds are directly utilised by the calf rather than converted to milk by the cow. Gunadi *et al.* (2015) subsequently evaluated the use of leucaena (*Leucaena leucocephala*) alone or a leucaena-cassava (*Manihot esculanta crantz*) mixture which would be more readily available to smallholder farmers in eastern Indonesia. There was no difference in liveweight gain of suckling calves supplemented with these diets (0.32 to 0.35 kg/day) so it is unknown if an energy or protein supplement may be more beneficial to young Bali calves that are still obtaining a significant proportion of their nutrients via the milk. It is therefore important to understand the relationships between energy and protein supplements and milk in the diet of pre-weaned Bali calves.

The objective of this study was to determine the effect of a protein supplement (sesbania) and an energy supplement (maize grain) on the preweaning growth of Bali cattle under field conditions.

Materials and Methods

Site, animals, management and treatments

The experiment was carried in a demonstration farm of Karang Kendal hamlet (8° 19'22"S 116° 12'33"E) North Lombok district, West Nusa Tenggara province, Indonesia between June 2014 and March 2015.

Singleton bearing Bali cows (n=24) were blocked into either young cow less than 4 years old of age or mature cow above 5 years old of age groups. At approximately 60 days of age Bali calves (29.0 ± 6.7 kg) within each of the

two age blocks were randomly allocated to one of three treatment groups. The three treatments were I. Control (n=8) within which cow-calf pairs were maintained under prevailing management conditions and calves received no supplement, II. Maize supplementation (n=8) within which calves had *ad libitum* access to maize grain (930 g DM/kg and 960 g organic matter (OM) and 94 g CP/kg DM) in a group pen between 800 and 1700 h each day, and III. Sesbania supplementation (n=8) within which calves had *ad libitum* access to sun-dried sesbania leaves (857 g DM/kg and 942 g OM and 279 g CP/kg DM) in a group pen between 800 and 1700 h each day. Calves in both supplementation groups had *ad libitum* access to drinking water. Cows in both supplementation groups were fed as farmer normal practice during the day and were reunited with their calves for suckling between 1700 and 800 h each evening. Experimental treatments continued until all calves, including controls, were weaned at 180 days of age. Calf liveweight and milk production of cows were measured every 30 days. Milk production were measured using the weigh-suckle-weigh technique. Fed and water intake of supplemented calves while in the group pen were measured over seven consecutive days at the end of experiment.

*Statistical analyse*s

Data were analysed using One Way ANOVA procedures in SPSS version 16.0.

Results and discussions

Liveweight gain and supplement and water intake

Calves offered supplements had average daily liveweight gain and were heavier at weaning at 180 days of age than calves that did not receive supplements (Table 1). However the type of supplement had no affect on average daily gain of calves or calf liveweight at weaning. The amount of supplement consumed to achieve these similar rates of liveweight gain was significantly less for calves fed the sesbania diet, suggesting calves may have utilised this feed source much more efficiently for liveweight gain than the energy dense maize grain. The type of supplement also had no affect on water intake by calves.

Table 1. Liveweight gain, weaning weight, supplement intake and imbibed water intake of preweaning calves fed no creep feed, maize and sesbania *ad libitum*

Parameters	Treatments*		
	Control	Maize	Sesbania
Birth weight (kg)	13.4 ± 0.67	13.9 ± 0.61	14.2 ± 1.1
Initial weight (kg/60 days)	30.7 ± 2.3	26.3 ± 1.6	30.2 ± 2.9
Final weight (kg/180 days)	59.2 ± 4.2^{a}	65.4 ± 4.7^{b}	66.6 ± 5.3^{b}
Total weight gain (kg/head)	28.5 ± 2.7^{a}	39.1 ± 3.3^{b}	36.4 ± 2.7^{b}
Average daily gain(kg)	0.24 ± 0.02^{a}	0.33 ± 0.03^{b}	0.30 ± 0.02^{b}
Intake of creep feed (g DM/kg LW.day)	-	9.1 ± 0.7^{a}	3.0 ± 0.2^{b}
Intake of imbibed water (g /kg LW.day)	-	19.6± 2.8	23.8 ± 1.6

**Values are means and standard error of the difference of the means (s.e.d). Within rows means with different uppercase letter are significantly different (P<0.05).*

The liveweight gain of un supplemented, control calves in the current study (0.24 kg/day) is higher than that reported for Bali calves managed under low-input systems elsewhere in eastern Indonesia (0.0 to 0.1 kg/day). This probably reflects the better management of cattle in the collaborating village site and may explain the modest response of calves to supplementation in the current study compared with other studies (Jelantik *et al*. 2008 DeAlmedia and Quigley, unpublished). Another reason for the modest response of liveweight gain to supplementation in the present study is the relatively low intakes of supplement measured (less than 10 g DM/ kg LW. day) compared with intakes of up to 30 g DM/kg LW. day reported by Jelantik *et. al*. (2008). Nevertheless, a growth rate of above 0.3 kg/day for suckling calves in the present study is higher than that reported elsewhere. This demonstrates that feeding and management systems can be successfully implemented on farm to reach potential maximum liveweight gain for this class of Bali cattle. The liveweight gain achieved in this study required far less feed than if a similar response was to be achieved through supplementation of the cow.

The reasons for the significantly higher intake of maize grain compared to sesbania are unknown. Sesbania is

a significant component of the diet of Bali cattle in West Nusa Tenggara and rejection of sesbania by cattle due to palatability or anti-nutritional factors is not previously reported. The sesbania used in the current study was dried prior to feeding and it is possible that this form of feeding was less palatable to young calves. Nevertheless, the fact that the liveweight gain response was similar, despite the different supplement, intakes may suggest that dietary protein is of greater importance for growth of this class of Bali cattle and that feeds that supply more protein will be used more efficiently for growth than those that supply energy. High protein tree legumes are readily integrated into existing farming systems and are more accessible to smallholder farmers than concentrates and grains where there is strong competition for human consumption or monogastric feeding.

Cow milk production and quality

The quantity and composition of milk produced by cows was unaffected by the treatment of the calves (control vs. supplemented and supplement type) (Table 2). Milk production declined from approximately 2.2 to 0.8 kg/day between days 60 and 160 after calving.

Table 2. Milk production and quality of respective dam from all treatments measured for 100 days over lactation period from day 60 to day 160

Parameters		Calve Groups		
		Control	Maize	Sesbania
Milk Production				
- Average over lactation	(kg/day)	1.5± 0.05	1.5± 0.08	1.5± 0.07
- Day 60 of lactation	(kg/day)	2.2± 0.15	2.2± 0.13	2.1± 0.08
- Day 160 of lactation	(kg/day)	0.8± 0.88	0.7± 0.09	0.7± 0.07
Average milk composition				
- Protein	(g/L)	82.3 ± 2.0	80.1 ± 1.8	75.3 ± 2.4
- IgG	(g/L)	22.8 ± 0.6	21.3 ± 0.8	17.6 ± 1.6
- Total solid	(%)	13.1 ± 0.3	14.1 ± 0.5	12.7 ± 0.4

It was expected that the production and composition of milk from cows in the current experiment would be unaffected by treatment of calves. This was evident and suggests that calves from all treatments received similar quantities of nutrients from milk through the experiment. This implies that any response observed in the current experiment was solely attributed to supplement intake in the creep feeding pen rather than any changes in milk intake.

Conclusions

Supplementation of suckling Bali calves with either a protein or energy based feed will promote growth above unsupplemented calves. The use of feeds with higher protein content such as tree legumes may promote more efficient growth than energy based supplements and will be more readily established and available within smallholder farming systems.

Acknowledgments

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KEYWORD：Bali cattle, milk production, supplementation, calf growth

REFERENCES

Darmadja, D., 1980. Half a century traditional cattle husbandry within agricultural ecosystem in Bali. PhD Thesis. Pajajaran University. Bandung.

Gunadi, S., DT. Panjaitan, D. Mayberry, K. McCosker, G. Fordyce and D. Poppi. 2013. Bali cattle breeding fed on low quality feed base, reproduction performance. Proseding Seminar IlmuTernakdanVeteriner, Medan, 3-5 September 2013.

Gunadi, S., Dahlanuddin, T. Panjaitan., 2015. Leucaena (Leucaenaleucocephala) and leucaena cassava

(*Manihotesculentacrantz*) mixture supplementation improved preweaning growth of Bali cattle (*Bosjavanicusd'alton*). Proceeding of ISAINI Seminar, Manado, 8-9 September 2015.

Jelantik, I. G. N., M. L. Mullik, C. Leo-Penu, J. Jeremias and R. Copland. 2008. Improving calf survival and performance by supplementation in Bali cattle. Australian Journal of Experimental Agriculture. Volume 48 Issue 6-7 pp. 950-953.

Jelantik, IG. N., M.L. Mullik, dan R. R. Copland., 2009. Cara Praktis Menurunkan Angka Kematian dan Meningkatkan Pertumbuhan Pedet Sapi Bali Melalui Pemberian Suplemen. Undana Press.

Martojo, H. 2012. Indigenous Bali cattle is the best suited cattle breed for sustainable small farm in Indonesia. Reproduction in Domestic Animals 47. 10-14

Moran, J.B. 1973. Heat tolerance of Brahman cross, buffalo, banteng and shorthorn steers during exposure to sun and as a result of exercise: J. Aust. Inst. Agric. Sci. 24 (5):775

Moran, J.B. 1978. Digestibility and water metabolism in Brahman cross, Buffalo, Banteng and Shorthorn steers fed on low quality roughages. : J. Aust. Inst. Agric. Sci. 44 (1):57-59

Supriyadi, M. 2015. Effect of body condition score during late pregnancy and lactation on preweaning calves growth performance of Bali cattle. Master Thesis. Mataram University.

Toelihere, M.Z. (2003). *Increasing the success rate and adoption of artificial insemination for genetic improvement of Bali cattle*. Entwistle, K and Lindsay, D (Ed). Strategies to improve Bali cattle in eastern Indonesia. ACIAR Proceedings No. 110: 48-53.

Wirdahayati, R.B., C. Liem, A. Pohan, J. Nulik,P. TH. Fernandez, Asnahdan A. Bamualim., 1997. Pengkajian teknologi usaha pertanian berbasis sapi potong di Nusa Tenggara Timur. Dalam Pertemuan Pra-Raker BadanLitbang Pertanian II. Manado, 3 — 4 Maret 1997.

O-25-1

Effect of Saccharomyces cerevisiae Fermented Cassava Bioethanol Waste on Feed Intake, Digestibility, Rumen Fermentation and Mocroorganism Diversity in Dairy Calf

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ABSTRACT: The objective of this experiment was to study effect of yeast (*Saccharomyces cerevisiae*) fermented cassava bioethanol waste, (YECAW) on feed intake, digestibility, rumen fermentation and mocroorganim diversity. Four, meal dairy calf with 109 ± 6.23 were randomly assigned to a 4x4 latin square design to receive YECAW at 0, 5, 10 and 20% in concentrate diets. Concentrate diet was fed at 1% BW and rice straw was fed ad libitum. The results shown that inclusion of YECAW were not altered on feed intake of rice straw, concentrate and total intake. Digestion coefficients of DM, OM, CP, NDF and ADF were ranged 69.4-66.0, 71.2-68.1, 66.8-65.6, 54.6-52.3 and 43.1-42.6% DM, respectively. Increasing of YECAW tend to reduce nutrient intake but there were not significantly different among treatments (P>0.05). OM and CP intake were ranged from 3.0-3.2 and 2.6-2.9 kg/d. Mean values of ruminal pH and temperature were not changed among treatment with ranged from 6.8-7.1 and 38.8-39.1℃ , respectively. Increasing of YECAW levels were not affect ruminal NH_3-N concentration, population of bacteria, protozoa and fungi. In conclusion, inclusion of YECAW at 20% in concentrate diet was not affect on feed utilization, rumen fermentation and ruminal microorganism.

INTRODUCTION

Cassava bioethanol waste (CBW) is a by-product of bioethanol production from whole cassava root as the raw material. CBW contains crude protein contents around 11-14% DM and some fibers, which are available nutrients for ruminant animals. Phoemchalard et al. (2014) reported that CBW can be used in the diet of heifer cattle without adverse effects on feed utilization and growth performance. Furthermore, Cherdthong et al. (2016) demonstrate that inclusion of 10 % CBW in TMR diets did not adversely affect feed intake, nutrient digestibility, ruminal fermentation characteristics, and blood metabolites in goat. Therefore, CBW is attractive for growing goat diets andmay be effectively used as an alternative roughage source in the diets of goats.

The process of protein enrichment of animal feed using microorganisms in a semi-solid culture to improve the nutritional value of ruminants feed has been evaluated (Boonnop et al., 2009 Polyorach et al. 2014). Incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition. Boonnop et al. (2009) showed that using fermented cassava chip with yeast increased crude protein from 2 to 30.4% CP, while Polyorach et al. (2014) reported that yeast fermented cassava chip protein could be prepared to increase crude protein level up to 47% CP and could inprove feed utilization and animal performance.

The objective of this experiment was to study effect of yeast (*Saccharomyces cerevisiae*) fermented cassava bioethanol waste, (YECAW) on feed intake, digestibility, rumen fermentation and mocroorganim diversity.

MADTERIALS AND METHODS

Yeast inoculum preparation was done according to the method of Polyorach et al. (2014) and some important details are as follows: activated yeast was prepared using 20 g of Bakers' yeast and 20 g cane sugar mixed with 100 mL distilled water, then mixed well and incubated at room temperature for 1 h (A). Liquid media was prepared using 8 g molasses and 100 mL distilled water, followed by addition of 64 g urea, then adjusting the pH of the solution using H_2SO_4 to achieve a final pH 3.5 to 5 (B). Mixed (A) and (B) at 1:1 ratio then flushed with air for 66 h at room temperature using an air pump (600 W). After 66 h, the yeast medium solution twas mixed with cassava bioethanol waste at a ratio of 1 mL: 1.3 g, then fermented in solid state under shade for 72 h, followed by sun-drying for 48 h. Cassava bioethanol waste was obtained from local ethanol plant and sun-dried for 3 d, then ground to pass a 0.5-mm sieve (Cyclotech Mill, Tecator, Sweden). The final product is stored in plastic bag for later

use as an ingredient in the concentrate supplement. The proportions of concentrated ingredients and the chemical composition of the concentrates YECAW and rice straw, are shown in Table1.

Four meal Holstein Fresian crossbred calf with 109 ± 6.23 were randomly assigned to a 4x4 latin square design to receive YECAW at 0, 5, 10 and 20% in concentrate diets. Concentrate diet was fed at 1% BW and rice straw was fed ad libitum. The experiment was conducted for 4 periods with 21 days per each. The first 14 days were for adaptation period and last 7 days were for samples collection (diets, feces, blood, and rumen fluid). Experimental diets were sampled daily during the collection period and were composited by period prior to chemical analyses. Feed offered and refusals samples were collected during the last 7 days of each period at morning and afternoon feedings. Fecal samples were collected at 09.00 or 12.00 h by rectal sampling. The samples were dried at 60℃ and ground (1 mm screen using a Cyclotech Mill, Tecator, Sweden) and analyzed using AOAC (1995) method for DM, N, ash. ADF and NDF were estimated according to Van Soest et al. (1991). Acid-insoluble ash (AIA) was used to estimate the digestibility of nutrients (Van Keulen and Young, 1977). Approximately, 45 mL of rumen fluid was taken from the rumen by a stomach tube connected to a vacuum pump at 0 and 4 h after feeding on the last day of each period. Ruminal pH and temperature were determined. Ruminal NH_3-N concentration was analyzed using Kjeltech Auto 1030 Analyzer (AOAC, 1995). Rumen fluid was used for direct counts of protozoa, bacteria and fungal zoospores using methods of Galyean (1989) by haemacytometer (Boeco, Singapore). A blood sample (about 10 mL) was collected from the jugular vein for analysis of plasma urea N according to Crocker (1967). Statistical analysis accounted for the 4 × 4 Latin square design using the GLM procedure of SAS (1996). Differences between treatment means were determined by Duncan's New Multiple Range Test and differences among means with P.

RESULTS AND DISCUSSION

Table 1 shows ingredient and chemical composition of experimental diets. Crude protein in YECAW was 21.5% DM, while concentrates diets contain 14.6-14.9% DM and to meet requirements of dairy calf. Effect of levels of YECAW in concentrate diets on intake and digestibility of feed in dairy calf are showed in Table 2. Inclusion of YECAW did not altered on feed intake of rice straw, concentrate and total intake (P>0.05). Total intake was ranged from 87.1 to 89.8 g/kg $BW^{0.75}$. Similarly, Phoemchalard et al. (2014) reported that no difference on dry matter intake was found when supplementing with either 15 or 30 % unfermented cassava bioethanol waste in yearling heifers. However, Cherdthong et al. (2016) indicated that fattening goats fed with unfermented cassava bioethanol waste in total mixed ration higher than 10% DM could reduce nutrient intake and digestibility. This might be due to the quality of cassava bioethanol waste that contains higher fiber and lower CP when compared to YECAW and may be related to differences in animal species. Increasing of YECAW tend to reduce nutrient intake but there were not significantly different among treatments (P>0.05). Intakes of OM and CP were 3.0 to 3.2 kg/d. and 2.6 to 2.9 kg/d, respectively. Digestion coefficients of DM, OM, CP, NDF and ADF were not changed among YECAW levels and ranged from 69.4-66.0, 71.2-68.1, 66.8-65.6, 54.6-52.3 and 43.1-42.6% DM, respectively. In addition, feeding of YECAW could improved DM digestibility when compared to those reported by Phoemchalard et al. (2014) who found that DM digestibility were ranged from 49 to 53% DM when yearling heifers fed unfermented cassava bioethanol waste. Improving of cassava bioethanol waste by yeast could be increase quality of by-product and enhance feed utilization.

Effect of YECAW levels on ruminal fermentation, NH_3-N concentration and microorganism population of dairy calf is presented in Table 3. Ruminal pH, and temperature were not changed among treatments with ranged from 6.8-7.1 and 38.8- 39.0 ℃ . Average ruminal pH values ranged from 6-7 and these ranges were considered as optimal levels for microbial digestion of fiber and microbial protein synthesis. Rumen NH_3-N concentration were not significantly different among treatments (P>0.05) with values ranging from 15.0-15.7 mg/dl, respectively. This is in agreement with results from Cherdthong et al. (2016), who reported no differences in ruminal NH_3 in goats when 10-20% cassava bioethanol waste was added to the basal diet. Increasing of YECAW levels did not adversely affect population of bacteria, protozoa and fungi and values ranging from 6.5 to 7.0 x 10^{12}, 3.2 to 4.0 x 10^5 and 6.9 to 7.4 x 10^3 cell/ml, respectively.

CONCLUSION

Inclusion of YECAW at 20% in concentrate diet was not affect on feed utilization, rumen fermentation and ruminal microorganism. Thus, feeding of YECAW is recommended since it has a positive economic impact, controlled environmental pollution and might be alternative protein sourse for ruminants.

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KEYWORD：Ethanol waste, Yeast fermented, Dairy calf, Feed intake, Fermentation

Table 1 Ingredient and chemical composition of experimental diets (% of dry matter)

Item	Levels of yeast fermented cassava bioethanol waste (YECAW), %DM				YECAW	Rice straw
	0	5	10	20		
Ingredients, %DM						
Cassava chip	45.0	43.0	41.0	39.0		
Soybean meal	14.0	12.0	10.0	8.0		
YECAW	0.0	5.0	10.0	20.0		
Rice bran	12.6	11.7	12.7	9.0		
Palm kernel meal	11.0	11.0	10.0	8.9		
Coconut meal	11.0	11.0	10.0	9.0		
Urea	1.4	1.3	1.3	1.1		
Molasses	2.0	2.0	2.0	2.0		
Salt	1.0	1.0	1.0	1.0		
Sulfur	1.0	1.0	1.0	1.0		
Mineral premix	1.0	1.0	1.0	1.0		
Chemical composition						
Dry matter, %	90.6	90.1	90.8	91.3	93.4	92.4
Organic matter, %DM	94.8	93.0	91.5	90.4	87.5	86.5
Ahs, %DM	5.3	7.0	8.5	9.6	12.5	13.8
Crude protein, %DM	14.9	14.6	14.6	14.7	25.1	2.1
Neutral detergent fiber, %DM	14.6	20.5	24.1	27.6	65.2	79.5
Acid detergent fiber, %DM	8.9	12.1	14.4	17.0	40.6	54.5

Table 2 Effect of levels of yeast fermented cassava bioethanol waste (YECAW) in concentrate diets on intake and digestibility of feed in dairy calf

Item	Levels of yeast fermented cassava bioethanol waste (YECAW), %DM				SEM	P-value
	0	5	10	20		
DM intake						
Rice straw						
g/kg $BW^{0.75}$	54.5	53.9	52.3	52.1	2.45	0.66
Concentrate						
g/kg $BW^{0.75}$	35.2	35.2	35.0	35.0	2.55	0.77
Total intake						
g/kg $BW^{0.75}$	89.8	89.1	87.3	87.1	2.98	0.44
Nutrient intake, kg/d						
Organic matter	3.0	3.2	3.1	3.0	2.05	0.26
Crude protein	2.7	2.9	2.7	2.6	1.98	0.39
Neutral detergent fiber	0.2	0.2	0.2	0.2	0.02	0.48
Acid detergent fiber	1.6	1.8	1.7	1.6	1.05	0.12
Apparent digestibility						
Dry matter, %	69.4	67.4	66.7	66.0	3.01	0.15
Crude protein, %DM	66.8	67.2	65.6	66.1	2.49	0.37
Organic matter, %DM	71.2	69.5	68.6	68.1	3.68	0.26
Neutral detergent fiber, %DM	54.6	52.3	53.0	52.7	2.06	0.09
Acid detergent fiber, %DM	43.1	42.6	42.7	43.0	1.98	0.11

Table 3 Effect of yeast fermented cassava bioethanol waste (YECAW) levels on ruminal fermentation, NH_3-N concentration and rumen microbes of dairy calf

Item	Levels of yeast fermented cassava bioethanol waste (YECAW), %DM				SEM	P-value
	0	5	10	20		
Ruminal pH	6.8	6.9	6.9	7.1	0.87	0.84
Ruminal temperature, °C	39.0	38.9	38.8	39.1	2.65	0.21
NH_3-N concentration, mg/dl	15.0	15.4	15.0	15.7	1.12	0.58
Ruminal microbes, cell/ml						
Bacteria, x 10^{12}	6.6	6.8	6.5	7.0	2.58	0.09
Protozoa, x 10^5	3.7	3.2	4.0	3.7	1.98	0.26
Fungal zoospore, x 10^3	7.4	7.4	6.9	7.4	3.99	0.17

REFERENCES

AOAC. 1995. Official Methods of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Boonnop, K., M. Wanapat, N. Nontaso, and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. Sci. Agric. 66:629-633.

Cherdthong, A., B. Pornjantuek and C. Wachirapakorn. 2016. Effect of feeding cassava bioethanol waste on nutrient intake, digestibility, and rumen fermentation in growing goats. Trop. Anim. Health Prod. DOI:10.1007/s11250-016-1094-6

Galyean, M. 1989. Laboratory procedure in animal nutrition research. Las Cruces (NM): Department of Animal and Life Science, New Mexico State University; p 107–122.

Phoemchalard, C., S. Uriyapongson, and E.P. Berg. 2014. Effect of cassava bioethanol by-product and crude palm oil in Brahman x Thai native yearling heifer cattle diets: I. Nutrient digestibility and growth performance. Trop. Anim. Health Prod. 46:663–668.

Polyorach, S., M. Wanapat, and A. Cherdthong. 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using In vitro gas production technique. Asian Australas. J. Anim. Sci. 27: 36-45.

Statistical Analysis System, 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc., Cary, NC, USA.

Van Keulen, J., and B.A. Young. 1977. Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies. J. Anim. Sci. 44:282–287.

Van Soest, P. J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

O-25-2 The effects of feeding methods during pre-weaning period on the fattening performance of male calves

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Abstract

Total 28 male calves were used to test 2 feeding systems in pre-weaning period (TMR: total mixed ration containing 10% alfalfa hay sized 1.5-2 cm and choice feeding with feed ingredients) on fattening performance of calves. The calves were housed individually. The choice fed calves received barley, wheat bran, soybean meal and alfalfa hay as free choice and ad libitum. All calves consumed 4 L whole milk in two meals daily. The choice fed calves preferred 5.56 % alfalfa hay, 29.60% wheat bran, 49.43 % soybean meal and 12.20 % barley and they formulated the diet containing 2.60 Mcal ME/kg, 30.79% CP, 27.18% NDF and 12.04% ADF, while control TMR contained 2.59 Mcal ME/kg, 17.94% CP, 24.99% NDF and 11.85% ADF.

After weaning all calves were monitored under research farm rearing practice until fattening stage of the study. Both groups were fattened with standard TMR containing 85% concentrate having 12.45% CP and 2.65 Mcal ME/kg and 15% wheat straw during the fattening period. All calves were housed individual fattening paddocks sized 3mx6 m. Weight, feed intake and feed to gain were recorded biweekly for 12 wks. Initial body weights of choice fed calves and TMR fed calves were 221.2 ± 12.5 and 219.0 ± 12.0 kg, respectively.

There were no differences in feed intake (9.83 ± 0.28 kg/day for TMR *vs.* 9.39 ± 0.24 kg/day for choice fed calves), daily gain (1.79 ± 0.06 kg/day for TMR *vs.* 1.74 ± 0.06 kg/day for choice fed calves), and feed to gain ratio (5.52 ± 0.17 for TMR *vs.* 5.44 ± 0.15 for choice fed calves).

In conclusion, the high protein preference or choice feeding of the calves during pre-weaning period does not improve future fattening performance of male calves.

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Introduction

Adult ruminants can make a diet from offered ingredients (Forbes, 2001 Gorgulu et al. 2008, Boga et al 2014). Pre-ruminant calves are usually kept separately from their mothers, Consequently, they have not a chance to learn the organoleptic and metabolic properties of feedstuffs under supervision of their elder partners or parents. However Simitzis et al. (2008) reported young animals have some degree of innate ability to choose and consume feed to meet their nutrient requirements, in addition to avoiding certain toxins and antinutritional factors in feeds (eg. tannins).

Young calves have been shown to preferentially consume protein source during pre-weaning period when feed ingredient supplied as free choice and selected a diet containing higher crude protein (CP) (31 to 35%) than a standard starter protein level (18%) and this preference was not changed after 2 weeks from weaning as well (Gorgulu et al. 2012). Similar diet preferences were demonstrated by Miller-Cushon et al. (2014) and Montoro and Bach (2012), where choice-fed calves preferentially consumed soybean meal and/or full-fat soybean and consumed a diet containing high protein content (27-32%) compared to single base starter diet as well. High protein level in starter diet did not affect pre-weaning growth performance (Gorgulu et al. 2012 Montoro and Bach, 2012 Miller-Cushon et al. 2014). However Drackley et al. (2002) found that calves fed with starter feed containing 22.00% CP were more efficient than those fed 18.00% CP.

Currently, there is some information related to the effects of colostrum and milk feeding on lactation performance of female calve (Terré et al., 2009 Moallem et al. 2010 Soberon et al., 2012). However, insufficient data is available on the effect of starter protein content on post-weaning and future performance of female and male calves. Nutrition and management practice during the backgrounding phase (the time period between weaning and finishing) may be a major contributors to finishing performance and carcass characteristics (Loken et al. 2009) of fattening cattle. The aim of this study was, therefore, to examine the effect of choice feeding and/or high protein content of starter on fattening performance of male calves during finishing phase.

Material and methods

Animal material used in the study was obtained from MSc Thesis of Hassani (2014). A total of 28 male calves

having 38.09 ± 1.07 kg initial body weight were allocated to one of two feeding methods (TMR-feeding and choice feeding with feed ingredients in the TMR) according to their birth weights. The calves in the TMR group received a diet containing concentrate and 10% ground alfalfa hay (1.5-2 cm chop length) *ad libitum*. Calves in the choice-fed group received all feed ingredients used in the TMR on a free-choice basis, *ad libitum*. Feed ingredients used in the choice-fed groups, except alfalfa hay, were supplemented with limestone, salt and vitamin mineral premix with an amount of TMR and offered as grounded form. The TMR were formulated with barley, wheat bran, soybean meal, and alfalfa hay. The ingredients and nutrient content of the TMRs and the diets selected by choice-fed calves are shown in Table 1.

The calves were housed in a semi-open barn, and each calf was kept in an individual pen (1.5 × 1.5 × 1.5 m) with straw bedding. Conventionally, each calf was offered whole milk daily, in a plastic bucket 2 L in the morning and 2 L in the evening, during an 8 week pre-weaning period. The chemical composition of the milk was 12.2% DM, 3.3% fat, 3.1% total protein, 2.6% casein, and 4.74% lactose. Post-weaning, all calves were fed with the same TMR containing 50% calf grower (16.7% CP, 2.66 Mcal ME/kg) and 50% alfalfa hay (1.5-2 cm chop length) mixed with wagon, ad libitum for 8 weeks (Table 1), to evaluate the effect of pre-weaning feeding methods on post-weaning performance of the calves (Table 2).

After finishing Hassani study (2014), all calves were kept under practical management condition of Research and Application Farm of Faculty of Agriculture until fattening study. Male calves reaching about 10 months and about 212.8 ± 8.7 kg grouped according the feeding methods during pre-weaning period. They were fattened with a standard TMR having 85:15 concentrate roughage ration (Table 1) for a total 12 weeks.

Live weight, live weight gain, and feed intake were monitored biweekly and evaluated 4 week interval during studies. The feeds were offered ad libitum (refusals on the last day of the week were noted) and given to the animal by adding fresh feed. The chemical composition of the feeds was analyzed using AOAC (1998) procedures. ADF and NDF analyses were based on the method of Van Soest et al. (1991). The study was performed according to a completely randomized design. Data were analyzed with One-way ANOVA using SPSS (SPSS, 1999). Statistical significance was set at a value of $P < 0.05$.

Results and Discussion

The findings obtained pre-weaning period revealed that calves preferred diets containing higher protein than NRC (2001) recommendation (18% vs. 30.79%CP), gained more weight during pre-weaning period but improvement in daily gain disappeared after weaning.

High differences in protein preferences before weaning may improve future performance of calves. It is, therefore, important to investigate the effects of high protein preferences during pre-weaning period on fattening performance of calve during finishing phase (Table 3).

The results in the present study indicated that the choice fed calves selecting a diet containing high protein during pre-weaning period had similar fattening performance ($P>0.05$) with the calves receiving standard starter during all finishing period. There were no differences in feed intake (9.83 ± 0.28 kg/day for TMR vs. 9.39 ± 0.24 kg/day for choice fed calves), daily gain (1.79 ± 0.06 kg/day for TMR vs. 1.74 ± 0.06 kg/day for choice fed calves), and feed to gain ratio (5.52 ± 0.17 for TMR vs. 5.44 ± 0.15 for choice fed calves) throughout experiment. It could be said that high protein content of starter had no effect on fattening performance of male calve during finishing phase, although some researcher reported that increase in nutrient intake from milk or milk replacer from birth up to 56 days of life, may result in increase in milk yield during first lactation compared with the calves on a restricted diet during the same period (Foldager and Krohn, 1994 Bar-Peled et al., 1997 Terré et al., 2009 Moallem et al. 2010 Soberon et al., 2012). All these studies include high colostrum, milk or milk replacer intake and high intake of these may supply protein, energy and other nutrient simultaneously as colostrum, milk and milk replacer were nutrient dense products. However, the calves selected a diet high protein by consuming soybean meal and they did not have any alternative to increase energy intake along soybean meal simultaneoussly. It is well known that soybean is best alternative (NRC 2001) for increasing energy and protein content of the diet among the feed ingredients supplied to the calves. Limited study is available for the effects of starter nutrient content (protein, energy) on adult performance of calves. Sexten et al. (2004) reported that providing additional dietary CP in creep feeds may improve lactation performance of female calve.

On the other hand daily gain obtained in the present study was quite higher (1.77 kg/day) than the usual values (1.0-1.3 kg/day, Gungor et al., 2004 Ozkutuk and Goncu, 1996 Kirkland et al. 2007, Ayasan et al. 2012) for Holstein bulls. This probably related to previous nutritional status of the bulls. After 16th week age, calves kept in research farm rearing condition and average daily gain of the bulls were about 0.58 ± 0.02 kg/day before finishing phase. Before finishing, backgrounding is used to prepare animal for finishing. Backgrounding may increase mature size (Owen et al. 1993), allowing maturation muscle and bone while restricting fat deposition (Block et al. 2001) with low body weight gain. Nutrition and management practice during backgrounding phase are major contributors for finishing performance and carcass merits (Raltson et al. 1966). Low daily gain such as in our calves during backgrounding may cause compensatory growth and animal may increase daily gain especially in the first months of finishing phase. Hornick et al. (2000) reported that catch up growth increase during first month and reach a maximum close to 2 kg/day in cattle and the maximal growth rate lasts for another month and sharply decrease later and reach minimum at about 4 month after beginning of finishing phase. The high live weight gain obtained in the present study could be explained with compensatory growth. Lower daily gain and/or restricted feeding may decrease basal metabolism (NRC 2016) due to lower body weight and vital organ size and this may explain that a relatively higher proportion of nutrient consumed (eg. energy, protein) can be supplied and used more body weight gain (Hornick et al. 2000).

CONCLUSIONS

The previous study (Hassani, 2014) revealed that during the pre-weaning period calves selected a diet containing higher CP (28-31%), when feed ingredients were supplied as a choice, compared with the standard starter protein level (18% CP). Choice fed calves had also higher daily weight gain during the pre-weaning, but the high protein consumption during pre-weaning period did not affect fattening performance of calves during finishing phase.

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KEYWORD : Male Calve, Choice Feeding, Starter Protein Content, Fattening, Future Performance

Table 1. Ingredients and nutrient contents of TMRs and the diets selected by calves of Hassani Study (2014).

	TMR	Choice Feeding	Postweaning TMR	Finishing TMR
Wheat straw, %	--	--	--	15.00
Corn, %	--	--	--	12.80
Wheat bran , %	17.28	29.60	--	27.20
Wheat midds, %		--	--	8.50
Wheat, %		--	--	12.60
Soybean meal, %	17.73	49.43	--	--
Cotton seed meal, %	--	--	--	1.60
Barley, %	52.29	12.20	--	17.00
Vitamin-Mineral Premix¥,%	0.08	0.09	--	0.09
Vinas,%	--	--	--	2.60
Vegetable oil, %	--	--	--	0.60
Limestone, %	1.52	1.70	--	1.40
Salt, %	0.56	0.60	--	0.70
Alfalfa hay, %	10.00	5.56	50.00	--
Calf Grower,%*			50.00	--
Compositions;				
DM, %	90.77	89.69	89.15	93.16
CP, %	17.94	30.79	13.41	12.08
EE, %	2.23	2.44	2.39	3.81
ADF, %	11.85	12.04	27.67	13.96
NDF, %	24.99	27.18	34.87	33.45
Ash, %	7.20	8.28	5.67	6.82
ME, Mcal/kg ***	2.59	2.60	2.19	2.54

* contains limestone, salt and vitamin mineral premix.
** Calf grower contained 16.7% CP and 2.66 Mcal ME/kg.
***concentrate ME content was calculated according to TSE (1991). Alfalfa hay ME content was calculated according to Schroeder (1994).
¥1 kg contains 8.000.000 IU vitamin A, 10.000.000 IU vitamin D3, 2.000 mg vitamin E, 30.000 mg Mn, 50.000 mg Zn, 50.000 mg Fe, 50.000 mg Cu, 10 mg Co, 150 mg I and 800 mg Se.

Table 2. Pre-and Postweaning feed and nutrient intakes, daily gain and feed to gain ratios calves fed with different feeding methods during the preweaning period (Hassani, 2014).

Feeding Methods	TMR	Choice Feeding
Preweaning (1-8 weeks)		
Birth weight, kg	34.83	36.26
Feed intake*, g/day	670.46	682.79
Daily gain, g/day	564.16	632.27
Weaning weight, kg	69.75	73.29
CP intake, g/day	120.31	212.17
Feed to gain**	1.21	1.09
Postweaning (9-16 weeks)		
Final weight, kg	98.78	100.22
Daily gain, g/day	554.20	493.37
Feed intake*, g/day	2894.20	2767.61
Feed to gain	5.32	5.70

*as fed basis.

**calculated from solid feed only (not included solid from milk).

Table 3. Fattening performance during finishing phase of the calve fed different feeding systems in pre-weaning period.

Properties	TMR	Choice Feeding	P<
Birth weight, kg	34.83±1.49	36.26±1.82	0.55
Weaning weight, (kg)	69.75±2.08	73.29±6.95	0.47
16th week old weight, kg	98.78±3.30	100.22±3.13	0.75
Daily gain from 16th week old to 43th week old, g/day	0.60±0.03	0.55±0.04	0.25
0-4 weeks			
Age at the begining of fattening, month	10.05±0.33	10.12±0.30	0.87
Intial body weight, kg	218.35±12.31	207.53±12.56	0.54
Daily gain, kg/day	2.08±0.07	1.96±0.08	0.26
Dry matter intake, kg/day	8.40±0.24	8.40±0.24	0.16
Feed to gain ratio	4.06±0.12	4.02±0.13	0.85
5-8 weeks			
Daily gain, kg/day	1.65±0.08	1.62±0.07	0.82
Dry matter intake, kg/day	10.13±0.36	9.61±0.26	0.24
Feed to gain ratio	6.27±0.26	6.02±0.23	0.47
9-12 weeks			
Daily gain, kg/day	1.65±0.09	1.64-±0.07	0.93
Dry matter intake, kg/day	10.96±0.27	10.75±0.21	0.53
Feed to gain ratio	6.93±0.47	6.70±0.28	0.67
Overall			
Daily gain, kg/day	1.79±0.06	1.74±0.06	0.55
Dry matter intake, kg/day	9.83±0.28	9.39±0.24	0.24
Feed to gain ratio	5.52±0.17	5.44±0.15	0.73
Final body weight, kg	369.14±15.87	354.00±15.67	0.50

REFERENCES

Association of Official Analytical Chemists. 1998. Official method of analysis. 16th ed. AOAC, Washington, DC.

Ayasan, T., Gok, K., Asarka, A., Hizli, H., Görgülü, M., Karakozak, E., Coskun, M.A., Segmenoglu, M.S. 2012. Mısır Silajı ve Seker Pancarı Posasının Erkek Danaların Besi Performansı, Kan Parametresi ile Kesim Ölçütleri Üzerine Etkisi. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi 7 (1):64-73, 2012

Bar-Peled, U., Robinzon, B., Maltz, E., Tagari, H., Folman, Y., Bruckental, I., Voet, H., Gacitua, H. and Lehrer, A.R. 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. Journal of Dairy Science 80:2523–2528.

Block, H. C., J. J. McKinnon, A. F. Mustafa, and D. A. Christensen 2001. Manipulation of cattle growth to target carcass quality. J. Anim. Sci. 79:133–140.

Boga, M., Gorgulu, M. and Sahin, A. 2014. Effects of feeding methods, season and production level on lactation performance and feeding behaviour of dairy cows. Bulgarian Journal of Agricultural Science, 20, 915-923.

Drackley, J.K., Bartlett, K.S. and Blome, R.M. 2002. Protein content of milk replacers and calf starters for replacement calves. http://www.livestocktrail.uiuc.edu/dairynet/paper Display.cfm?ContentID=339 (Retrieved 27 November 2012)

Foldager, J. and Krohn, C.C. 1994. Heifer calves reared on very high or normal levels of whole milk from birth to 6–8 weeks of age and their subsequent milk production. Proceedings of the Society of Nutrition Physiology 3:301. (Abstr.).

Gorgulu, M., Akyol, M.A., Boga, M. and Goncu, S. 2012. The effects of choice feeding and season on feeding behavior and growth performance of calves. Journal of Animal and Feed Sciences, 21, 263–275

Gorgulu, M., Boga, M., Sahin, A., Serbester, U., Kutlu, H.R. and Sahinler, S. 2008. Diet selection and eating behaviour of lactating goats subjected to time restricted feeding in choice and single feeding system. Small Ruminant

Research, 78(1-3), 41-47.
Gungor, M., Alcicek, A. 2004. Siyah alaca ve farkli etçi ırk melezlerinin besi performanslarının araştırılmasi. Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi, 14(1).
Hassani, M.W. 2014. Buzağılarda Sütten Kesim Öncesi Tercihli Yemlemenin Sütten Kesim Sonrası Dönemde Performans Üzerine Etkileri. Ç.Ü. Fen Bilimleri Enstitusu. Zootekni Anabilim Dalı. Yüksek Lisans Tezi. Adana.
Hornick, JL; Van Eanaeme, C; Gerard, O; Dufrasne, I and Istasse, L. 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol., 19: 121- 132.
Kirkland, R.M., Patterson, DC., Keady, T.W.J., Moss, B.W., Steen, R.W.J. 2007. Beef production potential of Norwegian Red and Holstein–Friesian Bulls slaughtered at two ages. Animal. 1:1506–1514
Loken, B. A., R. J. Maddock, M. M. Stamm, C. S. Schauer, I. Rush, S. Quinn, and G. P. Lardy. 2009. Growing rate of gain on subsequent feedlot performance, meat, and carcass quality of beef steers. J. Anim. Sci. 87:3791-3797.
Miller-Cushon, E.K., Terré, M., DeVries, T.J. and Bach, A. 2014. The effect of palatability of protein source on dietary selection in calves. Journal of Dairy Science. 97:4444-4454.
Moallem, U., Werner, D., Lehrer, H., Zachut, M., Livshitz, L., Yakoby, S. and Shamay, A. 2010. Long- term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650.
Montoro, C., Bach, A. 2012. Voluntary selection of starter feed ingredients offered separately to nursing calves. Livestock Sci.149:62–69.
Forbes, J.M., 2001. Consequences of feeding for future feeding. Comp. Biochem. Physiol. Part A (128) 463-470.
National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 8th Edition. National Academy Press. Washington, DC, USA.
National Research Council. 2016. Nutrient Requirements of Beef Cattle. 7th Edition. National Academy Press. Washington, DC, USA.
Owens, F. N., Dubeski, P. and Hanson, C. F. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. 71: 3138–3150.
Ozkutuk, K. Goncu, S. 1996. Sıcaklık stresinin, süt sığırcılığı ve besi üzerine etkisi konusunda Çukurova bölgesinde yapılan çalışmalar. Hayvancılık'96 Ulusal Kongresi,18-20 Eylül,1996, Izmir,Sf.37-44.
Ralston, A. T.-, W. H. Kennick, T. P. Davidson, and K. E. Rowe 1966. Effect of prefinishing treatment upon finishing performance and carcass characteristics of beef cattle. J. Anim. Sci. 25:29–33
Sexten, W.J., Faulkner, D.B. Dahlquist, J.M. and Ireland, F.A. 2004. Influence of creep feeding and protein level on growth and maternal performance of replacement beef heifers. Prof. Anim. Sci. 20:211-217.
Simitzis, P.E., Bizelis, J.A., Deligeorgis, S.G. and Feggeros, K. 2008. Effect of early dietary experiences on the development of feeding preferences in semi-intensive sheep farming systems-a brief note. Applied Animal Behaviour Science 11, 391-395
Soberon, F., Raffrenato, E., Everett, R.W. and Van Amburgh, M.E. 2012. Pre-weaning milk replacer intake and effects on long term productivity of dairy calves. Journal of Dairy Science 95:783–793
SPSS, 1999. SPSS for Windows, Rel. 10.0.0. 1999. Chicago: SPSS Inc.
Terré, M., Tejero, C. and Bach, A. 2009.Long-term effects on heifer performance of an enhanced growth feeding programme applied during the pre-weaning period. Journal ofDairy Research 76:331–339
VanSoest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-359.

O-25-3

The utilization of yeast fermented cassava ethanol waste (YFCEW) from bio-ethanol plant in dairy cattle ration

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Abstract: The purpose of this study was to investigate the addition of yeast fermented cassava ethanol waste (YFCEW) in total mixed ration (TMR) on feed intake, nutrient digestibility and milk production in dairy cows. Four crossbred Holstein Friesian cows with initial weight 431 kg and days in milk 99 days were randomly allotted according to a 4 × 4 Latin square design (21 day/period). The cows were assigned to receive one of four dietary treatments: 1) total mixed ration (TMR) without YFCEW, 2) TMR with YFCEW at 5 %, 3) TMR with YFCEW at 10 % and 4) TMR with YFCEW at 20 %. The rations were formulated containing 14 percent of crude protein and 10.0 MJ ME/kgDM. The TMR consisted of roughage to concentrate at ratio of 40:60 with rice straw used as main source of roughage. The results showed that feed intake (kgDM/d, %BW and g/kg$W^{0.75}$) and digestibility of CP of cows fed TMR with YFCEW was linearly decreased (P<0.05), while digestibility of EE was significantly increased (P<0.01) as YFCEW in TMR was increased. However, digestion coefficients of DM, OM, CP, NDF and ADF were not significantly different among dietary treatments (P>0.05). Nevertheless, milk production in cows fed TMR consisted of YFCEW at 0 to 10% was not significantly different but was significantly higher (P<0.01) than cows fed TMR consisted of YFCEW at 20%. Milk fat was linearly increased (P<0.01) as YFCEW in TMR was increased. It was concluded that addition of YFCEW at 10% in TMR for lactating cows appeared to be promising.

Introduction

Recent year, alternative energy sources are focus due mainly to high price of fuel. Ethanol from biomass has become increasingly popular as alternative source replacing gasoline. In Thailand, main materials for bio-ethanol industry were cassava tuber. Approximately, distillery slop left from ethanol plants was about 1,400-1,600 tonnes (5-7% total solid) per year. Distillery slop so-called cassava ethanol waste may have impact to environment. Laorodphan et al. (2013) showed that dried cassava ethanol waste (DCEW) can be used as ruminant feeds. It contained 92.7% dry matter (DM), 19.5% ash, 7.49% crude protein (CP), 5.46% ether extract (EE), 41.5% nitrogen free extract (NFE), 57.0% neutral detergent fiber (NDF), 49.7% acid detergent fiber (ADF) and 13.9% acid detergent lignin (ADL). In addition, previous works have been shown that inclusion of DCEW has been used upto 30% in concentrate for beef (Phoemcharad et al., 2015) and upto 10% in total mixed ration (TMR) for growing goat (Cherdthong et al., 2016). Moreover, Wachirapakorn et al. (2016) also suggested that DCEW can be used 10% in or 17% in concentrate for lactating cows. However, DCEW seems to have a limitation to use in ruminant feeds. Phonsaen et al. (2016) has found way of improving nutritive value of DCEW by using yeast to enrich DCEW. Yeast fermented cassava ethanol waste (YFDCEW) contained high protein, 25%CP, it might be used as protein sources in dairy diet. Thus, the objective of the present experiment was to investigate the addition of YFCEW in diet of dairy cows on feed intake, nutrient digestibility, milk composition and milk production.

Materials and Methods

Four multiparous Holstein Friesian crossbred cows with initial weight 431 kg and day-in-milk 99 days, were randomly assigned to receive dietary treatments in a 4×4 Latin square design with four 21-d periods each comprising 14 d for dietary adaptation and 7 d for data collection. Dietary treatments were TMR with YFCEW inclusion at 0, 5, 10 and 20 %, respectively. Diets were formulated for isocarlolic and isonitrogenous diet. TMR was offered *ad libitum*, after milking at 0700 and 1700 h. Water and mineral block were free access at all times. Cows were milked two times a day at 0600 and 1500 h. Milk yield was recorded daily.

Feed and refusals were recorded daily. Dietary treatments were weekly sampled and were composited by period prior to analyses. Fecal samples were collected by rectal sampling during the last 7 days of each period. Composited samples were dried at 60℃, ground (1 mm screen) and analyzed using the standard methods of AOAC (1997) for DM, Ash, EE and CP, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). Apparent digestion of nutrients was used acid-insoluble ash (AIA) as marker

according to Van Keulen and Young, (1977).

Rumen fluid samples were collected by stomach tube, approximately 500 ml of rumen fluid was immediately measured for pH and then filtered through four layers of cheesecloth. 10 ml of 50% H_2SO_4 solution was added to 100 ml of rumen fluid. The mixture was centrifuged at 16,000 × g for 15 minutes and supernatant was stored at -20 ℃ prior to NH_3-N measurement (Bremner and Keeney, 1965).

A blood sample was taken from the jugular vein at the same time as rumen fluid sampling, and then separated by centrifugation at 3000 × g for 15 minutes and then plasma was sampled and stored at -20℃ until analysis for blood urea nitrogen (BUN) (Crocker, 1967). Milk samples were collected and pooled on the last 5 consecutive days) of each period by equal volume (50 ml/d and collected for two 50-ml aliquots of milk for analyzed milk composition.

Statistical analysis

Data were subjected to analysis of variance according to a 4 × 4 Latin square design using the PROC GLM (SAS, 1996). Significant differences between treatments were determined using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Result and Discussion

TMRs used in this experiment contained similar crude protein (14%) which was recommended by Wachirapakorn et al. (2014). NDF and ADF contents were slightly increased as YFCEW was increased in TMR. Voluntary feed intake (kg/d, %BW and g/kg $BW^{0.75}$) of cows fed TMRs containing YFCEW at 0, 5 and 10% was not significant different ($P>0.05$) but higher that of cows fed TMR containing YFCEW at 20% ($P<0.01$). It was observed that digestibilities of DM, OM, CP, NDF and ADF has no different among dietary treatments ($P>0.05$). However, digestibility of EE linearly increased ($P<0.01$) when increased level of YFCEW in diets (Table 1). Milk yield of cows fed TMR containing 20% YFCEW was significant lower ($P<0.05$) than that of cows fed TMR containing 0, 5 and 10% YFCES. However, milk yield of cows fed TMR containing 0, 5 and 10% YFCES was not different ($P>0.05$). Milk fat, total solids and F:P ratio were highest ($P<0.05$) in dairy cows fed TMR containing YFCEW at 20 %, while, 4% fat-corrected milk (FCM) and other milk composition were not significantly different among dietary treatments ($P>0.05$). F:P ratios in cows fed TMR containing YFCEW were in optimum ratio indicating TMRs in this experiment were balance between energy and protein in diets. Moreover, inclusion of YFCEW at any level did not affect means of ruminal pH, ammonia-nitrogen, and blood urea nitrogen.

Conclusion

From current experiment, it was concluded that YFCEW can be included 10% in TMR for lactating cows without any reverse effect on milk production. .

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KEYWORD : yeast fermented cassava ethanol waste (YFCEW), feed intake, milk yield, dairy cow

Table 1 Effects of level of dried cassava pulp from bio-ethanol industry (YFCEW) supplementation on voluntary intake, nutrient digestibility, rumen fermentation, feed efficiency, milk yield and milk composition in dairy cows

item	**YFCEW** (%)					*P*-
	0	5	10	20	SEM	value
Initial body weight, kg	455.8	455.6	466.8	458.4	4.23	0.31
Body weight change, kg	14.0	21.8	10.5	1.25	1.76	14.0
Voluntary feed intake						
kg	14.7^{a}	15.3^{a}	14.6^{a}	12.9^{b}	0.23	<0.01
% BW	3.2^{ab}	3.4^{a}	3.1^{ab}	2.8^{c}	0.05	<0.01
g/kg $BW^{0.75}$	149.4^{ab}	154.8^{a}	144.7^{ab}	130.1^{c}	2.09	<0.01
Nutrient digestibility, %						
DM	63.0	64.3	65.0	60.9	1.69	0.40
OM	67.8	69.2	69.4	65.4	1.63	0.37
CP	72.8	70.4	69.0	66.0	1.73	0.13
EE	81.6^{a}	82.5^{a}	85.9^{b}	85.4^{b}	0.58	<0.01
NDF	49.7	52.0	54.3	49.9	2.87	0.66
ADF	40.6	44.4	48.6	46.8	2.32	0.18
ME intake, MJ/d	138.5^{a}	147.3^{a}	139.3^{a}	115.5^{b}	4.60	0.01
ME, MJ/kgDM	9.4	9.6	9.5	9.0	0.21	0.37
MCP, kg/d	1.1^{a}	1.2^{a}	1.1^{a}	1.0^{b}	0.04	0.01
Milk yield, kg/d	13.2^{a}	13.3^{a}	12.6^{ab}	11.8^{b}	0.27	0.03
4% FCM, kg/d	12.6	13.1	12.7	12.2	0.33	0.39
ECM, kg/d	12.5	12.9	12.5	11.9	0.31	0.29
Milk composition, %						
Milk fat	3.7^{a}	3.9^{b}	4.1^{ab}	4.2^{b}	0.06	<0.01
Milk protein	3.3	3.3	3.3	3.3	0.01	0.69
lactose	4.8	4.7	4.7	4.7	0.02	0.31
Solid not fat	8.7	8.7	8.7	8.7	0.03	0.70
Total solid	12.4^{a}	12.5^{ab}	12.7^{b}	12.9^{c}	0.10	0.03
Fat : protein	1.1^{a}	1.2^{b}	1.2^{b}	1.3^{c}	0.02	<0.01
Rumen fermentation						
pH	6.8	6.8	6.8	6.9	0.06	0.89
NH_3-N, mg%	12.4	11.4	10.9	10.7	1.29	0.80
BUN, mg%	22.6	21.5	20.9	20.9	1.20	0.74

L = linear, Q = quadratic, C = cubic

ECM = energy corrected milk = milk x (0.38*%fat+0.24x%protein+0.17x%lactose)/3.17

NH_3-N =ammonia-nitrogen, BUN = blood urea nitrogen

REFERENCES

AOAC. 1997. Official Method of Analysis. 16th Ed., Association of Official Agriculture Chemicals, Washington, DC., USA.

Bremner, J.M., and D.R. Keeney. 1965. Steam distillation methods of determination of ammonium, nitrate and nitrite. Anal. Chem. Acta. 32:218.

Cherdthong, A., B. Pornchantuke and C. Wachirapakorn. 2016. Effect of feeding cassava bioethanol waste on nutrient intake, digestibility, and rumen fermentation in growing goats. Tropical Animal Health and Production. pp 1-6. First online: 20 June 2016.

Crocker, C.L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33: 361-365.

Huber, J.T. and L. Kung. 1981. Protein and Nonprotein Nitrogen Utilization in Dairy Cattle. J. Dairy Sci. 64: 1170-1195.

Laorodphan, N., C. Mikled, N. Chongkasikit, and C. Phatsara. 2013. Effects of the level of dried cassava pulp from ethanol process in the ration on rumen fermentation and digestibility in fistulated Thai native bull. J. Agric. 29: 89-98.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th Revised Edition. National Academic Press, Washington, D.C. 408 p.

Phoemchalard, C., S. Uriyapongson and E.P. Berg. 2014. Effect of cassava bioethanol by-product and crude palm oil in brahman x Thai native yearling heifer cattle diets: i. Nutrient digestibility and growth performance. Trop Anim Health Prod 46:663-668.

Phonsean, K., C. Wachirapakorn, C. Wongnen and W. Daenseekaew. 2016. Effect of Yeast-fermented Bio-ethanol Waste on Feed Intake, Digestibility and Rumen Fermentation in Thai Native Cattle. J. AGT RMU. (in press).

SAS. 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc., Cary, NC: USA.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co., New York: USA.

Van Keulen, J., and B.A. Young. 1977. Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies. J. Anim. Sci. 44:282–287.

Van Soest, P.J., J. B. Robertson, and B. A. LEWIS. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wachirapakorn, C., P. Parkmaluk, M. Wanapat, P. Pakdee, and A. Cherdthong. 2014. Effects of levels of crude protein and ground corn cobs in total mixed ration on intake, rumen fermentation and milk production in crossbred Holstein Friesian lactating dairy cows. J. Applied Anim Res. 42:263-268.

Wanapat, M. 1999. Feeding of ruminants in the tropics based on local feed resources. Khon Kaen Publishing Company Ltd., Khon Kaen, Thailand.

Zinn, A.R., and F.N. Owen. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-167.

O-25-4 Effects of exogenous fibrolytic enzyme (EFE) supplementation on rumen fermentation and fibrolytic bacteria population in ruminants fed rice straw-based diet

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Abstract: The objective of this experiment was carried out to investigate the use of exogenous fibrolytic enzyme (EFE) to improve feed efficiency in ruminants fed rice straw as roughage source. Three rumen-fistulated animals in each species, crossbred dairy (>75% Holstein Friesian) cattle, crossbred beef (>75% Brahman) cattle, and swamp buffalo steers were used in this experiment. The dietary treatments were a diet without EFE supplementation (control) and a diet with EFE supplementation at 500 mg/kg DM diets. The results revealed that the supplementation of EFE by direct-fed in dairy cattle, beef cattle and swamp buffalo, reduced total feed intake (P<0.01). Moreover, fibrolytic bacteria population (*Ruminococcus albus, Butyrivibrio fibrisolvens* and *Prevotella ruminicola)* were diversified among ruminant species by EFE supplementation. Based on current observation, it could be concluded that EFE supplementation seemed to unchanged rumen fermentation but changed fibrolytic bacteria population of ruminants.

INTRODUCTION

In general, rice straw is main roughage source for ruminants in Thailand (Wanapat et al., 2009). However, it is characterized by low levels of crude protein and high level of structural carbohydrates (cellulose, hemicellulose and lignin), which drastically affected the dry matter (DM) intake, digestibility and animal performance (Safari et al., 2011). Improving the utilization of rice straw could be by supplementation of exogenous fibrolytic enzymes (cellulose and xylanase EFE) resulting in improved fiber utilization and animal performance. There are several previous studies shown that EFE have been used to improve the nutritive value of fiber-rich diets and the performance of cattle (Elwakeel et al., 2007). Although the measurements of total tract digestibility in dairy cows have generally shown positive responses to EFE with variable (Dean et al., 2005 Knowlton et al., 2007). Sutton et al. (2003) noted that while an EFE product applied to a total mixed ration (TMR) was only slightly increased ruminal fiber digestion and total tract digestibility.

Therefore, the objective of this experiment was to investigated the use of EFE to improve feed efficiency of rice straw in ruminants.

MATERIALS AND METHODS

Nine rumen fistulated steer, 3 heads from Holstein-Friesian crossbred dairy cattle (>75% HF), 3 heads from beef cattle (Brahman), and 3 heads from Thai swamp buffaloes steers, were used in this study. Animals were fed at 2% of BW on a standard diet (concentrate: roughage=50:50) with rice straw was used as a roughage source and the concentrate were formulated to be at 11% of CP. The dietary treatments were a diet without (control) and with EFE supplementation at 500 mg/kg DM diets.

The experiment was conducted over 3 periods with 21 days, feeds were regularly sampled and feces samples were collected of each individual animal at the last 7 days of each period and analyzed for DM by the AOAC (1997) procedures. Feed intake were recorded daily throughout 21-d of each period. On the last day of each period, 200 ml rumen fluid samples were collected via fistula at 0 and 4 h. post-feeding and then measured for the pH immediately. The first portion of rumen fluid samples were added 5 mL of 1 *M* H_2SO_4 to 50 mL of rumen fluid then centrifuged at 16,000×g for 15 minutes, the supernatant was stored at -20℃ prior analyzed for (NH_3-N) (Bremner and Keeney, 1965) and volatile fatty acids (VFAs) according to Mathew et al. (1997). Another portion was

immediately placed on ice, and then store at -20℃ for DNA extraction (Yu and Morrison, 2004) prior estimated for the number of total bacteria (Suzuki et al., 2000 Nicol et al., 2008), Archaea (Takai and Horikoshi, 2000) and cellulolytic bacteria there are *Fibrobacter succinogenes, Ruminococcus albus, R. flavefaciens, Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Anaerovibrio lipolytica, Megasphaera elsdenii* and *B. proteoclasticus* (present studied) by using qPCR analysis (Biorad).

A blood sample was taken from the jugular vein at the same time as rumen fluid sampling into 6-ml Vacutainer tubes without anticoagulant, refrigerated for 1 h. and then centrifugation at 3,000 × g for 15 minutes, the supernatant was stored at -20℃ until analysis of blood urea nitrogen (BUN) according to Crocker (1967).

All data were analyzed as a cross-over designs using PROC GLM of SAS (1996). The significant group differences were compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Feed intake and apparent digestibility of nutrients

EFE supplementation reduced total feed intake ($P<0.01$) confirmed with previous research that EFE supplementation enhanced digestibility of dietary (Llewellyn et al*.,* 2010). There are show positive correlation between feed intake and digestibility, in other words, more digestibility has received the connotation of carrying less indigestible matter and leading to reduced incubation time of digestion (Togtokhbayar et al., 2015), increased particle out flow rate from the rumen (Alvarez et al., 2009) and gain more ruminal fill for more feed intake by short-term intake that was affected by the ruminal fill (Gregorini et al., 2007).

EFE supplementation were not affected on ruminal pH, NH_3-N, concentration of VFAs, BUN and ruminal microorganism population, similarly with Shekhar et al. (2010) that blood glucose and blood urea nitrogen concentration were not affected by EFE supplementation. However, total VFAs and the number of total bacteria and *R. flavefaciens* of EFE treatment was higher than the control but not significantly different ($P>0.05$). In agreement with Giraldo et al. (2007) and Arriola et al. (2011) who demonstrated that treatment of wheat straw with an EFE preparation containing xylanase and β -gluconase activities increased fiber digestibility and VFAs production. In addition, EFE supplementation was released more reducing sugars from forage and can stimulate a greater concentration of volatile fatty acids and microbial protein synthesis (Mendoza et al. 2014) and resulting in greater attachment and colonization of rumen microorganisms to the plant cell wall (Nsereko et al. 2002), changed ruminal fermentation, increase particle outflow rate from the rumen associated with a reduction in rumen liquid viscosity (Alvarez et al. 2009) and improved fiber digestion (Nsereko et al. 2002).

Among species of ruminants, buffalo had shown lower molar proportion of propionic acid (C_3) and butyric acid (C_4), but higher concentration of BUN, total VFAs, acetic acid (C_2) and ratio of C_2:C_3 than those in beef and dairy cattle. These results were concomitant with higher amount of population of *R. flavefaciens* and *R. albus* that significantly different among each species. In accordance with Xie et al. (1997), Wora-anu et al. (2006) and Khejornsart et al. (2011) who reported that ruminal bacteria of buffalo contained high amount of fibrolytic bacteria (*B. fibrisolvens, F. succinogenes* and *R. flavefaciens)*. Leading to higher fiber utilization and degradation in buffalo than cattle (Kennedy et al. 1992), any variations between cattle and buffalo in the nature of the rumen microbial population and numbers of ruminal bacteria might contribute to an explanation of the differences in the type of fermentation occurring, the end-products resulting from fermentation and digestive capability (Wanapat et al. 2000).

CONCLUSION

The present study observed that the supplementation of EFE by direct-fed to ruminants, particularly in swamp buffalo, resulted in changed rumen fermentation by increasing the number of fibrolytic bacteria.

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KEYWORD : Exogenous fibrolytic enzymes (EFE), rice straw, dairy cattle, beef cattle, buffalo

Table 1 Effect of exogenous fibrolytic enzymes (EFE) supplementation and species of ruminants on voluntary dry matter intake and nutrients digestibility

Items	Treatment		SEM	P-value	Species			SEM	P-value
	Control	EFE			Dairy	Beef	Buffalo		
Body weight, kg	508	510			604	509	415		
Total feed intake (kg/day)	10.5^{a}	9.78^{b}	0.14	<0.01	13.2^{a}	9.46^{b}	8.37^{c}	0.17	<0.01
Concentrate intake (kg/day)	5.59	5.07	0.44	0.43	6.32	5.31	4.36	0.52	0.07
Rice straw intake (kg/day)	4.90	4.71	0.42	0.76	5.12	5.09	4.19	0.50	0.37
Ruminal pH	6.76	6.98	0.04	0.75	7.08^{a}	7.12^{a}	6.75^{b}	0.08	0.02
NH_3-N, mg%	8.25	12.8	0.35	0.60	13.2	11.4	14.0	0.87	0.35
BUN, mg %	11.9	9.78	0.27	0.82	7.83^{b}	8.92^{b}	14.4^{a}	0.90	<0.01
Volatile fatty acids (VFAs)	40.4	40.8	0.05	0.36	40.4	40.5	40.9	0.20	0.19
Total VFAs, mM									
Acetic acid (C_2), %	97.2	101.4	2.14	0.14	93.6^{b}	88.4^{b}	115.9^{a}	4.37	<0.01
Propionic acid (C_3), %	63.4	65.6	0.42	0.22	63.2^{b}	61.1^{b}	69.3^{a}	1.09	<0.01
Butyric acid (C_4), %	27.1	25.1	0.46	0.22	26.9^{a}	28.0^{a}	23.3^{b}	0.77	<0.01
C_2:C_3 ratio	9.55	8.97	0.21	0.34	9.82^{a}	10.1^{a}	7.82^{b}	0.59	0.05
Ruminal bacteria population									
Total bacteria ($\times 10^{11}$)	2.51	3.32	0.34	0.54	2.52	2.87	3.35	0.57	0.59
Archaea ($\times 10^{8}$)	2.01	2.11	0.35	0.54	1.71	2.38	2.09	0.39	0.71
*F. succinogen*es ($\times 10^{8}$)	6.62	5.32	1.28	0.42	6.42	8.45	3.04	2.93	0.45
R. flavefaciens ($\times 10^{10}$)	2.25	2.33	0.01	0.06	2.27^{b}	1.71^{b}	2.89^{a}	0.34	0.12
R. albus ($\times 10^{10}$)	2.05^{b}	4.04^{a}	0.64	0.25	2.15^{b}	3.16^{ab}	3.82^{a}	0.57	0.17
B. fibrisolvens ($\times 10^{7}$)	8.37	7.45	1.76	0.88	6.44	7.20	10.1	2.16	0.79
P. ruminicola ($\times 10^{8}$)	1.79^{b}	3.78^{a}	1.15	0.44	3.48	2.25	2.63	1.31	0.79
A. lipolytica ($\times 10^{5}$)	4.83	4.83	1.92	0.85	3.70	4.76	6.03	1.73	0.64
M. elsdenii ($\times 10^{5}$)	1.35	1.57	0.24	0.91	1.51	2.00	0.87	0.59	0.37
B. proteoclasticus ($\times 10^{9}$)	1.39^{b}	2.14^{a}	0.46	0.66	1.89	1.53	1.86	0.34	0.71

^{ab} Means in the same row with different superscript differ (P<0.05)

NH_3-N = ammonia nitrogen,

BUN = blood urea nitrogen,

REFERENCE

Alvarez, G., J.M. Pinos-Rodriguez, J.G. Herrera, J.C. Garcia, S.S. Gonzalez, and R. Barcena. 2009. Effects of exogenous fibrolytic enzymes on ruminal digestibility in steers fed high fiber rations. Livest. Sci. 121: 150–154.

AOAC. 1997. Official Method of Analysis. 16th Ed., Association of Official Agriculture Chemicals, Washington, DC.: USA.

Arriola, K.G., S.C. Kim, C.R. Staples, and A.T. Adesogan. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J. Dairy Sci. 94:832–841.

Bremner, J.M., and D.R. Keeney. 1965. Steam distillation methods of determination of ammonium, nitrate and nitrite. Anal. Chem. Acta. 32:218.

Crocker, C.L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33: 361-365.

Dean, D.B., A.T. Adesogan, N. Krueger, and R.C. Littell. 2005. Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. J. Dairy Sci. 88: 994–1003.

Elwakeel, E.A., E.C. Titgemeyer, B.J. Johnson, C.K. Armendariz, and J.E. Shirley. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci. 90: 5226–5236.

Giraldo, L.A., M.L. Tejido, M.J. Ranilla, and M.D. Carro. 2007. Effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters. *J. Anim. Sci*. 85: 1962-1970.

Gregorini, P., S.A. Gunter, C.A. Masino, and P.A. Beck. 2007. Effect of ruminal fill on short-term intake rate and grazing dynamics of beef heifers. Grass Forage Sci. 62: 346-354.

Kennedy, P.M., A.N. Boniface, Z.J. Liang, D. Muller, and R.M. Murray. 1992a. Intake and digestion in swamp buffaloes and cattle. 2. The comparative response to urea supplements in animals fed tropical grasses. J. Agric. Sci. 119:243–254.

Khejornsart, P., M. Wanapat, and P. Rowlinson. 2011. Diversity of anaerobic fungi and rumen fermentation characteristic in swamp buffalo and beef cattle fed on different diets. Livest. Sci. 139:230–236.

Knowlton, K.F., M.S. Taylor, S.R. Hill, C. Cobb, and K.F. Wilson. 2007. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. J. Dairy Sci. 90: 4356–4360.

Llewellyn, D.A., T.T. Marston, K.L. Teutemacher, J.J. Higgins, T. Melgarejo. 2010. Evaluation of low molecular weight fractions and crude enzyme preparation from a Trichoderma cellulase complex as a treatment for fibrous feeds. Anim. Feed Sci. Technol. 160: 39–48.

Mendoza, G.D., O. Loera-Corral, F.X. Plata-Pérez, Pe.A. Hernández-García, and M. Ramírez-Mella. 2014. Considerations on the use of exogenous fibrolytic enzymes to improve forage utilization. Sci. World J. ID 247437.

Nicol, G.W., S. Leininger, C. Schleper, and J.I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ. Microbiol. 10: 2966-2978.

Nsereko, V.L., K.A. Beauchemin, D.P. Morgavi, L.M. Rode, A.F. Furtado, T.A. McAllister, A.D. Iwaasa, W.Z. Yang, and Y. Wang. 2002. Effect of a fibrolytic enzyme preparation from Trichoderma longibrachiatum on the rumen microbial population of dairy cows. Canadian J. Microbiol. 48: 14-20.

Safari, J.G., D.E. Mushi, L.A. Mtenga, G.C. Kifaro, and L.O. Eik. 2011. Growth, carcass yield and meat quality attributes of Red Maasai sheep fed wheat straw-based diets. Trop. Anim. Health Prod. 43: 89–97.

Samuel, M., S. Sagathewan, J. Thomas, and G. Mathen. 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian J. Anim. Sci. 67: 805-807.

SAS. 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc., Cary, NC: USA.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co., New York: USA.

Sutton, J.D., R.H. Phipps, D.E. Beever, D.J. Humphries, G.F. Hartnell, J.L. Vicini, and D.L. Hard. 2003. Effect of method of application of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows. J. Dairy Sci. 86: 546–556.

Suzuki, M.T., L.T. Taylor, and E.F. DeLong. 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 50-nuclease assays. Appl. Environ. Microb. 66: 4605-4614.

Takai, K., and K. Horikoshi. 2000 Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. App. Environ. Microbiol. 66:5066-5072.

Togtokhbayar, N., M.A. Cerrillo, G.B. Rodríguez, M.M.M.Y. Elghandour, A.Z.M. Salem, C. Urankhaich, S. Jigjidpurev,

N.E. Odongo, and A.E. Kholif. 2015. Effect of exogenous xylanase on rumen *in vitro* gas production and degradability of wheat straw. Anim. Sci. J. 86: 765-771.

Wanapat, M., S. Chumpawadee, and P. Paengkoum. 2000. Utilization of urea-treated rice straw and whole sugar cane crop as roughage sources for dairy cattle during the dry season. Asian-Aust. J. Anim. Sci. 13:474-477.

Wanapat, M., S. Polyorach, K. Boonnop, C. Mapato, and A. Cherdthong. 2009. Effect of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. Livest. Sci. 125: 238–243.

Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Bio. Technique. 36:808-812.

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Effects of Dried Cassava Pulp from Bio-Ethanol Industry Supplementation on Rumen Fermentation, Rumen Cellulolytic and Xylanolytic Bacteria in Dairy Cows

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ABSTRACT

The purpose of this study was to investigate the effects of addition of dried cassava pulp from bio-ethanol industry (DCPE) in dairy cows on rumen fermentation, rumen cellulolytic and xylanolytic bacteria in dairy cows. Four crossbred Holstein Friesian cows with initial weight 407 kg and days in milk 90 days were randomly allotted according to a 4 × 4 Latin square design (21 day/period). Four dietary treatments were total mix ratio (TMR) without DCPE (control) and with DCPE addition at 10, 20 and 30 % dietary DM. The ration was formulated to contain 12 percent crude protein and 2.2Mcal ME/kg.DM. Rice straw was used as the roughage source. The TMR consisted of roughage and concentrates at ratio 40:60. The results showed that total volatile fatty acid (TVFA), acetic acid (C2), propionic acid (C3), butyric acid (C4) and C2:C3 ratio were not different among dietary treatments (P>0.05). Moreover, Enumeration of therumen cellulolytic bacteria (DNA copy number) such as *Fibrobactor succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* were not different among dietary treatments (P>0.05). However, DNA copy number of xylanolytic bacteria such as *Prevotella ruminicola* tend to be cubic increased (P=0.07) when the level of DCPE in diets was increased. Moreover, increased the level of DCPE in diets, *Butyrivibrio fibrisolvens* tend to be quadratic decreased (P=0.09).

INTRODUCTION

In the tropic and sub-tropic area, feed resources and crop-residues are enormously available locally for use to increase livestock production (Wanapat, 1999). Currently, ethanol from biomass of agriculture products have become increasingly popular as alternative sources of gasoline. The most prospective material for bio-ethanol production was cassava (*Manihot esculenta*Crantz). Cassava was a major biomass resource in Thailand, cassava pulp produced in large amounts as by-product of ethanol production manufacturing. Approximately 350-370 tonnes of cassava chips are converted to 150,000 litters ethanol and 1,400-1,600 tones distillery slop (5-7% total solid). The distillery slop was dried and converted to a waste to dry cassava pulp from bio-ethanol industry (DCPE). It has been shown that DCPE contained 92.7% dry matter (DM), 80.4% organic matter (OM), 19.5% ash, 7.49% crude protein (CP), 5.46% ether extract (EE), 41.5% nitrogen free extract (NFE), 57.0% neutral detergent fiber (NDF), 49.7% acid detergent fiber (ADF) and 13.9% acid detergent lignin (ADL) (Jintawanit et al., 2008 Laorodphan et al., 2013). DCPE contains a high amount of neutral detergent fiber (NDF) and acid detergent fiber (ADF) content. NDF is less digestible than non-structural carbohydrates (NSC), therefore, the concentration of NDF in feeds or diets is negatively correlated with energy concentration. In the rumen, the prominent bacteria are cellulolytic bacteria (Hungate, 1960). The cellulolytic bacteria includes *Ruminococcus albus, Ruminococcus flavefaciens* and *Fibrobacter succinogenes*. Moreover, xylanolytic bacteria includes *Butyrivibrio fibrisolvens* and *Prevotella ruminicola* (Wanapat, 1990 Wachirapakorn, 1998). Increasing the feed efficiency by with enhance the cellulolytic and xylanolytic activity meaning to increase energy available of DCPE and lead to decrease feed cost. The objective of the present experiment was to investigate the effects of addition of DCPE in diet on rumen fermentation and rumen cellulolytic microorganism in dairy cows.

MATERIALS AND METHODS

Four multiparous Holstein Friesian crossbred cows, 77 day in milk and 407 kg body weight, were randomly assigned to receive dietary treatments in a 4 × 4 Latin square design with four 21-d periods each comprising 14 d for dietary adaptation and 7 d for data collection. Dietary treatments were based on total mixed ration (TMR) with DCPE supplementation at 0, 10 20 and 30%, respectively. Oil (palm oil) was used as energy source to make isocaloric dietary treatments when DCPE was added. TMR were offered *ad libitum*, at approximately 07.00 and 17.00 h. Water and mineral block were available at all times. At the last day of each period, the rumen fluid

was collected by used stomach tube at 0, 2 and 4 h-post feeding and filtered through four layers of cheesecloth. The first portion of rumen fluid was added 10 ml of 1M H_2SO_4 solution to 100 ml of rumen fluid to stop rumen bacterial fermentation. The mixture was centrifuged at 16,000 x *g* for 15 minutes and supernatant was stored at -20℃ prior to VFA analyzed with High Performance Liquid Chromatography (HPLC) according to Mathew *et al.*(*1997*). The second portion of 2 h-post feeding rumen fluid sample was collected by used stomach tube and frozen immediately on ice to stop fermentation process of microbe activity. Rumen fluid samples were stored at -20 ℃ until bacterial analysis (Gudla et al., 2012). The samples was extracted from 500 µl of rumen fluid content by the RBB+C method (Yu and Morrison, 2004) and used High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20℃ for subsequent studies. The concentration of the genomic DNA was determined with a Nanodrop spectrophotometer. The targeted rumen cellulolytic bacteria including *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes* and xylanolytic bacteria including *Butyrivibrio fibrisolvens, Prevotella ruminicola* were determined by real-time PCR analysis and used fluorescence detection of SYBR green mix described by Potu et al. (2011). New designed 16S rRNA gene primers and annealing-temperature optimization for cellulolytic bacteria primer sets were performed use pure-culture genomic DNA as the template are shown in Table 1. The optimized annealing temperature was selected base on the presence of one PCR product by qPCR. The specificity of cellulolytic primers and xylanolytic primers were confirmed by use the BLAST program in the Gene-Bank Database of National Center for Biotechnology Information(NCBI).

Statistical analysis

All data were statistically analyzed as a 4 × 4 Latin square design using the PROC MIXED (SAS 1996). Significant differences between treatments were determined using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie 1980).

RESULT AND DISCUSSION

TMR used in this experiment contained similar crude protein (12%) and 2.2 Mcal ME/kg.DM in all dietary treatments. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were slightly increased as DCPE was increased in TMR. Ether extract of TMR was increased as DCPE increased. Concentration of EE in TMR with inclusion of DCPE at 0, 10, 20 and 30% were 2.5, 4.5, 6.0 and 7.2%, respectively. The primary end products of fiber fermentation are volatile fatty acids (VFAs), which a major metabolic energy for the ruminant, (Krause et al., 2003). Inclusion of DCPE at any level did not affect means of total volatile fatty acid (TVFA), acetic acid (C2), propionic acid (C3), butyric acid (C4) and C2:C3 ratio (Table 2). DNA copy number of therumen cellulolytic bacteria such as *F. succinogenes*, *R. flavefaciens* and *R. albus* were not different among dietary treatments (P>0.05). However, DNA copy number of xylanolytic bacteria such as *P. ruminicola* and *B. fibrisolvens* tend to slightly difference when the level of DCPE in diets was increased (Table 3). *P. ruminicola* in cows fed 20% DCPE in TMR tend to be cubic increased (P=0.07). Wallace et al. (1997) reported *P.ruminicola* was among the most numerous microbes cultivable from the rumen of ruminant animals, where they help the breakdown of protein and carbohydrate (Dodd et al., 2009). Therefore, population of *P. ruminicola* in the rumen increased when the substrate such as NDF and ADF increased in the diet. However, increased levels of DCPE in TMR trend to decrease *B. fibrisolvens* population. *B.fibrisolvens* are involved in degradation of plant structural carbohydrates, protein breakdown and biohydrogenation of lipids (Shingfield and Wallace, 2014). However, high noble of lipids in ruminant diets has an adversely affect to rumen fermentation and microbial population. Consequently, high level of palm oil in the diet (5.1%) resulted in decrease *B.fibrisolvens* population in the rumen. Palmquist (1988) suggested that high intake dietary lipids affect rumen microorganism population and reduce rumen fermentation efficiency, fiber digestibility. Moreover, lipids have higher proportions of unsaturated fatty acids (USFA) are adversary effects to the permeability of microbial membrane and inhibit activity of bacteria, protozoa, fungi and manipulate rumen fermentation (Van Soest, 1994 Nagaraja et al., 1997).

CONCLUSION

From this experiment, it was concluded that inclusion of DCPE in TMR did not alter rumen fermentation and cellulolytic bacteria. However, high fat in diet may interfere rumen xylanolytic bacteria population.

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KEYWORD : Dried Cassava Pulp from Bio-Ethanol Industry (DCPE), Rumen, Bacteria, Dairy cows

Table 1. qPCR primer designed for rumen cellulolytic and xylanolytic bacteria

Target bacterial	Gram	Gene	Primers sequences (5'–3')	Annealing temp. (°C)	Product size (bp)
Ruminococcus albus	+	16S rRNA	F- GCTTACTGGGCTTTAACTGA R- CCCACACCTAGTAATCATCG	55	103
Ruminococcus flavefaciens	+	16S rRNA	F- GTAGCCGGACTGAGAGGTTG R- ATCGCTGCATCAGGGTTTC	56.9	113
Fibrobacter succinogenes	-	16S rRNA	F- CAACCCACGTTTCCAGTT R- TGTGTAGCCCAGGATGTAA	55	119
Butyrivibrio fibrisolvens	-	16S rRNA	F- TGTTGGCTTCCATAGGGAGT R- CCCGTCAATTCCTTTGAGTT	55.2	97
Prevotella ruminicola	-	16S rRNA	F- GGAAGTCTGAACCAGCCAAG R- TACCTACAAACGGGGACACG	53.7	103

Table 2. Effects of levels of dried cassava pulp from bio-ethanol industry (DCPE) on rumen fermentation in dairy cows

Item	h.	Ethanol waste (%)				SEM	p-value	Contrast		
		0	10	20	30			L	Q	C
Total VFA mmol/l	0	39.7	40.5	38.7	39.4	2.06	0.55	0.61	0.35	0.59
	2	45.5	54.9	36.4	47.9	4.74	0.28	0.6	0.94	0.1
	4	51.5	47.7	44.2	50.1	7.73	0.88	0.66	0.63	0.74
	average	45.6	43.4	39.3	44.6	4.51	0.77	0.73	0.43	0.59
Acetic acid	0	77.2	68.3	75.2	78.3	3.20	0.62	0.93	0.34	0.38
(C2) m/100m	2	68.9	63.6	68.9	67.0	3.10	0.49	0.79	0.35	0.17
	4	68.7	65.9	67.5	48.9	1.62	0.35	0.29	0.54	0.14
	average	71.4	66.4	71.1	63.3	2.67	0.19	0.15	0.6	0.11
Propionic Acid	0	17.0	21.6	19.4	17.2	1.85	0.77	0.58	0.54	0.65
(C3) m/100m	2	21.4	24.4	20.9	24.5	1.78	0.37	0.67	0.74	0.14
	4	20.2	23.4	20.6	17.6	1.52	0.55	0.6	0.66	0.31
	average	19.7	23.1	20.2	20.6	1.71	0.54	0.97	0.41	0.25
Butyric acid	0	5.75	10.07	5.43	4.45	1.36	0.36	0.57	0.19	0.19
(C4) m/100m	2	9.73	12.03	10.21	8.57	1.64	0.44	0.37	0.18	0.31
	4	11.1	10.8	11.8	11.3	0.80	0.22	0.23	0.09	0.21
	average	8.92	10.48	8.67	8.24	1.50	0.66	0.47	0.45	0.54
C2:C3 ratio	0	4.95	3.40	3.90	4.61	0.71	0.79	0.55	0.59	0.71
	2	3.32	2.76	3.33	2.82	0.31	0.36	0.76	0.51	0.12
	4	3.43	2.95	3.29	2.80	0.24	0.49	0.34	0.9	0.29
	average	3.81	2.99	3.56	3.35	0.32	0.39	0.59	0.37	0.17

L = linear, Q = quadratic, C = cubic

Table 3. Effects of levels of dried cassava pulp from bio-ethanol industry (DCPE) on rumen cellulolytic and xylanolytic microorganism in dairy cows (DNA copy number)

Item	Ethanol waste (%)				SEM	p-value	Contrast		
	0	10	20	30			L	Q	C
Ruminococcus albus (10^8)	5.66	4.39	5.88	5.14	0.64	0.11	0.97	0.56	0.02
Ruminococcus flavefaciens (10^8)	4.33	3.73	4.64	3.59	0.53	0.18	0.44	0.54	0.04
Fibrobactor succinogenes (10^7)	4.04	3.30	4.58	2.81	0.91	0.24	0.41	0.43	0.09
Butyrivibrio fibrisolvens (10^6)	1.40^{a}	0.62^{b}	1.00^{ab}	1.05^{ab}	0.29	0.09	0.48	0.05	0.11
Prevotella ruminicola (10^7)	1.01^{ab}	0.36^{b}	1.25^{a}	1.02^{ab}	0.34	0.07	0.39	0.38	0.01

L = linear, Q = quadratic, C = cubic

REFERENCES

Dodd, D., S.A. Kocherginskaya, M.A. Spies, K.E. Beery, C.A. Abbas, R.I. Mackie, and I.K.O. Cann. 2009. Biochemical analysis of a β -D-xylosidase and a bifunctional xylanase-ferulic acid esterase from a xylanolytic gene cluster in *Prevotella ruminicola* 23. J. Bacteriol. 191 (10): 3328-3338.

Gudla, P., A.A. AbuGhazaleh, A. Ishlak, and K. Jones. 2012. The effect of level of forage and oil supplement on biohydrogenation intermediates and bacteria in continuous cultures. Anim Feed Sci Technol. 171 (2-4): 108-116

Hungate, R.E. 1966. The rumen and its microbes. New York academic press, New York, NY, USA.

Jintawanit, W., S. Jatuporrapong, and A. Kanto. 2008. Study composition cassava pulp from ethanol manufacturing use for animal feed and crops fertilizer. Suwanvajokkasikit Animal R&D Institutute. Kasetsart University. Kamphaeng Saen Campus, Nakhon Pathom. Thailand. (In Thai)

Krause, D.O., S.E. Denman, R.I. Mackie, M. Morrison, A.L. Rae, G.T. Attwood, and C.S. McSweeney. 2003. Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. Microbiol. Rev. 27: 663-693.

Laorodphan, N., C. Mikled, N. Chongkasikit, and C. Phatsara. 2013. Effects of the level of dried cassava pulp from ethanol process in the ration on rumen fermentation and digestibility in fistulated Thai native bull. J. Agric. 29: 89-98.

Mathew, S., S. Sagathevan, J. Thomas, and G. Mathen. 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian. J. Anim. Sci. 67: 805-807.

Nagaraja, T.G., C.J. Newbold, C.J. Van Nevel, and D.I. Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523-632 in The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Chapman and Hall, London, UK.

Palmquist, D.L. 1988. The feeding value of fats. In Feed science (ed. ER Orskov), pp. 293–311. Elsevier Science Publisher, Amsterdam, Netherlands.

Potu, R.B., A.A. AbuGhazaleh, D. Hastings, K. Jones, and S.A. Ibrahim. 2011. The effect of lipid supplements on ruminal bacteria in continuous culture fermenters varies with the fatty acid composition. J. Microbiol. 49: 216–223.

SAS. 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc., Cary, NC: USA.

Shingfield, K.J., and R.J. Wallace. 2014. CHAPTER 1: Synthesis of Conjugated Linoleic Acid in Ruminants and Humans. *Conjugated Linoleic Acids and Conjugated Vegetable Oils*. pp. 1-65.

Steel, R.G.D, and J.H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co., New York: USA.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd Edition. Cornell University. Press, USA.

Wachirapakorn, C. 1998. An Introduction to Ruminant Nutrition and Feeding. Department of Animal Science, Faculty of Agriculture, Khon Kaen University. (In Thai)

Wallace, R.J., N. Mckain, G.A. Broderick, L.M. Rode, N.D. Walker, C.J. Newbold, and J. Kopecny. 1997. Peptidases of the rumen bacterium, *Prevotella ruminicola*. Anaerobe. 3 (1): 35-42.

Wanapat, M. 1990. Ruminant Nutrition. Funny Publishing: Bangkok. (In Thai)

Wanapat, M. 1999. Feeding of ruminants in the tropics based on local feed resources. Khon Kaen Publishing Company Ltd., Khon Kaen, Thailand.

Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques. 36, 808-812.

O-25-6 Effect of Cassava Ethanol Byproducts Fermented with Yeast on Rumen Fermentation and Feed digestion

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The cassava ethanol byproducts was one main products that need to be make as value added because it was remaining valuable nutrients. The objective of this study was to improve protein content by fermented with yeast. The experiment was separated into 2 experiments.

The experiment1, the effect of carbohydrate source and level of urea on protein content in fermentation cassava ethanol byproducts with yeast (Y), The design was 3x2x3 factorial in completely randomized design (CRD), 18 treatments were allocated by three factors, the first factor was the three ratios of cassava ethanol waste(EW) by products to cassava pulp (CPL)(EW100:CPL0, EW60:CPL40 and EW40:CPL60, respectively.) the second factor was the levels of urea (U) (U 1% and U 2%) and the third factor was the 3 levels of sugar (S) (S3, S6 and S9%, respectively) as T1 = EW + U 1% + S 3% , T2 = EW + U 1 % + S 6% , T3 = EW + U 1% + S 9% ,T4 = EW + U 2 % + S 3 %, T5 = EW +U 2 % + S 6 % ,T6 = EW+ U 2 % + S 9%, T7 = EW60 : CPL 40 + U 1% + S 3 %, T8 = EW60 : CPL 40 + U 1% + S 6%, T9 = EW60 : CPL 40 + U 1% + S 9 %, T10 = EW60 : CPL 40 + U 2% + S 3 % , T11 = EW60 : CPL 40 + U 2% + S 6%, T12 = T10 = EW60 : CPL 40 + U 2% + S 9 %, T13 = EW40 : CPL 60 + U 1% + S 3 %, T14 = EW40 : CPL 60 + U 1% + S 6 %, T15 = EW40 : CPL 60 + U 1% + S 9 %, T16 = กากEW40 : CPL 60 + U 2% + S 3 %, T17 = EW40 : CPL 60 + U 2% + S 6 %, T18 = EW40 : CPL 60 + U 2% + S 9 %. All means output were analyzed using Analysis of variance and Duncan's multiple range test by SAS, 1988.

Level of Ethanol Waste Protein

Normal analyzed of ethanol waste showed the nutrient valued as 25.1%DM, 9.3 %CP, 1.2 %fat, 51.3 %NDF, 37.8 % ADF and 8.8 % ash. When EW fermented with yeast within 15 day, the protein valued was increased highest 25.4% in T5 (EW +U 2 % + S 6 %) (Table 1). Kitsada (2009) did mother culture by using *S. cerevisiae* 20g , molasses 24%, urea 48% and incubation for 48 hr with oxygen ,then used mother culture improved cassava from 3.2 % crude protein to 30.4%. EW to CPL ratio was 100:0, 60:40 and 40:60. The highest protein valued was found on 100:0 levels (Table 2). The protein content was increased by yeast fermented the rest of sugar and starch available in EW. Thongkratok et al. (2010) studied fermented cassava with yeast and urea as 0, 0.25, 0.5, 0.75, 1.0 and 1.25% for 7 day. The data showed at 1.25 urea level, protein was increased up to 21.2%. Consequently, EW can be improved by yeast and the best treatment result will be use in next experiment.

The experiment 2, the effect of fermented cassava ethanol byproducts with yeast on feed and fiber digestion of TMR diets in *in vitro*. Five treatments were allocated into completely randomized design with an amount of fermented cassava ethanol byproducts (FEW) at the levels 0, 12.5, 25, 37.5, and 50% of DM in TMR diets. The FEW was prepared as experiment 1 of T5 (22.9 %DM, 1.2% fat, 25.4% CP, 9.2% ash, 45.5% NDF, 29.0% ADF). All diets had the same level of nutrients as 14.5 % CP, 67.5%TDN (table 3). The ration combinations were treatment1: FEW 0%, treatment2: FEW 12.5%, treatment3: FEW 25%, treatment4: FEW37.5%, treatment5: FEW 50%. Mean differences were analysis using proc GLM and Duncan's multiple range test by SAS, 1988 program.

The result showed that increasing levels of fermented cassava ethanol byproducts had a quadratic response effect on feed digestibility (57.4, 58.9, 61.5, 62.5 and 56.7% of DM, respectively) ($P<0.01$), but it was not effect on NDF and ADF digestibility ($P>0.05$).

Feed Digestion: DM (IVDMD), NDF (IVNDFD), ADF (IVADFD)

The feed dry matter digestion had a quadratic significance, $P<0.01$) at FEW 25 and 37.5 % ration. Increasing rate of FEW inclusion up 50 % had decreased DM digestion (57.4, 58.9, 61.5, 62.5 and 56.7%, respectively, $p<0.01$) (table 3). Using FEW at 50% had high proportion of NDF and ADF compared other level. High NDF and ADF might affect fermentation rate. NRC (1988) reported high proportion of NDF and ADF related to lignin content in ration. FEW at 25 and 37.5 % had higher DM digestion because of fermentation process made fiber more degradable to microbial organisms and had optimum moisture content than others. Pattama et al. (2008) used fermented

cassava pulp in ration, they found a quadratics response of feed digestibility at level 0, 20, 40 and 54 % (70.8, 76.7, 81.0 and 86.8 %, respectively). All level FEW had not significant difference to NDF and ADF digestibility. Also Khampa et al. (2009b) studied it did not significant in NDF , ADF . Data indicated that at 25 and 37.5 % would be improve DM digestion without any affects to NDF, ADF.

CONCLUSION

This data indicated that fermented cassava ethanol byproducts fermentation with yeast had improved in nutrient digestibility and can be applied for protein source in dairy diets at level of 37.5% of DM in TMR diet.

KEYWORD : Protein feed, Feed improvement, Ethanol waste, high moisture feed

Table 1 Fermented ethanol waste with cassava pulp, yeast, urea and sugar for value added of EW. (% DM)

EW:Pulp[1]	100 : 0						60 : 40						40 : 60						
urea	1.0%			2.0%			1.0%			2.0%			1.0%			2.0%			
sugar	3.0%	6.0%	9.0%	3.0%	6.0%	9.0%	3.0%	6.0%	9.0%	3.0%	6.0%	9.0%	3.0%	6.0%	9.0%	3.%	6.0%	9.0%	S
day/treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	
0	14.2^{g}	14.0^{g}	13.2^{gh}	20.7^{ab}	20.1^{bcd}	19.0^{de}	14.2^{g}	14.2^{g}	11.8^{ij}	21.4^{a}	20.5^{abc}	19.1^{d}	13.3^{gh}	12.8^{hi}	11.1^{ij}	19.5^{cd}	17.8^{f}	18.0^{ef}	0
5	14.8^{g}	15.6^{fg}	15.9^{f}	17.7^{e}	21.8^{b}	23.8^{a}	11.0^{i}	13.4^{h}	14.5^{g}	21.8^{b}	22.9^{a}	20.7^{c}	9.4^{j}	13.3^{h}	13.3^{h}	19.7^{cd}	20.6^{c}	19.4^{d}	0
10	14.9^{f}	16.4^{e}	16.6^{e}	23.8^{ab}	23.2^{b}	24.4^{a}	15.4^{f}	15.8^{ef}	14.9^{f}	22.0^{c}	21.8^{c}	22.2^{c}	11.6^{h}	12.6^{g}	11.5^{h}	19.1^{d}	20.0^{d}	20.0^{d}	0
15	16.3^{g}	17.1^{g}	17.1^{g}	24.3^{b}	25.4^{a}	24.2^{b}	15.1^{h}	14.5^{hi}	14.7^{hi}	22.6^{cd}	23.4^{bc}	21.2^{ef}	13.9^{ij}	13.9^{ij}	13.2^{j}	21.7^{de}	21.0^{ef}	20.6^{f}	0

^{a,b,c,d,e,f,g,h,i,j} Means within a row without a common superscript letter differ (P<0.05)
[1]CEB:Pulp = cassava ethanol byproduct: cassava pulp

Table 2 Fermented ethanol waste with cassava pulp, yeast, urea and sugar for value added of EW. (by factors, % DM)

Day	CEB:Pulp(C)			Urea (U)		Sugar (S)			SEM	P-value						
	100:0	60:40	40:60	1.0	2.0	3.0	6.0	9.0		C[1]	U[2]	S[3]	C*U	C*S	U*S	C*U*S
0	16.8^{a}	16.8^{a}	15.4^{b}	13.2^{b}	19.5^{a}	17.2^{a}	16.5^{b}	15.3^{c}	0.14	<0.01	<0.01	<0.01	0.06	0.09	0.07	0.34
5	18.2^{a}	17.4^{b}	15.9^{c}	13.4^{b}	20.9^{a}	15.7^{b}	17.9^{a}	17.9^{a}	0.14	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
10	19.8^{a}	18.6^{b}	15.7^{c}	14.4^{b}	21.8^{a}	17.8^{b}	18.2^{a}	18.2^{a}	0.13	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.14
15	20.7^{a}	18.5^{b}	17.3^{c}	15.0^{b}	22.7^{a}	18.9^{a}	19.2^{a}	18.4^{b}	0.14	<0.01	<0.01	<0.01	0.63	0.04	0.03	0.11

^{a,b,c} Means within a row without a common superscript letter differ (P<0.05)
[1]CEB:Pulp = cassava ethanol byproduct: cassava pulp, [2]U = urea, [3]S = sugar

Table 3 Feed ration and feed nutrient composition (%DM)

Ingredient, %DM	level of fermented cassava ethanol byproduct				
	0	12.5	25.0	37.5	50.0
Rice straw	26.0	26.0	26.0	26.0	26.0
Corn	2.0	0	3.0	8.5	13.0
Cassava chip	10.5	10.5	10.5	10.5	10.5
Cassava pulp	35.0	30.8	22.6	12.3	0
Soybean meal	25.4	19.2	11.9	4.2	0
FEW[1]	0	12.5	25	37.5	50
Urea	0.6	0.5	0.5	0.5	0
Mineral premix	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Analyzed composition (%)					
DM	91.9	92.7	93.1	93.4	93.7
CP	14.5	14.5	14.5	14.5	14.5
TDN	69.5	67.8	66.7	66.0	66.0
NDF	36.1	38.6	40.3	41.0	42.5
ADF	23.4	24.5	26.0	27.2	28.0
EE	1.1	1.1	1.2	1.2	1.2

[1]FEW = fermented cassava ethanol waste

Table 4 Level of FEW in ration on feed digestion in *in vitro* (%)

	level of FCEB[1]						Orthogonal polynomial			
Item	0%	12.5%	25%	37.5%	50%	SEM	L	Quad	C	Quart
IVDMD[2]	57.4[c]	58.9[bc]	61.5[ab]	62.5[a]	56.7[c]	0.36	<0.01	<0.01	<0.01	<0.01
IVNDFD[3]	37.2	36.8	44.1	37.5	40.2	1.38	0.52	0.53	0.87	0.14
IVADFD[4]	24.3	24.9	23.3	23.6	20.0	1.07	0.25	0.50	0.84	0.65

[a,b,c] Means within a row without a common superscript letter differ (P<0.05)
[1]FCEB = fermented cassava ethanol byproduct
[2]IVDMD = *in vitro* DM digestion at 48 hr.
[3]IVNDFD= *in vitro* NDF digestion at 48 hr.
[4]IVADFD = *in vitro* ADF digestion at 48 hr.

REFERENCES

Pattama Viyaboonya.2008). Effect of fermented cassava pulp on intake fertility of dairy heifer. MS-Thesis of Animal Science Major. Graduate School . Khon Kaen University.

Batajoo, K. K., and R. D. Shaver. 1994. Impact of nonfiber carbohydrate on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 77:1580-1588.

Beauchemin, K. A., B. I. Farr, L. M. Rode, and L. G. B. Schaalje. 1994. Optimum neutral detergent fiber concentration of barley-based diets for lactating dairy cows. J. Dairy Sci. 77:1013-1029.

Besong, S., J. A. Jackson, C. L. Hicks, and R. W. Hemken. 1996. Effect of a supplement liquid yeast product on feed intake, ruminal profiles, and yield, composition, and organoleptic characteristics of milk from lactating Holstein cows. J. Dairy Sci. 79:1654-1658.

Cherney, D. J., J. H. Cherney, and L. E. Chase. 2003. Influence of dietary nonfiber carbohydrate concentration and supplementation of sucrose on lactation performance of cows fed fescue silage. J. Dairy Sci. 86:3983-3991.

Felton, C. A., and T.J. Devries. 2010. Effect of water addition to a total mixed ratio on feed temperature, feed intake, sorting behavior, and milk production of dairy cows. J. Dairy Sci. 93:2651–2660.

Haddad, S. A., and S. N. Goussous. 2005. Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lamp. Anim. Feed Sci. Technol. 118:343-348.

Halasz, A., and R. Lasztity. 1991. Use of yeast biomass in food production. CPC Press, Inc. pp.11-12, 23-41, 45-105.

Khampa, S., S. Ittharat, and U. Koatdoke. 2011. Enrichment value of yeast-malate fermented cassava pulp and cassava hay as protein source replace soybean meal in concentrate on rumen ecology in crossbred native cattle. Pak. J. Nurt., 10:1126-1131.

Khampa, S., P. Chaowarat, R. Singhalert, and M. Wanapat. 2009a. Supplementation of yeast fermented cassava chip (YFCC) as a replacement concentrate and ruzi grass on rumen ecology in native cattle. Pak. J. Nurt., 8:597-600.

Khampa, S., P. Chaowarat, R. Singhalert, R. Pilajun, and M. Wanapat. 2009b. Supplementation of yeast fermented cassava chip as a replacement concentrate on rumen fermentation efficiency and digestibility of nutrient in heifer. J. Anim. Vet. Adv., 8:1091-1095.

National Research Council (NRC). 2001. Nutrient Requirement of Dairy cattle. 7th rev. ed. National Academy Press, Washington, DC.

Oboh, G., and A. A. Akindahunsi. 2003. Biochemical changes in cassava products (flour & gari) subjected to *Saccharomyces cerevisae* solid media fermentation. Food Chem. 82:599–602.

Pattarajinda, V. 2001. Formulating rations according to a ratio of metabolizable protein to net energy for lactating daily cow. Ph. D. dissertation. The University of Georgia Athens, Georgia.

Sommart, K., M. Wanapt, D. S. Parker, and P. Rolinson. 1996. Cassava chip as an energy source lactating dairy cows fed rice straw. The 8th AAAP animal science congress, Processdings. Vol.2. Tokyo.

Sriroth, K., R. Chollakup, S. Chotineeranat, K. Piyachomkwan, and C. G. Oates. 2000. Processing cassava waste for improve biomass utilization. Bioresource Technology. 71:63-69.

Walker, G. M. 1998. Yeast Physiology and Biotechnology. England, John Wiley and Sons Ltd. pp.12-219.

O-25-7

Adopting local best practice in reformulating dairy cow silage-based ration to improve cow fs performances

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INTRODUCTION

Smallholders dairy farming in humid tropic and developing countries commonly undertake with quite rudimentary facilities (Andrews and Davison 2002), low-input farming systems (Andrieu et al. 2007), lack of skills (Despal and Afnan, 2005) and limited access to information. It was established as part of social welfare and rural development schemes, to provide a regular cash flow for poor farmers (Moran, 2005). Now, it is developing toward rural industry and therefore requires a business approach to manage farm such as improving feeding management.

Based on National Dairy Survey, DGLS (2012) reported that in average, Indonesian dairy farmer kept 6.07 AU cattle, occupied 0.44 ha land which could only provide 62.7% of forage required to produce an average 13.5 l/head daily milk. The farmer did not have capacity to plan sustainable forage availability through conservation (silage, hay, fermentation). To fulfill cattle's requirements on forage, the farmer used natural grass, rice straw, corn stover, banana leaves or vegetable plant byproducts (Despal et al., 2014) depending on their daily availability. Such forage inbound logistic system influenced forage availability and quality greatly. During drought season, forage availability become scarce due to slower grow of cultivated and natural grass as a result of limited water available. Feed quality decreased faster due to higher sunlight intensity which reduced protein and increased fiber fractions (Narvaez et al. 2010) and influenced nutrient intake. The low nutrient intake of dry season diet resulted in an energy deficit characterized by a massive body reserves mobilization (Bartl et al. 2009) and decreasing milk production (Bernabucci et al 2015). There is a need for feeding strategy in coping with the difficult time which will determine survival of a dairy business by maintaining performance and farmer income (Hardie et al., 2014).

Beside sustainable availability of high quality feed, milking animals should be fed nutritionally balanced ration in an amount that provides nutrients to express their genetic potential (Garg et al 2013). To be able to balance the ration, information of feed qualities and nutrient requirements are needed. The effort to provide local dairy feed quality information have been done through several projects and continuously identified. Although dairy nutrient requirement fail to be estimated accurately, several best feeding practice have been identified, reconstituted and tested. The formula will be distributed to the local farmer as best practice through action research.

Silage is one of forage preservation technique which employed spontaneous fermentation activity of epiphytic lactic acid bacteria under anaerobic condition (Merry et al., 1997). The technique could provide forage with stabile quality and independent to climates or seasons (Cavallarin et al. 2005). Forage conserved as silage had better quality than hay (Regan 2000). Silage have been used as 90% of forage supply for dairy cattle in Netherland, Germany and Denmark. Although expert in tropical countries have been familiar with the technique, however, their implementation by smallholder dairy farmer still limited (Wilkins et al. 1999). Through several projects, authors introduced the technique to several dairy farmers in Indonesia. Some farmers have tried to produce and used it as daily feed.

The experiment were aimed at comparing two silage based ration (local best practice and the current farmer formulas) at two levels of milk production (low = 10 l/head/d and high = 13.5 l/head/day) on nutrient intake, milk production and component as well as body condition score.

METHODS

The experiment have been conducted in 2 traditional dairy farms (A1 = low production and A2 = high production), members of South Bandung Dairy Cattle Cooperative (KPBS), Pangalengan, West Java. The experiment was conducted during drought season 2015 to test two types of ration (B1 = control and B2 = reformulated). Ration compositions and their nutrient content are shown in Table 1.

Twelve multiparous cows with initial body weight 393 ± 17 kg, 5 - 6 months in milk, initial milk production (9.8 ± 1.3 l/d for A1 and 13.5 ± 1.1 l/d for A2) have been used in 2 x 2 block factorial design. Control rations (B1) were rations based on the current condition in each farm, while reformulated rations (B2) were rations that have

been formulated based on individual requirement of the cows. The cows were fed three or four times daily based on the farmer's feeding practices for 30 days of preliminary phase and 5 days of collecting data. The amount of feed offered and refusal were recorded and sampled for proximate analysis. Manure were scored daily during collecting data period according to Wells (2013). Milk production were measured from morning and afternoon milking and sampled for fat, protein, lactose, and SNF contents scanning using laktoscan S_L type. Body weight were estimated using Schoorl formula (Sudono 1999) and Body weight gain were calculted by substracting body weight at the end of experiment from the initial body weight. *Body Condition Score* (BCS) were evaluated according to Edmonson *et al*. (1989).

RESULTS AND DISCUSSIONS

Feed and nutrient intakes are shown in Table 2 and the cow's performances are shown in Table 3. Although A1 cows initially produced less milk, however, their feed and nutrient intake were higher than A2. Reformulated rations were consumed more than current ration. Increasing intake were more vocal for low production cows which experienced nutrients deficiencies during lactation stages which were showed by lower initial body score.
There were no significant impact of treatments on cow's performances except for manure score and BCS. Manure score in A1 improved after the experiment higher than A2 due to its lower initial score. Reformulated ration tent to increase manure score better than control ration. Improvement of manure score in all cow used were also influenced by lactation stage, the cows moved from mid to late lactation (Roche et al. 2013). Reformulated rations improved BCS and prepared the cows for the next lactation cycle (Roche et al. 2015).

CONCLUSIONS

It is concluded that reformulated ration improves feed consumption and body score which will be used to persist milk production for the next lactation cycle.

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KEYWORD : Dairy Cows, Local Feed, Milk, Reformulation, Silage

Table 1 Ration composition and nutrient contents

Ingredients/Nutrient contents	A1		A2	
	B1	B2	B1	B2
Ingredients (%)				
Cooperative concentrate	8.57	11.00	00.00	00.00
DDGS	0.00	0.00	1.90	15.00
Dried cassava waste	5.71	0.00	0.00	0.00
Brewery waste	7.14	10.00	0.00	8.00
Cabbage plant byproduct	7.71	0.00	0.00	0.00
Tofu waste	0.00	0.00	15.08	7.00
UPBS TMR waste	0.00	0.00	75.90	0.00
Pollard	10.29	9.00	0.00	0.00
Natural grass	32.00	0.00	0.00	0.00
Rice straw	0.00	0.00	7.12	0.00
Corn cob silage	28.57	70.00	00.00	70.00
Nutrient contents				
DM (%)	36.03	38.99	30.67	33.29
Ash (% DM)	10.37	11.48	8.45	10.72
Crude lipid (% DM)	2.99	3.60	3.75	3.64
Crude protein (% DM)	12.48	12.99	8.73	13.26
Crude fiber (% DM)	19.23	19.75	24.06	20.45
NFE (% DM)	54.89	52.19	55.01	51.19
TDN (% DM)	66.76	66.22	63.96	66.94

Table 2 Feed and nutrient intakes

Daily Intake (kg)	A1		A2		Significant Test		
	B1	B2	B1	B2	A	B	AB
DM	12.13 ± 0.00	15.91 ± 0.95	10.92 ± 0.26	13.79 ± 1.30	P<0.05	P<0.05	NS
Ash	1.20 ± 0.01	1.83 ± 0.10	1.10 ± 0.02	1.48 ± 0.14	P<0.05	P<0.05	NS
Crude Lipid	0.56 ± 0.01	0.57 ± 0.03	0.37 ± 0.01	0.50 ± 0.04	P<0.05	P<0.05	P<0.05
Crude Protein	1.67 ± 0.01	2.07 ± 0.12	0.83 ± 0.03	1.83 ± 0.17	P<0.05	P<0.05	P<0.05
Crude Fiber	1.79 ± 0.01	3.14 ± 0.19	2.58 ± 0.05	2.82 ± 0.26	P<0.05	P<0.05	P<0.05
NFE	6.91 ± 0.00	8.30 ± 0.49	6.00 ± 0.14	7.06 ± 0.66	P<0.05	P<0.05	NS
TDN	8.74 ± 0.00	10.62 ± 0.63	6.92 ± 0.17	9.15 ± 0.86	P<0.05	P<0.05	NS

Table 3. Cow's performances

Daily Intake	A1		A2		Significant Test		
(kg)	B1	B2	B1	B2	A	B	AB
Manure score							
before	1.67 ± 0.58	2.00 ± 0.00	2.92 ± 0.14	2.42 ± 0.52	P=0.006	NS	NS
after	3.00 ± 0.50	3.50 ± 0.50	3.13 ± 0.33	3.21 ± 0.97	NS	NS	NS
Delta	1.33 ± 0.29	1.50 ± 0.50	0.21 ± 0.26	0.79 ± 0.83	P=0.006	NS	NS
Milk production, liter							
before	10.33 ± 0.28	9.33 ± 1.89	13.70 ± 1.47	13.39 ± 1.07	P=0.006	NS	NS
after	10.38 ± 0.90	8.86 ± 0.85	13.14 ± 2.35	9.69 ± 2.21	NS	NS	NS
Delta	0.05 ± 1.01	-0.48 ± 0.77	-0.56 ± 2.34	-3.70 ± 1.89	NS	NS	NS
Fat, %	5.86 ± 0.52	5.34 ± 1.11	4.80 ± 0.28	5.18 ± 0.90	NS	NS	NS
SNF, %	7.82 ± 0.16	7.76 ± 0.49	7.76 ± 0.24	7.81 ± 0.39	NS	NS	NS
Lactose	4.33 ± 0.09	4.29 ± 0.28	4.25 ± 0.18	4.36 ± 0.20	NS	NS	NS
Protein, %	2.91 ± 0.06	2.88 ± 0.19	2.82 ± 0.07	2.89 ± 0.18	NS	NS	NS
BW							
Before	384.67 ± 33.76	379.15 ± 21.42	402.87 ± 22.27	406.90 ± 22.38	NS	NS	NS
After	411.58 ± 20.14	438.92 ± 7.27	408.46 ± 14.21	414.58 ± 24.10	NS	NS	NS
Gain	26.91 ± 29.82	59.77 ± 15.78	5.59 ± 10.04	3.18 ± 7.29	NS	NS	NS
BCS							
Before	2.67 ± 0.14	2.75 ± 0.00	2.41 ± 0.38	2.00 ± 0.00	P=0.008	NS	NS
After	2.41 ± 0.29	3.00 ± 0.25	2.33 ± 0.14	2.83 ± 0.14	NS	P=0.002	NS
Gain	-0.26 ± 0.43	0.25 ± 0.25	-0.08 ± 0.29	0.83 ± 0.14	NS	P=0.008	NS

REFERENCES

Andrews J and Davison T. 2002. Dairy Farm Layout and Design: Building and Yard Design, Warm Climates. Elsevier Ltd.

Andrieu N, Poix C, Josien E, Duru M. 2007. Simulation of forage management strategies considering farm-level land diversity: Example of dairy farms in the Auvergne. Computers and Electronics in Agriculture 55: 36–48

Bartl K, Gómez CA, Aufdermauer T, Garcia M, Kreuzer M, Hess HD, Wettstein HP. 2009. Effect of diet type on performance and metabolic traits of Peruvian local and introduced cow types kept at 200 and 3600 m of altitude. Livestock Science 122 (2009) 30–38

Bernabucci U, Basiricò L, Morera P, Dipasquale D, Vitali A, Cappelli FP, and Calamari L. 2015. Effect of summer season on milk protein fractions in Holstein cows. J. Dairy Sci. 98:1–13

Cavallarin, L., S.Antoniazzi., G. Borreani and E. Tobacco (2005). Effects of wilting and mechanical conditioning on proteolysis in sainfoin (Onobrychis viciifolia Scrop) wilted herbage and silage. *J. Sci. Food Agric*. 85: 831 – 838.

Despal & R. Afnan. 2005. Kebijakan Pengembangan Peternakan Skala Kecil. Dalam: Yustika, A.E. Meninjinakkan Liberalisme: Revitalisasi Sektor Pertanian & Kehutanan. Pustaka Pelajar. Yogyakarta. Pp. 141 – 159.

Despal, Malyadi, J., Destianingsih, Y., Lestari, A., Hartono, H., and Abdullah, L. 2014a. Natural Grass and Plant Residue Qualities and Values to Support Lactating Cows Requirement on Forage at Indonesian Small Scale Enterprise and Traditional Dairy Farming. Proceeding of International Workshop on Tropical Bio-resources for Sustainable Development, Bogor 13 – 15 August 2014

DGLS, 2012. National Dairy Competitiveness Study: Performance Evaluation as Basic for Sustainable Dairy Development Program toward Domestic Contribution Strengthening. Project Report. Directorate General for Livestock Services and Animal Health in Collaboration with Faculty of Animal Science, Bogor Agricultural University

Edmonson AJ, Lean LJ, Weaver LD, Farver T, Webster G. 1989. A body condition chart for Holstein dairy cows. *J. Dairy Sci*. 72: 68-70.

Garg MR, Sherasia PL, Bhanderi BM, Phondba BT, Shelke SK, Makkar HPS. 2013. Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. Animal Feed Science and Technology 179: 24– 35

Hardie, C.A., M. Wattiaux, M. Dutreuil, R. Gildersleeve, N.S. Keuler, V.E. Cabrera. 2014. Feeding strategies on certified organic dairy farms in Wisconsin and their effect on milk production and income over feed costs. Journal of Dairy Science, 97 (7):4612-4623

Merry, R.J., K.F. Lowes and A.Winter. 1997. Current and future approaches to biocontrol in silage. p. 17 – 27. In: Jambor, V., L. Klapil, P.Chromec and P. Prochazka (eds) Proc. 8th Int. Symposium Forage Conservation. Brno, Czech Republic, 29 September – 1 Octover 1997. Pohorelice, Czech Republic: Research Institute of Animal Nutrition.

Moran J. 2005. "Tropical dairy farming: Feeding management for small holder dairy farmers in the humid tropics". Landlink Press.

Regan, C. 2000. Comparison of the nutritive value of cavalcade and pangola grass forages preserved as silage or hay. In: Mannetje, L.T. Silage Making in the Tropics with Particular Emphasis on Smallholders. Proceedings of the FAO Electronic Conference on Tropical Silage 1 September to 15 December 1999.

Roche JP, Meier S, Heiser A, Mitchell MD, Walker CG, Crookenden MA. Riboni MV. 2015. Effects of precalving body condition score and prepartum feeding level on production, reproduction, and health parameters in pasture-based transition dairy cows J. Dairy Sci. 98:1–19

Roche JR, Kay JK, Friggens NC and Loor JJ. 2013. Assessing and Managing Body Condition Score for the Prevention of Metabolic Disease in Dairy Cows. Veterinary Clinics of North America: Food Animal Practice 29(2):323–336

Sudono A. 1999. Ilmu Produksi Ternak Perah. Bogor (ID): IPB Press.

Wells R. 2013. Manure scoring determines supplementation needs. http://www.noble.org/Global/ag/news-views/2013/10/manure-scoring.pdf. The Samuel Roberts Noble Foundation, Inc. AG News and Views.

Wilkins, R.J., L. Syrjälä-Qvist and K.K. Bolsen. 1999. The future role of silage in sustainable animal production. p. 23 – 35, In: Pauly, T (ed) 1999. Proc. 12th Int. Silage Conference. Swedish University of Agriculture Science, Uppsala, Sweden, 5 – 7 July 1999.

O-25-9

Effect of Yeast Fermented and Physical forms of Corn Husk on Digestibility and Fermentation by Using In vitro Gas Techniques

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ABSTRACT

This study aimed to investigate the effect of yeast fermented levels and physical forms of corn husk on gas kinetics, digestibility, and fermentation using *in vitro* techniques. The experimental design was a 2×3 factorial arrangement in a completely randomized design. Factor A was two physical forms of corn husk (Long form and chopped 4 cm) and factor B was three rations of yeast medium solution fermented corn husk (YMS:C 1:0, 1:0.5, 1:1). Results revealed that yeast medium solution fermented corn husk at the ratio 1:0.5 and 1:1 influenced the gas kinetics. Cumulative gas production was increase when increasing levels of yeast medium solution ($P<0.01$). *In vitro* true digestibility was higher in 1:1 yeast fermented ration with chopped corn husk ($P<0.05$). Bacterial population was higher in yeast fermented group ($P<0.01$) while protozoal population and fungal zoospores were similar among treatments ($P>0.05$). It could be concluded that ration of yeast medium solution fermented corn husk at 1:1 improved gas production, *in vitro* true digestibility and rumen fermentation. These results revealed a potential use of yeast fermented corn husk to improve rumen fermentation and potentially improved ruminant production efficiency in further *in vivo* experiment.

INTRODUCTION

The security of livestock feed has been becoming critical in terms of both quantity and quality, particularly the protein sources, which results in low productivity. Researchers have been trying to find alternative protein sources which could help to increase livestock productivity and efficiency (Guglielmelli et al., 2010). Most of the farmers in the northern part of Thailand use rice straw and others crop residues as roughage sources fed to ruminants. Corn husk is one of those which available in the dry season. However, there are limitation of using due to the low quality in terms of protein content and digestibility. Therefore, improving of corn husk is very interesting to be an alternative roughage sources for ruminants. Recently, incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition (Campanile et al., 2008). In addition, the use of yeasts in the ruminal production is a good alternative to replace antibiotics as growth promoters (Marrero et al., 2015). Moreover, supplementation of yeast culture in rice straw and maize stover improved the rate of gas production, DM and OM disappearances (Tang et al., 2008) and improved rumen fermentation (Wang et al., 2016). However, there are limited studies on the use of yeast fermented with physical forms of corn husk using an *in vitro* fermentation technique. Therefore, the aim of this study is to investigate the effect of yeast fermented and physical forms of corn husk on digestibility and rumen fermentation characteristics using *in vitro* gas technique.

Materials and methods

An *in vitro* study based on the technique described by Menke et al. (1979) was conducted to evaluate effect of yeast fermented and physical forms of corn husk using dairy cattle rumen fluids. The experimental design was a 2×3 factorial arrangement in a completely randomized design. Factor A was two physical forms of corn husk (C) (Long form (L) and short form (S) chopped 4 cm) and factor B was three rations of yeast medium solution (YMS) fermented corn husk (YMS:C 1:0, 1:0.5, 1:1), therefore, treatments were as follows T1 to T3 were LC and T4 to T6 were SC fermented with YMS at the ratio (1:0, 1:0.5, 1:1), respectively. The YMS would be replaced by the water to adjust similar total volume of the condition. The gas production was measured at 0, 1.5, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation according to Foiklang et al. (2016). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Nutrient compositions of substrates were analyzed according to the standard methods (AOAC, 1995). Inoculum's ruminal fluid was collected at 0, 2, 4 and 6 h of incubation for pH and NH_3-N analysis. *In vitro* true digestibility (TD) measurement based on Van Soest and Robertson (1985). Data

were analyzed by using the GLM procedures of the Statistical Analysis System Institute (SAS, 1996).

RESULTS AND DISCUSSIONS

Corn husk contained 3.5% of CP, 87.2% of NDF and 48.9% of ADF while YMS:C at 1:0.5 ratio cotained higher level of CP (4.6%) and lower level of NDF (72.7%). YMS:C at 1:1 ratio contained 5.8 % of CP and 70.1% of NDF. Gas kinetic was different among YMS rations (P<0.01 Table 1).

Supplementation of YMS affected the immediately soluble fraction (a), insoluble fraction (b), and potential extent of gas production (a+b) (P<0.05), but did not affect the gas production rate (c) (P>0.05). Cumulative gas production (96 h) was higher in the YMS:C ratio at 1:0.5, 1:1 (P<0.05) especially YMS:C ratio at 1:1 with short form corn husk (P<0.01). Marrero et al. (2015) reported that yeast addition with alfalfa hay (*Medicago sativa*) as substrate could increase accumulated gas production. These similar results were agreed with Tang et al. (2008) who supplemented yeast culture in rice straw and maize stover that could improve the rate of gas production, DM and OM disappearances. In addition, *in vitro* true digestibility at 24 and 48 h of incubations were shown to have high correlation with gas volume which was significantly higher in supplementation of YMS at the ration of YMS:C 1:1 (P<0.05). The pH value was significantly reduced when supplemented with YMS (P<0.01). The result was similar to Wang et al. (2016) who reported that addition of yeast species in maize stover and rice straw could decrease pH especially, *S. Cerevisiae* in rice straw base substrate. However, pH value is still in the optimum levels for ruminal microbes. NH_3-N concentration in the YMS supplemented group has shown lower than in control group (P<0.05 Table 2) and ranged from 8.5 to 12.1 mg/dL which higher than the result of Wang et al. (2016) which ranged from 5.24 to 8.83 mg/dL. The NH_3-N concentration was close to the report of Wanapat (1990) (15 to 30 mg/dL) which was reported to be suitable for microbial protein synthesis. Bacterial population was higher in yeast fermented group (P<0.01) while protozoal population and fungal zoospores were similar among treatments (P>0.05). This could be explained by the report of Newbold et al. (1995) that dietary inclusion of *S. cerevisiae* NCYC 240, NCYC 1026 and Yea-Sacc stimulated total and cellulolytic bacterial numbers *in vitro.* It has been confirmed that yeast culture supplementation benefits digestion and metabolism of ruminants in several aspects, such as the improvement of nutrient digestibility, decrease in the ruminal ammonia nitrogen, alleviation of pH fluctuation, and stimulation of ruminal microorganism population (Chaucheyras-Durand et al., 2008). Furthermore, it has been verified that yeast culture inclusion in the diets of ruminants can provide various growth factors, pro-vitamins and other stimulants to rumen microorganisms, and balance the ruminal fluid redox potential to create the optimal fermentation conditions for the rumen bacterial microflora (Jouany, 2001).

Conclusions and recommendations

Based on this study it could be concluded that the ration of yeast medium solution fermented corn husk at 1:1 improved gas production, *in vitro* true digestibility and rumen fermentation. These results revealed a potential use of yeast fermented corn husk to improve rumen fermentation and potentially improved ruminant production efficiency. However, *in vivo* feeding trial should be subsequently conducted.

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KEYWORD : Yeast fermented corn husk, Physical form, Digestibility, Rumen Fermentation, *In vitro* gas technique

Table 1 Effect of yeast fermented and physical forms of corn husk on gas kinetics, true digestibility and microbial mass using *in vitro* incubation

Treatment[1]	Gas Kinetics[2]				Gas (96 h) mL/0.2 g	TD[3], %	
	a	b	c	a+b	DM	24h	48h
LC +0 YMS	-3.8	56.9	0.046	53.0	56.0	60.14	67.35
LC +0.5 YMS	-3.7	63.9	0.048	60.2	66.3	64.29	69.16
LC +1 YMS	-3.2	67.5	0.051	64.2	72.7	68.57	74.31
SC +0 YMS	-4.2	61.2	0.046	56.9	59.9	62.79	77.45
SC +0.5 YMS	-4.2	72.3	0.052	68.1	70.5	69.88	79.03
SC +1 YMS	-3.3	77.8	0.056	74.5	78.4	68.28	82.12
SEM	0.11	1.9	0.004	2.0	2.1	1.5	1.4
Comparison							
LC vs SC	ns	ns	ns	ns	ns	ns	ns
YMS ration	*	*	ns	*	**	*	*
Interaction	ns	ns	ns	ns	ns	ns	*

[1]LC=long form corn husk, SC= short form corn husk, YMS= yeast medium solution. [2]a= the gas production from the immediately soluble fraction, b= the gas production from the insoluble fraction, c= the gas production rate constant for the insoluble fraction (b), a+b = the gas potential extent of gas production. [3]TD = true digestibility. *P<0.05, **P< 0.01, ns = non-significant. SEM=standard error of the mean.

Table 2 Effect of yeast fermented and physical forms of corn husk on rumen microorganisms and fermentation

Treatment[1]	Rumen microorganisms, cfu/mL			pH	NH_3-N, mg/dL
	Bacteria, $\times 10^{10}$	Protozoa, $\times 10^{6}$	Fungal zoospores,$\times 10^{5}$		
LC +0 YMS	5.9	4.4	3.9	6.50	11.4
LC +0.5 YMS	8.2	5.2	4.1	6.45	11.2
LC +1 YMS	12.1	5.1	4.0	6.42	8.5
SC +0 YMS	6.8	4.6	3.5	6.52	12.
SC +0.5 YMS	9.5	5.0	3.1	6.49	10.9
SC +1 YMS	13.3	4.7	3.3	6.38	10.4
SEM	0.47	0.33	0.41	0.02	0.4
Comparison					
LC vs SC	ns	ns	ns	ns	**
YMS ration	**	ns	ns	**	*
Interaction	ns	ns	ns	ns	*

[1]LC=long form corn husk, SC= short form corn husk, YMS= yeast medium solution.
*P<0.05, **P< 0.01, ns = non-significant. SEM=standard error of the mean.

REFERENCES

AOAC. 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Campanile, G., F. Zicarelli, D. Vecchio, C. Pacelli, G. Neglia, A. Balestrieri, R. Di Palo, and F. Infascelli. 2008. Effects of *Saccharomyces cerevisiae* on *in vivo* organic matter digestibility and milk yield in buffalo cows. Livest. Sci. 114:358-361.

Chaucheyras-Durand, F., N. D. Walker, and A. Bach. 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Technol. 145:5-26.

Foiklang, S., M. Wanapat and T. Norrapoke. 2016. *In vitro* rumen fermentation and digestibility of buffaloes as influenced by grape pomace powder and urea treated rice straw supplementation. Animal Sci. J. 87(3):370-377.

Guglielmelli, A., S. Calabrò, M. Cutrignelli, O. Gonzalez, F. Infascelli, R. Tudisco, and V. Piccolo. 2010. *In vitro* fermentation and methane production of fava and soy beans. EAAP Scientific Series 127:457-460.

Jouany, J. P. 2001. A new look at yeast cultures as probiotics for ruminants. Feed Mix. 9:17-19.

Marrero Y., Y. Castillo, O. Ruiz, E. Burrola, and C. Angulo. 2015. Feeding of yeast (*Candida* spp.) improves *in vitro* ruminal fermentation of fibrous substrates. J. Integr. Agric. 14(3): 514-519.

Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro.* J. Agric. Sci. (Camb.). 92:217-222.

Newbold, C. J., R. J. Wallace, X. B. Chen, and F. M. Mcintosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. J. Anim. Sci. 73:1811-1818.

Ørskov, E.R., and I. McDonal. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

SAS, 1996. User's Guide: Statistics, Version 5th Edition. SAS. Inst, Inc.,Cary, NC., U.S.A.

Tang, S. X., G. O. Tayo, Z. L. Tan, Z. H. Sun, L. X. Shen, C. S. Zhou, W. J. Xiao, G. P. Ren, X. F. Han, and S. B. Shen. 2008. Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. J. Anim. Sci. 86:1164-1172.

Van Soest, P. J., and J. B. Robertson. 1985. A laboratory manual for animal science. Cornell University Press, Ithaca, NY.

Wanapat, M. 1990. Nutritional Aspects of Ruminant Production in Southeast Asia with Special Reference to Thailand. Funny Press, Ltd., Bangkok, Thailand.

Wang Z., Z. He, K.A. Beauchemin, S.Tang, C. Zhou, X. Han, M. Wang, J. Kang, N. E. Odongo, and Z. Tan. 2016. Evaluation of Different Yeast Species for Improving *In vitro* Fermentation of Cereal Straws. Asian Australas. J. Anim. Sci. 29: 230-240.

O-25-10

Structural Features of Condensed Tannins Affect In Vitro Rumen C18:3n-3 Biohydrogenation in Dairy Cows

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1. Introduction

Condensed tannins (CT) in the plants have been used in the diets of ruminants for inhibiting the ruminal biohydrogenation (BH) process of C18:3n-3 and increasing the concentration of unsatuated fatty acids in drived products ruminants as meat and milk (Vasta et al., 2009b Dschaak et al., 2011). The study of (Jones et al., 1994) showed that condensed tannins (CT) from various legume forages inhibit the growth of many ruminal bacteria, including bacteria associated with BH (Vasta et al., 2010). Sivakumaran et al. (2004) demonstrated that CT from *Dorycnium rectum* forage inhibited growth of *Butyrivibrio fibrisolvens* bacteria which are involved in the BH process. Khiaosa-Ard et al. (2009) reported that addition of CT (78.9 g/kg DM) inhibited the last step of C18:3n-3 biohydrogenation in Rusitec. The addition of quebracho tannins in the diet of sheep resulted in an increased concentration of *trans-11*-C18:1 in the rumen (Vasta et al., 2009a Vasta et al., 2010) and an increased concentration of *cis-9,trans-11*-C18:2 and PUFAs in lamb meat (Vasta et al., 2009b). Dschaak et al. (2011) found that supplementation with quebracho tannin extract (containing 75% CT) at 30g/kg DM diet, increased the content of C18:3n-3 in milk, compared to a control diet.

The effect of CT on ruminant fermentation depends not only on the CT concentration but also on their structure and molecular weight (Wang et al., 1996). Structural properties of CT including: i) mean degree of polymerization (mDP), ii) prodelphinidins (PD):procyanidins (PC) ratio (%PD) and iii) ratio between *cis:trans* flavan-3-ols (%*cis*) within CT (Gea et al., 2011) may, however, be equally or more important for the mode of action. Sivakumaran et al. (2004) found that a CT extract from *Dorycnium rectum* with mDP of 10.3 monomeric units was more inhibitory for the growth of *Clostridium aminophilum, B. proteoclasticum* and *B. fibrisolvens*, compared to *D. rectum* CT fractions of medium and high molecular weight (mDP = 41 and 127 monomeric units, respectively). The hypothesis of this study was that one of the three CT structural properties (mDP, %PD, %*cis*) affect ruminal fermentation and BH. Therefore, the relationship between tannin structural properties and BH of C18:3n-3 as well as fermentation during *in vitro* incubation was investigated. Eight different tannin extracts were used with a wide range of structural properties.

2. Materials and Methods

2.1. Condensed tannin sources and extraction of condensed tannins

Black (*Ribes nigrum*), red currant (*Ribes rubrum*) leaves, goat willow (*Salix caprea*) leaves and twigs, weeping willow (*Salix babylonica*) catkins, white clover (*Trifolium repens*) flowers, whole sainfoin (*Onobrychis viciifolia*, var. Esparsette) and pine bark (*Pinus sylvestris)* were collected and after collection, the plant materials were freeze-dried and ground to pass through a 1-mm sieve using an impeller mill. Then the CTs were extracted according to the method of Williams et al. (2014).

2.2. Analysis of condensed tannin extracts

The extracts were analysed for CT structural properties by thiolytic degradation, followed by HPLC analysis (Novobilský et al., 2011 Gea et al., 2011). The method provides information on the CT concentration, percentage of flavan-3-ols (catechin, epicatechin, gallocatechin and epigallocatechin), CT terminal and extension units (Figure 1). In addition, it allowed calculation of the mDP, %PD and %*cis* flavan-3-ol in the CT polymers based on the formulae in Figure 1 (Gea et al., 2011) (Table 1).

2.3. Experimental design

The effects of CT structural properties on biohydrogenation of C18:3n-3 during *in vitro* incubation were evaluated

using a tannin-free total mix ration (TMR) as a control substrate. The TMR was composed of grass silage (600 g/kg dry matter = DM), maize silage (100 g/kg DM), concentrates (240 g/kg DM) and linseed (60 g/kg DM). The chemical composition of the TMR was: DM = 938.1 g/kg TMR in g/kg DM: organic matter (OM) = 918.9 crude protein (CP) = 162.7, neutral detergent fibre (NDF) = 395.7 acid detergent fibre (ADF) = 236.7 starch = 97.9 C18:3n-3 = 13.6. Condensed tannin extract was added to the TMR at an effective concentration of 40 g CT/kg in the presence (+PEG to inactivate the tannins, Makkar et al., 1995) or absence (-PEG) of polyethylene glycol (PEG 6000, Merck). The treatments consisted of 8 CT extracts from different plants as mentioned above. Each extract (to obtain 10 mg CT) and TMR (250 mg) -/+ PEG (100 mg CT:PEG = 1:10, w/w) (Pellikaan et al., 2011) were weighed into 250 mL incubation flasks (Schott bottle, GL45, Mainz, Germany). Each treatment was incubated in duplicate over 2 separate runs conducted on separate days. The average amount of OM and C18:3n-3 incubated in each flask in run 1 was 219.7 and 3.4 mg, and in run 2, 218.8 and 3.4 mg, respectively.

2.4. In vitro incubation

The rumen fluid from three cannulated cows per run with the lactation stage of cows was 118.6 ± 76.7 days in lactation and fat and protein-corrected milk was 30.1 ± 2.8 kg/d. The cows were fed *ad libitum* a grass-corn silage mixture and concentrate according to their requirements in the morning and in the afternoon for 10 days before the experiment started. The rumen fluid was pooled during each run and filtered through double layers of cheese cloth under continuous flushing with CO_2 and then diluted thoroughly with a phosphate buffer (per L Millipore water: 28.8 g $Na_2HPO_4.12H_2O$, 6.1 g $NaH_2PO_4.H_2O$, and 1.4 g NH_4Cl, adjusted to pH 6.9 by adding NaOH solution). The ratio of rumen fluid and phosphate buffer was 1:4 (v/v) (Sterk et al., 2010).

The incubation flasks containing an accurately weighed amount (~0.25 g) of TMR/CT extract (-/+PEG) mixture were flushed with CO_2 before rumen fluid/phosphate buffer mixture (30 mL) was added. Flasks containing only buffered rumen fluid -/+ PEG (blanks) were included. All flasks were incubated in shaking water baths at 39 ℃ at 40-50 movements per minute for 0, 12 and 24 h. At the end of each incubation period, flasks were removed sequentially and immediately placed on ice before flasks were opened and pH measured (Mettler Toledo FE20/EL20 pH meter, Schwerzenbach, Switzerland). Fermentation fluid from each flask was collected for determination of volatile fatty acid (VFA) and ammonia (NH_3) concentration. The incubation residue from each flask was collected, stored at -20℃ , and freeze dried and ground manually in a mortar before FA analysis. The FA concentrations in the blanks -/+PEG at 0, 12 and 24 h were used to correct the FA concentrations in flasks with TMR/CT extract (-/+PEG) of the corresponding time.

2.5. Chemical analysis

Grass silage, corn silage, concentrate and linseed were freeze dried, ground over a 1-mm sieve using an cross beater mill and analysed for DM (ISO 6496 ISO, 1999), ash (ISO 5984 ISO, 2002), N (ISO 5983 ISO, 2005) and starch (ISO 15914 ISO, 2004). Crude protein content was calculated as: CP = 6.25 × N. Neutral detergent fibre was analysed according to Van Soest et al. (1991) after a pre-treatment with a heat stable amylase and corrected for residual ash. Acid detergent fibre was determined according to Van Soest (1973).

Fermentation fluid, sampled for VFA and NH_3 were analysed according to Pellikaan et al. (2011). The total VFA (tVFA) and NH_3 concentration in the fermentation fluid was expressed as mmol/g incubated OM.

Fatty acids (FA) in the individual feed ingredients of the TMR and the incubation residue samples were determined as described by Khan et al. (2009) with FA expressed in g/kg DM of samples.

2.6. Calculations and statistics

Total C18 FA concentration per unit incubated DM in each incubation flask were assumed to be 100% at 0, 12, 24 h incubation and, therefore, individual C18:0, *cis*-9-C18:1 *cis*-9,*cis*-15-C18:2 C18:3n-3 were calculated as proportions of total C18 FA. Disappearance of C18:3n-3 from each incubation flask at 12 and 24 h was calculated relative to the 0 h time point and used to estimate the fractional BH rate per hour.

The individual FA and fermentation end-product data in combination with the PEG treatment measured at the different sampling times were analysed using the MIXED procedure of SAS (2010) using the following model:

$$Y_{ijk} = \mu + T_i + P_j + R_k + (T \times P)_{ij} + \varepsilon_{ijk} \quad (4)$$

where Y_{ijk} = dependent variable, μ = overall mean, T_i = tannin extract source (i = 1 to 9, 8 CT extracts and 1 control), P_j = effect of PEG (j = 1 to 2), R_k = run (k = 1 to 2), $(T \times P)_{ij}$ = effect of tannin extract type and PEG interaction and ε_{ijk} = residual error term. The statistical unit was the average of replicated *in vitro* bottles within

run. Differences among main effects were analysed using Tukey-Kramer's multiple comparison procedure in the LSMEANS statement in SAS with effects considered significant at $P<0.05$, and a trend at $0.05 \leq P<0.10$.
The relationship between mDP, %PD or %*cis* and fermentation parameter estimates were analysed using the multiple stepwise regression procedure in SAS (2010) where mDP, %PD and %*cis* were included as independent variables in the model. The criteria to include variables in the model were a combination of a low value for the Mallow's Cp-criterion, a high coefficient of determination (R^2), and setting the residual degrees of freedom (df) in the regression model at > 65% of the total df.

3. Results

3.1. Effect of CT sources on fermentation end-products

The contents of fermentation end-products at 0 and 12h were similar among all CT extracts and the control treatments, therefore, data are not presented. Fermentation end-products produced after 24 h incubation, are presented in Table 2. The tVFA concentration was reduced ($P<0.0001$) with all CT extract additions, compared to the control. The CT extract from red currant leaves gave the lowest ($P<0.0001$) tVFA concentration (7.6 mmol/g OM incubated), followed by sainfoin (7.7 mmol/g OM incubated, $P=0.0002$). Addition of CT extract increased ($P=0.0012$) the proportion of propionate (HPr) with the highest proportion found with the addition of the sainfoin CT extract (25.7 % in tVFA, $P=0.011$). The proportion of HBc was decreased ($P<0.0001$) in all CT extracts, compared to the control. The lowest proportion of HBc was found for red currant leaf and sainfoin extracts (1.4 % in tVFA, $P<0.0001$). All CT extracts decreased ($P<0.0001$) the NH_3 concentration compared to the control. The largest reduction in NH_3 concentration was caused by the CT extract from sainfoin (1.7 mmol/g OM incubated, $P<0.0001$), followed by the red currant leaf extract (2.0 mmol/g OM incubated, $P<0.0001$). In general, PEG addition increased ($P \leq 0.0047$) tVFA, NH_3 concentration, proportions of butyric acid (HBu), proportions of HBc as well as the non-glucogenic to glucogenic VFA (NGR) ratio. However, PEG addition decreased the proportion of HPr ($P=0.0004$). There was an effect of run ($P<0.0001$) on all fermentation end-products.

3.2. Effect of CT source on C18:3n-3 biohydrogenation

The proportion of FA, after 24 h of incubation and fractional rate of BH of C18:3n-3, are presented in Table 3. After 24 h incubation, the proportion of *cis*-9-C18:1 *cis*-9, *cis*-12-C18:2 *cis*-9, *cis*-12, *cis*-15-C18:3 were numerically higher in all CT sources, compared to the control, except for the CT from weeping willow catkins. The proportion of C18:0 and the fractional rate of BH of C18:3n-3 were numerically lower in all CT sources compared to the control, except for the CT from weeping willow catkins. Addition of PEG decreased ($P \leq 0.0019$) the proportion of *cis*-9, *cis*-12-C18:2 and *cis*-9, *cis*-12, *cis*-15-C18:3, compared to when no PEG was added. However, PEG addition increased ($P \leq 0.012$) the proportion of C18:0 and the fractional rate of BH of C18:3n-3, compared to no PEG addition. An effect of run was found ($P<0.0001$) in the proportion of FA and fractional rate of C18:3n-3.

3.3. CT properties and end-products of fermentation and biohydrogenation

The mDP negatively affected ($P<0.05$) tVFA and HBc with coefficients of determination (R^2) of 0.660 and 0.519, respectively (Table 4). Moreover, mDP tended ($P=0.08$) to positively affect the proportion of *cis*-9, *cis*-12-C18:2 with an R^2 of 0.424. The %PD tended to ($P \leq 0.07$) negatively affect HBu and NGR with an R^2 of 0.462 and 0.443, respectively. In addition, %PD tended ($P=0.08$) to positively affect HPr with an R^2 of 0.417 while %*cis* tended ($P=0.06$) to negatively affect HAc with an R^2 of 0.470.

Conclusions

Condensed tannins extracts from different plants had diversity in mDP, %PD and %*cis*, which affected rumen fermentation and biohydrogenation in a different manner. The mDP and %PD were found to be the most important factors of the CT structural properties to affect rumen fermentation. The CT with %PD > 70% and $5.0 \leq$ mDP $\leq$ 10.0 had the largest effect on rumen fermentation. However, mDP was found to be the most important factor affecting rumen biohydrogenation.

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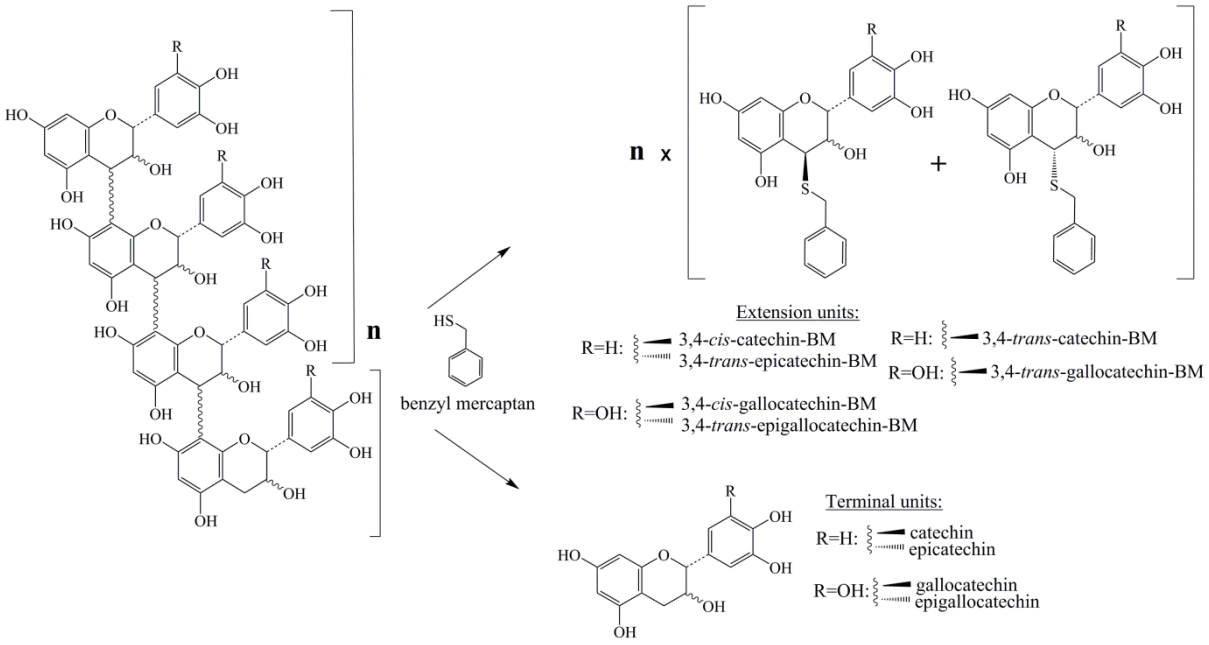
KEYWORD : Condensed tannins, Tannin structure, Biohydrogenation



$$\text{mDP} = \frac{\text{amount of extension and terminal flavan-3-ol units (mol)}}{\text{amount of terminal flavan-3-ol units (mol)}} \quad (1)$$

$$\%\text{PD} = \frac{\text{percentage of GC + EGC units}}{\text{percentage of C + EC + GC + EGC units}} \quad (2)$$

$$\%cis = \frac{\text{percentage of EC + EGC units}}{\text{percentage of C + EC + GC + EGC units}} \quad (3)$$

where C = catechin, EC = epicatechin, GC = gallocatechin and EGC = epigallocatechin flavan-3-ols with %PD + %PC = 100 and %*cis* + %*trans* = 100.

Figure 1. Thiolytic degradation of condensed tannin polymers. Extension subunits are released as flavan-3-ol benzyl mercaptan (BM) adducts, terminal subunits are released as the free flavan-3-ols (Gea et al., 2011).

Table 1. Condensed tannin (CT) content, mean degree of polymerization (mDP), percentage of prodelphinidins (%PD) and *cis* flavan-3-ols (%*cis*) in extracts obtained from eight plant sources

Plant source	CT content (g/100 g extract)	mDP	%PD (%)	%*cis* (%)
Black currant leaves	29.2 ± 1.2	5.4 ± 0.3	94.2 ± 0.1	12.9 ± 0.3
Goat willow leaves	29.9 ± 4.1	3.9 ± 0.0	3.3 ± 0.4	2.8 ± 0.1
Goat willow twigs	53.8 ± 2.1	4.3 ± 0.0	25.2 ± 0.2	53.0 ± 0.1
Pine bark	49.2 ± 0.9	2.5 ± 0.0	35.9 ± 0.3	79.6 ± 0.3
Red currant leaves	24.5 ± 1.4	9.8 ± 0.2	94.0 ± 0.2	77.3 ± 0.1
Sainfoin plants	12.6 ± 0.6	5.5 ± 0.0	74.7 ± 0.8	80.2 ± 0.1
White clover flowers	33.7 ± 1.1	4.4 ± 0.1	99.2 ± 0.0	65.7 ± 0.1
Weeping willow catkins	25.2 ± 0.4	2.3 ± 0.0	55.9 ± 0.1	77.7± 0.2

Values are means ± standard deviation.

Table 2. Concentration of fermentation end-products at 24 h *in vitro* incubation of a TMR to which different extracts containing condensed tannins with (+) and without (-) PEG were added.

Item	tVFA (mmol/g OM)		HAc (% of tVFA)		HPr (% of tVFA)		HBu (% of tVFA)		HBc (% of tVFA)		NGR Ratio		NH_3 (mmol/g OM)		pH	
	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG
TMR (control)	9.3^{a}	9.0	64.7	64.7	21.2^{a}	21.1	10.0^{ab}	10.0^{a}	2.2^{a}	2.2^{a}	3.6^{a}	3.6	2.8^{a}	2.8^{a}	6.5^{a}	6.5
Black currant leaves	7.9^{b}	8.8*	63.5	63.8	23.7^{ab}	21.8	9.4^{ab}	10.3^{a}	1.6^{c}	2.1^{ab***}	3.3^{ab}	3.6	2.1^{b}	2.4^{ab}	6.5^{a}	6.4
Goat willow leaves	8.4^{b}	8.7	64.2	64.1	21.9^{a}	21.9	10.3^{ab}	10.2^{a}	1.8^{bc}	2.0^{bc*}	3.6^{a}	3.6	2.1^{b}	2.3^{c}	6.4^{b}	6.4
Goat willow twigs	7.8^{b}	8.5	63.7	64.1	21.9^{a}	21.6	10.6^{a}	10.3^{a}	1.9^{b}	2.1^{ab*}	3.6^{a}	3.6	2.2^{b}	2.5^{ab}	6.5^{a}	6.5
Pine bark	8.3^{b}	8.5	62.2	64.2	24.5^{ab}	21.3	9.3^{b}	10.3^{a}	2.0^{b}	2.1^{ab}	3.1^{ab}	3.7	2.2^{b}	2.5^{ab}	6.4^{b}	6.5
Red currant leaves	7.6^{b}	8.3	62.8	63.8	24.6^{ab}	22.1	9.3^{b}	10.1^{a}	1.4^{d}	1.9^{bc***}	3.1^{ab}	3.5	2.0^{bc}	2.3^{bc}	6.5^{a}	6.4
Sainfoin plants	7.7^{b}	8.3	62.7	63.2	25.7^{b}	24.2	8.5^{b}	8.9^{b}	1.4^{d}	1.7^{c***}	3.0^{b}	3.1	1.7^{c}	2.0^{c}	6.4^{b}	6.4
White clover flowers	8.2^{b}	8.3	63.0	63.4	24.3^{ab}	22.0	9.2^{b}	10.3^{a*}	1.7^{c}	2.2^{a***}	3.2^{ab}	3.5	2.3^{b}	2.6^{ab}	6.4^{b}	6.4
Weeping willow catkins	8.2^{b}	8.4	63.8	63.7	22.3^{ab}	21.9	10.3^{ab}	10.3^{a}	1.9^{b}	2.1^{ab**}	3.5^{a}	3.6	2.4^{b}	2.7^{ab}	6.4^{b}	6.5
SEM	0.224		0.533		0.662		0.2159		0.031		0.130		0.067		0.026	
P-values																
Tannin source (T)	<0.0001		0.1025		0.0012		<0.0001		<0.0001		0.0067		<0.0001		0.0096	
PEG (P)	<0.0001		0.0687		0.0004		0.0006		<0.0001		0.0047		<0.0001		0.3695	
RUN	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
T×P	0.0355		0.6384		0.1882		0.0204		<0.0001		0.2899		0.1773		0.1454	

TMR = total mixed ratio (600 g/kg DM of grass silage, 100 g/ kg DM of maize silage, 240 g/ kg DM of concentrate, 60 g/kg DM of linseed); +PEG, -PEG = with/ without polyethylene glycol addition; tVFA = total volatile fatty acid (tVFA = HAc + HPr + HBu + HVa + HBc); HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; HBc = branched – chain volatile fatty acids (*iso*-butyric + *iso*-valeric acid); NGR= non–glucogenic to glucogenic VFA ratio [= (HAc + 2 × HBu + 2 × *iso*-butyric + HVa + *iso*-valeric)/(HPr + HVa + *iso*-valeric)]; NH_3 = ammonia; SEM = standard error of the mean.

^{abc}Different superscripts, indicate differences (P<0.05). per column for main effect TMR *versus* condensed tannin types.

*P<0.05; **P<0.01; ***P<0.001 indicates difference between –PEG and +PEG.

Table 3. Proportion of fatty acids at 24 h *in vitro* incubation and fractional rate of biohydrogenation of C18:3n-3 of a TMR to which different extracts of condensed tannins with (+) and without (-) PEG were added.

Item	C18:0 (% of total C18)		*Cis*-9-C18:1 (% of total C18)		*Cis*-9,*cis*-12-C18:2 (% of total C18)		*Cis*-9,*cis*-12,*cis*-15-C18:3 (% of total C18)		Rate of biohydrogenation (%/h)	
	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG
TMR (control)	51.4	45.4	22.3	26.3	8.5	9.2	17.6	18.9	2.6	2.6
Black currant leaves	30.5	41.5	26.1	25.1	13.7	10.9	29.6	22.4	1.6	2.4
Goat willow leaves	37.2	45.2	24.6	24.0	12.4	10.0	25.8	20.6	2.0	2.5
Goat willow twigs	37.4	44.5	24.2	25.3	11.8	9.9	26.4	20.2	2.0	2.5
Pine bark	32.9	66.2	26.3	21.0	12.4	4.9	28.3	8.0	1.9	3.5
Red currant leaves	29.7	58.1	26.8	22.8	14.7	6.6	28.7	12.4	1.7	3.1
Sainfoin plants	44.1	58.0	27.4	22.1	10.0	7.1	18.5	12.6	2.6	3.1
White clover flowers	40.6	51.6	25.9	25.8	11.6	7.7	21.8	14.9	2.3	2.9
Weeping willow catkins	54.1	55.0	23.3	24.0	8.1	7.3	14.5	13.5	3.0	3.0
SEM	7.11		2.21		1.64		4.36		0.36	
P-values										
Tannin source (T)	0.2934		0.9773		0.1703		0.1965		0.1677	
PEG (P)	0.0024		0.2720		0.0005		0.0019		0.0120	
RUN	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
T×P	0.2297		0.4601		0.2092		0.3410		0.3541	

TMR = total mixed ratio (600 g/kg DM of grass silage, 100 g/kg DM of maize silage, 240 g/kg DM of concentrate, 60 g/kg DM of linseed); +PEG, -PEG = with/ without polyethylene glycol addition; Values for C18:0, *cis*-9-C18:1; *cis*-9,*cis*-12-C18:2; *cis*-9,*cis*-12,*cis*-15-C18:3 are a percentage of total C18 FA (% of C18:0 + % of *cis*-9-C18:1 + % of *cis*-9,*cis*-12-C18:2 + % of *cis*-9,*cis*-12,*cis*-15-C18:3).

Table 4. Relationship between structural properties of condensed tannins (mDP, %PD, %*cis*) and end-products of fermentation and biohydrogenation at 24 h incubation as estimated by multiple stepwise regression.

End-products	α	mDP	%PD (%)	%*cis* (%)	R^2
tVFA, mmol/g OM	8.53***	-0.103**	-	-	0.660
HAc, % of tVFA	64.08***	-	-	-0.014*	0.470
HPr, % of tVFA	22.11***	-	0.025*	-	0.417
HBu, % of tVFA	10.43***	-	-0.013*	-	0.462
HBc, % of tVFA	2.03***	-0.065**	-	-	0.519
NGR Ratio	3.59***	-	-0.005*	-	0.443
Cis-9-C18:1, % **of ∑C18**	-	-	-	-	-
Cis-9,*cis*-12-C18:2, % **of ∑C18**	9.12***	0.573*	-	-	0.424
Cis-9,*cis*-12,*cis*-15-C18:3, % **of ∑C18**	-	-	-	-	-

- = parameters not selected; α = intercept; mDP = mean degree of polymerization (i.e. the average number of flavan-3-ol monomers per polymer); %PD = percentage of prodelphinidin subunits gallocatechin (GC) and epigallocatehin (EGC) units); %*cis* = percentage of flavan-3-ols with *cis* configuration; tVFA = total volatile fatty acid (tVFA = HAc + HPr + HBu+ HVa+ HBc); HAc = acetic acid; HPr = propionic acid; HBu = butyric acid; HBc = branched–chain volatile fatty acids (*iso*-butyric + *iso*-valeric acid); NGR= non–glucogenic to glucogenic VFA ratio [= (HAc + 2 × HBu + 2 × *iso*-butyric + HVa + *iso*-valeric)/(HPr + HVa + *iso*-valeric)].
*P<0.08; **P<0.05; ***P<0.001; R^2 = coefficient of determination.

REFERENCES

Dschaak, C.M., Williams, C.M., Holt, M.S., Eun, J.S., Young, A.J., Min, B.R., 2011. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J. Dairy Sci. 94, 2508-2519.

Gea, A., Stringano, E., Brown, R.H, Mueller-Harvey, I., 2011. In situ analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. J. Agri. Food Chem. 59, 495-503.

Jones, G.A., McAllister, T.A., Muir, A.D., Cheng. K.J., 1994. Effects of sainfoin (*Onobrychis viciifolia Scop*.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60, 1374-1378.

Khan, N., Cone, J.W., Hendriks, W.H., 2009. Stability of fatty acids in grass and maize silages after exposure to air during the feed out period. Anim. Feed Sci. Technol. 154, 183-192.

Makkar, H.P.S., Dawra, R.K., Singh, B., 1988. Changes in tannin content, polymerisation and protein precipitation capacity in oak (*Quercus incana*) leaves with maturity. J. Sci. Food. Agric. 44, 301-307.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. Vet. Parasitol. 182, 213-220.

Pellikaan, W.F., Stringano, E., Leenaars, J., Bongers, D.J.G.M., Schuppen, S.V.L.V., Plant, J., Mueller-Harvey, I., 2011. Evaluating effects of tannins on extent and rate of in vitro gas and CH_4 production using an automated pressure evaluation system (APES). Anim. Feed Sci. Technol. 166-167, 377-390.

SAS., 2010. SAS/STAT software, version 9.3. SAS Institute Inc., Cary, NC. USA.

Sivakumaran, S., Molan, A.L., Meagher, L.P., Kolb, B., Foo, L.Y., Lane, G.A., Attwood, G.A., Fraser, K., Tavendale, M., 2004. Variation in antimicrobial action of proanthocyanidins from Dorycnium rectum against rumen bacteria. Phytochem. 65, 2485-2497.

Sterk, A., Hovenier, R., Vlaeminck, B., Van Vuuren, A.M., Hendriks, W.H., Dijkstra, J., 2010. Effects of chemically or technologically treated linseed products and docosahexaenoic acid addition to linseed oil on biohydrogenation of C18:3n-3 i*n vitro*. J. Dairy Sci. 93, 5286-5299.

Van Soest, P.J., 1973. Collaborative study of acid-detergent fiber and lignin. J. Ass. Offic. Anal. Chem. 56, 781-784.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M., Priolo, A., 2009a. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. J. Anim Sci. 87, 2674-2684.

Vasta, V., Priolo, A., Scerra, M., Hallett, K.G., Wood, J.D., Doran, O., 2009b. Δ 9 desaturase protein expression and fatty acid composition of longissimus dorsi muscle in lambs fed green herbage or concentrate with or without added tannins. Meat Sci. 82, 357-364.

Vasta, V., Yáñez-Ruiz, D.R., Mele, M., Serra, A., Luciano, G., Lanza, M., Biondi, L., Priolo, A., 2010. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Appl. Environ. Microbiol. 76, 2549-2555.

Wang, Y., Douglas, G.B., Waghorn, G.C., Barry, T.N., Foote, A.G., 1996. Effect of condensed tannins in Lotus corniculatus upon lactation performance in ewes. J. Agric. Sci. 126, 353-362.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against Ascaris suum. PloS ONE. 9, e97053.

O-26-6 EVALUATION OF FULLY OXIDIZED BETA-CAROTENE (OXBC) AS AN ALTERNATIVE TO ANTIBIOTICS FOR GROWTH PROMOTION AND DIESEASE PREVENTION IN SWINE

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ABSTRACT:

A study was conducted to evaluate the effect of dietary supplementation with OxBC on productivity and clinical health of swine raised under commercial conditions in Vietnam. OxBC is a naturally occurring product, with reported host-directed immunomodulatory activities, and it is composed of compounds obtained by completely oxidizing beta-carotene.

Five hundred pigs (28 days old) were evenly and randomly divided into 5 treatment groups that included a non-supplemented negative control, a positive control receiving antibiotic growth promoters, and three groups receiving supplementation with OxBC at 2, 4 or 8 ppm in the feed. Diets were administered daily over the entire 140-day post-wean growth cycle.

Results showed that all three OxBC doses significantly improved overall growth rate, feed efficiency, and body weight relative to the negative control group. Furthermore, animals receiving 4 or 8 ppm OxBC also had better overall performance than the positive control group.

The clinical health benefits of OxBC supplementation were most evident in young piglets during the starter period. In starter pigs, the use of OxBC significantly and dose-dependently reduced the diarrhea rate from 7.9% in the negative control to 6.0% (2 ppm OxBC), 4.9% (4 ppm OxBC) and 3.7% (8 ppm OxBC). Diarrhea rates in the 4 and 8 ppm OxBC groups were also significantly lower than the 6.6% rate observed in the positive control group. Reductions in piglet diarrhea rate occurred concurrent with significant improvements in starter-pig growth performance and feed efficiency.

This study demonstrated the effectiveness of OxBC in enhancing productivity and reducing the incidence of disease in hogs raised under commercial conditions in Southeast Asia. Furthermore, the study showed that such benefits are attainable at economically viable doses for producers. The results reported here support the use of OxBC as a natural product alternative to antibiotics for growth promotion and diarrhea prevention in hogs.

INTRODUCTION:

The widespread routine use of antibiotics in animal feeds for the purposes of growth promotion and disease prevention has been linked to the rise of antibiotic resistant bacteria, which pose a serious threat to human and animal health. In response to this threat, governments around the world have begun to restrict and even ban the use of in-feed antibiotics for growth promotion and disease prevention. The goal of reducing antibiotic use in feeds without compromising animal productivity and producer economics has increased the need to identify suitable and efficacious alternatives to antibiotics whose use will not result in the rise of resistant bacteria.

A recently discovered family of compounds, beta-carotene-oxygen copolymers, derived from the complete oxidation of beta-carotene, has shown promise as a replacement for the growth promoting and disease preventing applications of in-feed antibiotics. The copolymers were discovered during research into the non-provitamin A activities of beta-carotene. The results of that research showed that the complete and spontaneous oxidation of beta-carotene yields a mixture of compounds consisting predominantly (85%) of beta-carotene-oxygen copolymers (Burton et al., 2014). The entire mixture, referred to as OxBC (Oxidized Beta-Carotene), has demonstrated biological activities that are attributed to the copolymers. Specifically, the biological activities relate to the ability to: 1) prime the innate immune system to more effectively detect and respond to pathogens and 2) to limit the extent of excessive late stage inflammation (Burton et al., 2014 Duquette et al., 2014 Johnston et al., 2014). Of further interest, the active copolymer compounds found in the OxBC product mixture occur naturally in various human foods and animal feeds (Burton et al., 2016).

Avivagen Inc., the Canadian-based company responsible for the discoveries, has developed a commercial product, OxC-beta™ for Livestock (10%), based on the immunomodulatory activities of its active OxBC ingredient. A

previous proof of concept study, conducted in Canada with starter pigs, showed that inclusion of OxBC in the feed provided significant benefits to piglet growth and feed utilization efficiency (Hurnik et al. 2011). The current study was conducted to more fully evaluate the efficacy of OxBC to enhance the growth performance and clinical health of pigs across the entire post-wean growth cycle in animals reared under typical commercial production conditions in Vietnam.

METHODS:

Five hundred (500) weaned barrows and gilts (aged 28 days at study day 0) were used in a 140-day complete wean-to-finish feeding study. The herd was PRRS asymptomatic, and pigs were vaccinated against hog cholera, foot-and-mouth disease and PRRS.

The study design consisted of five treatments with five pens (repetitions) per treatment and twenty pigs per pen. The five treatment groups were as follows: 1) negative control - 0 ppm OxBC with no medications, 2) positive control - 0 ppm OxBC + antibiotics, 3) low dose OxBC - 2 ppm OxBC with no antibiotics, 4) medium dose OxBC - 4 ppm OxBC with no antibiotics, and 5) high dose OxBC - 8 ppm OxBC with no antibiotics.

A commercial open feed formulation program was used in the study. No in-feed or water-administered medications were used in the trial, except for the positive control (group-2), which contained the following: study day 0 to 28 (starter stage) - 150 ppm chlortetracycline and 100 ppm colistin sulphate study day 29 to 84 (grower stage) - 150 ppm chlortetracycline study day 85 to 140 (finisher stage) - no antibiotics were used. OxBC was administered as the commercial product OxC-beta™ for Livestock (10%). The pigs had *ad libitum* access to feed and water throughout the trial.

Pigs were individually weighed at the start of the trial (day 0) and at days 28, 84, and 140. All feed given was weighed daily, the feeders were emptied weekly, and the feed inventory was weighed. Growth performance parameters were calculated for each stage of the production cycle (starter, grower, and finisher) as well as for the overall study period (day 0 to 140).

Statistical analyses were performed using the Statistical Analysis System for Personal Computers (SAS) Version 9.1 (SAS Institute, Cary, NC). Treatment effects were tested with a combination of analysis of variance for continuous variables using the MIXED procedure and Chi^2 analysis, including the Cochrane-Armitage trend test. Dichotomous variables (pigs clinically treated or removed) were created using the *post mortem* and clinical results from the data provided to analyze the number of pigs removed from or treated during the study. The data were analyzed by logistic regression using the GLIMMIX procedure.

RESULTS:

The effect of dietary supplementation with OxBC on the average daily weight gain (ADG), feed conversion ratio (FCR), and final body weight of hogs is shown in Table 1. At the beginning of the study, on day-0, there were no significant differences in body weights among any of the groups ($P > 0.05$). By the end of the starter period, on day-28 of the study, piglets in all three OxBC groups had gained significantly more weight than either the positive or the negative control group. Supplementation with OxBC at 2, 4, or 8 ppm improved the ADG of starter pigs by 21.1, 22.7 and 23.2%, respectively, compared to the non-supplemented negative control group. By comparison, the use of antibiotics in the positive control provided only a 6% improvement in ADG relative to the negative control group. Supplementation with OxBC also benefited the feed efficiency of starter pigs. Significant reductions in FCR for the three OxBC groups ranged from 5.4 to 8.3% compared to the negative control. The FCR of animals in the 4 and 8 ppm OxBC groups was also significantly lower than the positive control antibiotic group.

During the grower period (study days 29 to 84), supplementation with all three doses of OxBC significantly increased ADG by 5.3% relative to the non-supplemented negative control. The growth rate of animals in the positive control group was significantly higher than the negative control, and there was no difference in ADG between the OxBC groups and the positive control group during the grower stage ($p>0.05$). With respect to FCR in grower pigs, all three OxBC groups as well as the positive control group had significantly improved FCR when compared to the negative control. Improvements in FCR were 5.5, 8.7, and 10.7% for 2, 4 and 8 ppm OxBC, respectively. By comparison, the use of antibiotics within the positive control improved FCR by 5.5% relative to the negative control. Grower pigs in the 8 ppm OxBC group had the lowest (best) FCR of all groups, which was statistically lower than both control groups as well as the 2 ppm OxBC group.

During the finisher stage the ADG of animals in the three OxBC groups was numerically superior to that of either the negative or positive control groups, however, the differences did not reach statistical significance.

Supplementation with 4 or 8 ppm OxBC resulted in significant improvements in FCR compared to both control groups. Animals receiving 4 or 8 ppm OxBC had FCRs that were 7.2 and 8.1% lower than the negative control group, respectively. Animals in the 2 ppm OxBC group had an FCR that was 5.2% lower than the negative control, while animals in the positive control group had a 1.4% reduction compared to the negative control, although these differences were not significant. It should be noted that during the finisher stage, animals in the positive control group did not receive antibiotics. It is a common practice and legal requirement in many jurisdictions (including Vietnam), to remove antibiotics from feed during the finisher stage.

For the overall study period (study days 0 to 140) dietary supplementation with OxBC at 2, 4 or 8 ppm led to significant increases in ADG and final body weight relative to both the negative and positive control groups. Improvements with OxBC ranged from 4.5 to 5.2% for overall ADG and from 4.1 to 4.9% for final body weight, compared to the negative control group. The FCR of animals in all three OxBC groups was also significantly improved, by 6.5 to 10.4%, when compared to the negative control and both the 4 and 8 ppm OxBC groups also outperformed the antibiotic control group on FCR. By comparison, the use of antibiotics also provided significant benefits to the overall ADG (2.3%) and final body weight (2.2%) relative to the negative control, however, these benefits were less than those seen in the OxBC groups. The antibiotic control group did not differ significantly from the negative control on overall FCR.

The effect of dietary supplementation with OxBC on diarrhea incidence rate and mortality are shown in Table 2. For each of the 3 study periods and for the overall study, the highest diarrhea rates were observed in the negative control while the lowest rates were observed in the OxBC groups. The starter period (study day 0 to 28) had the highest incidence rate of diarrhea, and the use of OxBC resulted in a significant and dose-dependent reduction in diarrhea occurrence relative to the negative control. The incidence rate of diarrhea in the 8 ppm OxBC group was less than half that of the negative control, and both the 4 and 8 ppm OxBC groups had significantly reduced incidences of diarrhea relative to the positive control antibiotic group. Diarrhea rate in the 2 ppm OxBC group was statistically lower than the negative control group and did not differ from the rate observed in the positive control animals.

The grower and finisher periods of the trial were characterized by considerably lower rates of diarrhea compared to the starter period. Despite the lower background rate of diarrhea observed in these later periods of the trial, use of OxBC continued to provide significant protection from the disease. Furthermore the disease sparing effect of OxBC occurred in a dose-dependent manner.

For the overall study period, animals in the negative control group had the highest rates of diarrhea while animals in the 8 ppm OxBC group had the lowest rate of diarrhea. OxBC significantly and dose-dependently reduced the overall diarrhea incidence rate relative to both the negative and positive control groups. Analysis of overall mortality rates revealed that relatively few pigs died during the study and that the lowest number of deaths occurred in the OxBC groups. However, there were no significant differences in the rate of mortality among any of the groups. The ability of OxBC to reduce the incidence of diarrhea is consistent with the product's immunomodulatory modes of action, which include the priming of innate immune defenses to more readily detect and clear infections (Johnston et al., 2014). Previous studies in mice demonstrate that oral supplementation with OxBC results in increased levels of innate immune receptors in the gut (Johnston et al., 2014). These immune receptors are responsible for detecting and triggering innate immune responses to species of gram-negative bacteria including E. coli, the pathogen most commonly associated with post-wean diarrhea in young piglets (Francis 2002 Fairbother et al., 2005). The benefits to growth performance and clinical health observed with OxBC in this trial are consistent with those of previous trials in hogs, poultry and cattle (Hurnik et al., 2011 Duquette et al., 2014 Oh et al., 2015,).

Conclusion:

Results of this trial demonstrate the efficacy of OxBC in improving the growth performance and clinical health of pigs reared under commercial production conditions. Treatment with low ppm levels of OxBC, via the feed, led to significantimprovements in the final body weight, overall growth performance and clinical health. OxBC was of significant benefit to growth performance and clinical health during each of the three stages of the hog production cycle (starter, grower, and finisher) with the most apparent benefits observed in young starter piglets. The results of the current study combined with earlier research demonstrate that OxBC represents an efficacious, safe, natural product-derived alternative to the use of antibiotics for growth promotion and disease prevention in hogs. The ability of OxBC to reduce the incidence of diarrhea is clear and consistent for all stages of pig's growth.

Table 1: Effect of dietary OxBC on average daily gain (ADG), feed conversion ratio (FCR), and final body weight of hogs.

	Negative Control	Positive Control*	OxBC (2 ppm)	OxBC (4 ppm)	OxBC (8 ppm)
Body weight day-0 (kg)	7.7±0.13	7.8±0.07	7.6±0.14	7.6±0.11	7.8±0.09
Starter Period – study day 0 to 28					
ADG† (g/head/day)	358^{b}±7.8	381^{b}±11.9	435^{a}±20.0	440^{a}±19.3	442^{a}±14.7
FCR (Feed/Gain)	1.68^{a}±0.03	1.59^{b}±0.03	1.59^{bc}±0.01	1.54^{d}±0.02	1.55^{cd}±0.01
Grower Period – study day 29 to 84					
ADG† (g/head/day)	569^{b}±2.4	598^{a}±16.8	596^{a}±5.4	601^{a}±14.0	600^{a}±10.7
FCR (Feed/Gain)	2.89^{a}±0.14	2.73^{b}±0.03	2.73^{b}±0.05	2.64^{bc}±0.04	2.57^{c}±0.08
Finisher Period – study day 85 to 140					
ADG† (g/head/day)	892±12.6	889±7.6	901±9.1	902±7.1	904±9.2
FCR (Feed/Gain)	3.46^{a}±0.14	3.41^{a}±0.13	3.28^{ab}±0.08	3.21^{b}±0.05	3.18^{b}±0.03
Overall – study days 0 to 140					
ADG† (g/head/day)	656^{c}±4.1	671^{b}±3.4	686^{a}±3.6	690^{a}±1.4	690^{a}±3.2
FCR (Feed/Gain)	3.07^{a}±0.12	2.93^{b}±0.06	2.87^{bc}±0.05	2.80^{c}±0.04	2.76^{c}±0.04
Body weight day-140 (kg)	99.5^{c}±0.46	101.7^{b}±0.48	103.6^{a}±0.53	104.2^{a}±0.26	104.4^{a}±0.45

Values represent means ± SD
Values in the same row with different superscripts (^{a,b,c}) are significantly different ($P<0.05$)
*Animals in the positive control group received the flowing antibiotics in the feed:

- Day 0 – 28 (starter period) 150 ppm chlortetracycline + 100 ppm colistin sulphate
- Day 29 – 84 (grower period) 150 ppm chlortetracycline
- Day 85 – 140 (finisher period) no antibiotic

Table 2: Effect of dietary OxBC on diarrhea rate and overall mortality of hogs.

	Negative Control	Positive Control*	OxBC (2 ppm)	OxBC (4 ppm)	OxBC (8 ppm)
Starter Period – study day 0 to 28					
Diarrhea Rate (%)	$7.93^{a}\pm0.56$	$6.61^{b}\pm0.52$	$5.96^{b}\pm0.20$	$4.86^{c}\pm0.37$	$3.68^{d}\pm0.60$
Grower Period – study day 29 to 84					
Diarrhea Rate (%)	$2.67^{a}\pm0.22$	$2.12^{b}\pm0.12$	$1.97^{b}\pm0.19$	$1.57^{c}\pm0.10$	$1.40^{c}\pm0.12$
Finisher Period – study day 85 to 140					
Diarrhea Rate (%)	$0.87^{a}\pm0.06$	$0.70^{b}\pm0.06$	$0.64^{bc}\pm0.07$	$0.67^{b}\pm0.06$	$0.55^{c}\pm0.08$
Overall – study days 0 to 140					
Diarrhea Rate (%)	$3.09^{a}\pm0.14$	$2.49^{b}\pm0.14$	$2.26^{c}\pm0.06$	$1.89^{d}\pm0.08$	$1.53^{e}\pm0.08$
Mortality Rate (%)	5.0±3.5	3.0±2.7	2.0±2.7	2.0±2.7	2.0±2.7

Values represent means ± SD
Values in the same row with different superscripts (^{a,b,c}) are significantly different ($P<0.05$)
*Animals in the positive control group received the flowing antibiotics in the feed:

- Day 0 – 28 (starter period) 150 ppm chlortetracycline + 100 ppm colistin sulphate
- Day 29 – 84 (grower period) 150 ppm chlortetracycline
- Day 85 – 140 (finisher period) no antibiotic

REFERENCES

Burton G.W., Daroszewski J., Mogg T.J., Nikiforov G.B., and Nickerson J.G. Discovery and characterization of carotenoid-oxygen copolymers in fruits and vegetables with potential health benefits. *J Agric Food Chem* 2016, 64, 3767-3777.

Burton G.W., Daroszewski J., Nickerson J.G., Johnston J.B., Mogg T.J., and Nikiforov G.B. □ -Carotene autoxidation: oxygen copolymerization, non-vitamin A products, and immunological activity. *Can J Chem* 2014, 92, 305-316.

Duquette S.C., Fischer C.D., Feener T.D., Muench G.P., Morck D.W., Barreda D.R., Nickerson J.G., and Buret A. G. Anti-inflammatory effects of retinoids and carotenoid derivatives on caspased-3-dependent apoptosis and efferocytosis of bovine neutrophils. *Am J Vet Res* 2014, 75 (12), 1064-1075.

Fairbother J.M., Nadeau E., and Gyles C.L. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. 2005, *Anim Health Res Rev* 6, 17-39.

Francis D.H. Enterotoxigenic Escherichia coli infection in pigs and its diagnosis. *J Swine Health Prod* 2002, 10, 171-175.

Hurnik D., Daroszewski J., and Burton G.W. Determination of the effect of a fully oxidized □ -Carotene supplement on the immune system and growth performance of weaned pigs. American Association of Swine Veterinarians 42nd Annual Meeting, Phoenix Arizona 2011, Phoenix Arizona 2011, 277-279.

Johnston J.B., Nickerson J.G., Daroszewski J., Mogg T.J., and Burton G.W. Biologically active polymers from spontaneous carotenoid oxidation: a new frontier in carotenoid activity. *PLOS ONE* 2014, 9, (10), e111346.

Oh J-Y., Wei B., Roh J-H., Kang M., Seo H-S., Yoon R-H., Cha S-Y., and Jang H-K. Preventative effects of OxC-beta on a necrotic enteritis challenge model of broiler chickens. Conference abstract, Spring Academic Symposium of the Korean Society of Veterinary Science, Seoul, Korea. April, 2015.

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Effect of Human Isolated LAB Species as Probiotic Supplement for Piglet on Gut Microbiota and Intestinal Morphology

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ABSTRACT

Probiotic microorganisms have been widely used as a feed supplement. By far, species of probiotic microorganism and its concentration were known to affect the value of a probiotic supplement. However, not much has been known about the effect of microbial origin where it was isolated. It became curious whether human isolated LAB species could be good for animals. The aim of this study was to evaluate the value of human isolated LAB species as probiotic supplement for piglet. In trial 1 and 2, 8 different human isolated LAB species (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus rahmnosus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Streptococcus thermophilus*) were individually supplemented to piglet diet. Then diarrhea score, intestinal villus and microflora of large intestinal contents were measured. Trial 1 found that shape and height of villus of piglet in *Lactobacillus acidophilus* and *Streptococcus thermophilus* groups were less developed and more damaged than those of other groups. This was well associated with the increased diarrhea score in the both groups. Trial 2 found that there were no remarkable difference at height and shape of villus and diarrhea score of weaned piglet among 6 LAB species groups (*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*). This study implied that some of human isolated LAB strains would not be recommend as a probiotic feed supplement to weaned piglet.

INTRODUCTION

Early weanling of piglets is often associated with gut disorders such as mucosal inflammation (Pié et al., 2004), intestinal barrier dysfunction (Wijtten et al., 2011) and diarrhea (Caspary, 1992 van Beers Schreurs et al., 1992 Wu et al., 2015). Because weanling imposes tremendous stress on piglets and is accompanied by marked changes in gastrointestinal physiology, microbiology and immunology (Hampson, 1986 Pluske et al., 1997 Brooks et al., 2001 Heo et al., 2012). Especially, diarrhea during weanling phase has induced many negative effects on growth performance even during grower and finisher phases. One of traditional remedies to reduce diarrhea was the use of antibiotic supplement. Use of dietary antibiotic has been banned in most of the countries. Therefore, other strategies such as limiting feed consumption, regulating nutrient contents of diet, functional component and probiotic supplementation have been applied to reduce diarrhea. A one of these strategies is probiotic supplementation. Probiotic is the one of the most popular supplement in this kind. Use of probiotics has been known (Liong, 2007) to modulate gastrointestinal health, to improve lactose intolerance, to increase natural resistance against infectious diseases in the gastrointestinal tract, to suppress traveler's diarrhea and to reduce bloating. Most of probiotic supplements consisted of lactic acid bacteria combination. Microorganisms derived from *Lactobacillus, Streptococcus, Enterococcus, Bacillus, Clostridium, Bifidobacterium* species and *E. Coli* Niss 1917 were frequently used as probiotic components. Generally, these microorganisms were isolated from gut digesta or excreta of animals or humans. However, it is not well elucidated yet how the source and strains of probiotics affect the proposed benefit. It is also not known what about the use of probiotic strains isolated from different animals. Human isolated strains of LAB has been used in commercial probiotic product. Little has been known how the LAB species isolated from human as probiotic supplement for weaning pigs. Therefore this study was to evaluate the effect of human isolated LAB species as probiotic supplement for weaning piglet.

MATERIAL AND METHODS

Experimental Protocal: The experiment was approved by Institutional Animal Care and Use Committee (IACUC), Kangwon National University (KNU), Republic of Korea. This experiment was conducted in the research building and analysis laboratory at KNU.

Experimental design: Totally 32 of piglets [(Yorkshire × Landrace) × Duroc] were used for 14 days feeding trials. Two trials were conducted in this experiment. 8 treatments were designated according to LAB species and

culture broth supplementation. 6 LAB species in trial I were *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rahmnosus*, *Bifidobacterium bifidum*, *Bifidobacterium breve* and *Streptococcus thermophilus*. After trial I, we could hypothesize that LAB strains producing an amount of organic acid were able to have negative influence on development of gut. Thus, trial II was conducted for establishing the effect of low organic acid producing LAB species as probiotic supplement for piglet on gut microbiota and intestinal morphology. Trial II was designated as 8 treatments following to 6 LAB species (producing low organic acid) and culture broth. 6 LAB species (producing low organic acid) were *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Bifidobacterium breve*.

Diet and LAB species: Commercial mash form diet was used in this experiment. Human isolated LAB species respectively contained 10^6cfu/ml. LAB species were *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus rahmnosus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Streptococcus thermophiles.*

Diarrhea score: Diarrhea score of each feces was the numerical average score of four evaluators. Feces was visually inspected then a numerical score was given based on the following guideline: 1= Very hard and dry, 2= Firm, but not hard, 3= Looks like log, but little or no visible segmentation, 4= Very moisty, but has distinct log shape, 5= Have texture, but defined shape, 6= Have texture, but no defined shape 7= Watery, no texture.

Gut villus height and crypt depth: All pigs were sacrificed for this experiment. After slaughtering, duodenum, jejunum and ilium were surgically collected. Each intestine was carefully washed by 3% saline and stored in formalin solution. The samples were fixed using paraffin, then sliced 6 *u*m thick then dyed using AzureA and Eosine. Dyed samples were subjected to the microscopic inspection.

Intestinal microbiota: After slaughtering, fecal samples were collected from large intestine of each pigs. Fecal samples were diluted with 1 % peptone solution. Plate counting method was used to count *E. coil, Lactobacillus spp.* and *Salmonella spp.* after cultivating at EMB, MRS and XLD agars.

Statistical analysis: All the data were analyzed using a one-way analysis of variances. The means were subjected to test of significance by Duncan Multiple Range Test using SPSS 22.0 version.

RESULTS AND DISCUSSION

Diarrhea score: Diarrhea scores were varied according to LAB species (Table 1). *Lactobacillus acidophilus*, *Streptococcus thermophiles* and *Bifidobacterium breve* supplementation exerted higher ($P<0.05$) diarrhea scores comparing to other groups.

Table 1. Effect of dietary supplemented LAB species on diarrhea score in weaned piglets

	None	*L. acidophilus*	*L. plantarum*	*L. rhamnosus*	*B. bifidum*	*B. breve*	*S. thermophilus*	Culture media	SEM
Diarrhea score[1)]	3.25^{d}	6.13^{a}	4.17^{bc}	3.75^{cd}	3.25^{d}	4.86^{b}	5.92^{a}	3.50^{d}	0.217

[1)] Scored by the scales as: 1= Hard and dry, 2= Firm, but not hard, 3= Looks like log, but little or no visible segmentation, 4= Very moisty, but has distinct log shape, 5= Have texture, but defined shape, 6= Have texture, but no defined shape 7= Watery, no texture.
[abcd] mean values with different superscripts in the same row within the same parameters differ ($p<0.05$).

Gut villus height and crypt depth: Villus height of duodenum in none supplemented group was the highest ($P<0.05$) compared to LAB supplemented groups. There were no differences between none and LAB supplemented groups in villus height of jejunum and ilium. Crypt depth of duodenum in *L. plantartum* group was deeper ($P<0.05$) than those in *B. longum* group but with no differences compared to other LAB species groups. Crypt depth of the jejunum in *B. longum* group was higher than that in *L. fermentum, L. reuteri, B. bifidum* and none supplemented groups. Villus height: Crypt depth ratio in duodenum in *L. plantarum* supplemented group was the lowest ($P<0.05$) compared to that in other LAB species supplemented groups and none supplemented group.

Table 2. Effect of dietary supplemented LAB species on the morphological parameters of intestines in weaned piglets

	None	*L. plantartum*	*L. fermentum*	*L. reuteri*	*B. bifidum*	*B. lactis*	*B. longum*	Culture media	SEM
Villus height (*u*m)									
Duodenum	490.95^{a}	394.75^{bc}	323.83^{c}	395.39^{bc}	405.05^{b}	389.50^{bc}	405.42^{b}	417.77^{b}	10.209
Jejunum	339.90	313.89	342.32	359.87	341.26	332.77	334.45	332.86	4.382
Ilium	325.69	286.54	320.13	307.72	315.73	314.83	299.70	303.88	4.634
Crypt depth (*u*m)									
Duodenum	271.25^{ab}	305.66^{a}	231.33^{bc}	247.37^{b}	253.58^{b}	250.46^{b}	246.31^{b}	198.18^{c}	6.130
Jejunum	179.65^{b}	208.46^{ab}	182.16^{b}	187.49^{b}	199.08^{b}	213.15^{ab}	244.80^{a}	222.42^{ab}	5.424
Ilium	211.21	195.84	185.61	198.41	198.23	194.43	208.21	198.87	3.642
Villus height: crypt depth									
Duodenum	1.84^{ab}	1.29^{d}	1.41^{cd}	1.61^{bcd}	1.63^{bcd}	1.56^{bcd}	1.65^{bc}	2.14^{a}	0.050
Jejunum	1.99	1.57	1.89	1.99	1.77	1.56	1.39	1.54	0.059
Ilium	1.55	1.50	1.74	1.57	1.61	1.63	1.45	1.54	0.029

^{abcd} mean values with different superscripts in the same row within the same parameters differ ($p<0.05$).

Gut Microbiota: There were no significant differences in the population of *Lactobacillus spp*. and *E.coli.* in large intestinal content among all treatments. *Salmonella spp.* was not detected in all samples.

Table 3 Effect of dietary supplemented LAB species on microbial population in large intestinal content

	None	*L. plantartum*	*L. fermentum*	*L. reuteri*	*B. bifidum*	*B. lactis*	*B. longum*	Culture media	SEM
***Lactobacillus spp.* (log10 CFU/g)**	8.44	8.47	8.28	8.32	8.46	8.45	8.42	8.27	0.038
***E.coli* (log10 CFU/g)**	6.13	6.08	6.36	6.41	6.46	6.04	6.09	6.35	0.063
***Salmonella spp.* (log10 CFU/g)**	ND	ND	ND	ND	ND	ND	ND	ND	

CONCLUSION AND IMPLICATION

This study found that dietary supplemented LAB species affected both intestinal morphology and diarrhea scores. There was no remarkable differences in diarrhea score and intestinal morphology between none supplemented and human isolated LAB species supplemented groups. Therefore, it could be concluded that the human isolated LAB species in this experiment and their combination would not be recommended as a probiotic supplement for weaned piglets.

KEYWORD : LAB species, Piglet, Probiotic, Gut microbiota, Morphology

REFERENCES

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption. *The American Journal of Clinical Nutrition*,*55*(1), 299S-308S.

Hampson, D. J. (1986). Alterations in piglet small intestinal structure at weaning.*Research in veterinary science*,*40*(1), 32-40.

Heo, J. M., Opapeju, F. O., Pluske, J. R., Kim, J. C., Hampson, D. J., & Nyachoti, C. M. (2013). Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post]weaning diarrhea without using in]feed antimicrobial compounds.*Journal of animal physiology and animal nutrition*,*97*(2), 207-237.

Liong, M. T. (2007). Probiotics: a critical review of their potential role as antihypertensives, immune modulators, hypocholesterolemics, and perimenopausal treatments.*Nutrition reviews*,*65*(7), 316-328.

Pluske, J. R., Hampson, D. J., & Williams, I. H. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review.*Livestock production science*,*51*(1), 215-236.

Pié, S., Lallès, J. P., Blazy, F., Laffitte, J., Sève, B., & Oswald, I. P. (2004). Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets.*The Journal of nutrition*,*134*(3), 641-647.

Van Beers]Schreurs, H. M. G., Vellenga, L., Wensing, T., & Breukink, H. J. (1992). The pathogenesis of the post] weaning syndrome in weaned piglets a review.*Veterinary Quarterly*,*14*(1), 29-34.

Wu, Y., Jiang, Z., Zheng, C., Wang, L., Zhu, C., Yang, X. & Ma, X. (2015). Effects of protein sources and levels in antibiotic-free diets on diarrhea, intestinal morphology, and expression of tight junctions in weaned piglets.*Animal Nutrition*,*1*(3), 170-176.

Wijtten, P. J., van der Meulen, J., & Verstegen, M. W. (2011). Intestinal barrier function and absorption in pigs after weaning: a review.*British journal of nutrition*,*105*(07), 967-981.

O-26-10

Phytate-hydrolyzing Bacillus sp. Isolated from Pig Gastrointestinal Tract

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INTRODUCTION

Phosphorous is categorized as one of the essentials minerals besides calcium, potassium, sodium, chlorine, magnesium and sulfur for fundamental maintenance of life, growth and reproduction of livestock. Shortage of phosphorous confers high susceptibility to diseases, high mortality, poor growth, reproduction disturbances and fragile bones in livestock (McClure, 2008). Phosphorous available in grains are mainly stored in the form of phytic acid (Israr et al., 2013). However, feeds of plant origin hold 50% - 80% of their total phosphorous in the form of phytate (Harland and Morris, 1995). Phytase (myo-inositol hexakisphosphate phosphohydrolase) is an enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate).

Monogastric animals, such as swine, poultry and fish do not produce phytase naturally in their digestive system. Thus, the majority of the phosphorous in feedstuffs remains unavailable to the animals. In addition, the chelating effect of the phosphate groups causes phytic acid to exhibit anti-nutritive properties (Urbano et al., 2000). These setbacks create disadvantages to both farmers and consumers. Various strategies are sought after to develop efficient feeding such as through the supplementation of inorganic phosphorous, the addition of microbial phytases or phytase producing microbes as probiotics to animal feeds.

The supplementation of inorganic phosphorous in the animal feed used to be sought as a solution (Ravindran et al., 1999 Zyla et al., 1999). However, the utilization of inorganic phosphorous creates setbacks which include: cost ineffectiveness, phosphorous excretion into the environment and the increase in demand for rock phosphate (Xin et al., 2013). Phytase has proven itself beneficial to improve the efficiency of livestock feed. It was also reported that the supplementation of phytase can completely replace other inorganic phosphorous sources and resulted in the reduction of phosphorous discharge into environment (Hung et al., 2014). The addition of microbial phytases and phytase-producing microbes as probiotics to animal feeds have been extensively used in monogastric animal feeds in order to optimize dietary phosphorous availability and reducing phosphorous excretion into the environment (Selle and Ravindran, 2007).

The application of phytase-producing microbes as probiotics to animal feeds may confer an auxiliary benefit. The addition of various specific *Bacillus* sp. to animal feeds is proven to be beneficial. *Bacillus* sp. is able to survive through heat treatment in food processing (Nath et al., 2015). They are also known for its capability to produce essential enzymes that could support digestive function of the gut (Cui et al., 2013). However, there is a limited report available concerning *Bacillus* sp. isolated from pig gastrointestinal tract. Therefore, this study was aimed to isolate *Bacillus* sp. from pigs and to evaluate its potent to hydrolyze phytate.

MATERIALS AND METHODS

Fresh epithelial samples of gastrointestinal tract were collected immediately from local piglets. Approximately 3-5 cm of samples was used as source of isolation.

Species identification of isolates was performed by phenotypic, biochemical and genotypic methods. Phenotypic methods include cell morphology observation, endospore staining and Gram staining. Biochemical characterizations were performed where required. Catalase assay was performed by transferring the isolate on a wetted glass slide to observe release of oxygen-gas-forming bubbles. Genotypic methods performed by amplifying 16S rRNA gene by using a set of primers (27F and 1492R) and PCR product was sequenced. Homology searches were done to identify the species of bacteria.

Screening for phytate degrading bacteria was done by culturing the isolates on modified phytase screening medium (MPSM) agar medium containing 2% dextrose, 0.5% $(NH_4)_2SO_4$, 0.05% KCl, 0.05% $MgSO_4$, 0.001% $MnSO_4$, 0.2% $CaCl_2$, 0.4% phytic acid sodium salt and 1.5% bacteriological agar, pH 7.0. Clear halo zones were observed up to 72 hours of incubation at 37°C.

Other various enzyme assays were also conducted, which include amylase, cellulase and protease assay. Amylase assay was performed based on the enzyme hydrolysis starch. Microbial colonies capable to hydrolyze starch (1% soluble starch) on the media agar could be recognized by formation of their surrounding clear halo after staining

with Lugol solution. Cellulase assay was performed based on the enzyme hydrolysis of cellulose. Microbial colonies capable to hydrolyze cellulose (1% Carboxymethylcellulose) on the media agar can be recognized by formation of their surrounding clear halo after staining with Congo Red solution. Protease assay was performed using 3% skim milk agar. Microbial colonies capable to hydrolyze protein on the media could be recognized by formation of their surrounding clear halo.

RESULTS AND DISCUSSION

In this study, six isolates obtained from local piglets GI tract are identified to be rod-shaped, gram positive (Figure 1) with endospore and catalase positive. 16S rRNA gene analyses recognize isolates to be *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus aryabhattai*, *Bacillus cereus* and *Bacillus pumilus*.

The result of enzyme assays shows variable level of enzymes activities. All Bacillus tested exhibit variable phytase activities. *Bacillus amyloliquefaciens* exhibits highest phytase activity followed by *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus aryabhattai* and *Bacillus cereus* and *Bacillus pumilus* exhibit minimum or no phytase activity after 72 hours incubation at 37ºC (Table 1).

In addition to phytase activities, other biomolecule degrading enzyme activities were evaluated as shown in Table 1. All isolates are able to show enzymatic activities against starch, cellulose and protein except for *Bacillus pumilus*. *Bacillus pumilus* exhibits only proteolytic activities and no enzymatic activities against starch and cellulose.

Bacteria of the Bacillus genus are among the most widespread microorganisms in nature. Being ubiquitous in the environment they find their way easily into food products. *Bacillus* sp. consistently enters the gastrointestinal track of healthy organisms. It has also been suggested that gut microbiota is responsible for degradation of phytic acid. Bacillus bacteria could support digestive function of the gut producing essential enzymes that helps to promote efficient absorption of nutrients (Khan et al., 2013).

The results of present study supports the application of *Bacillus* sp. obtained may contribute to phosphorous availability for digestion in pigs especially *Bacillus amyloliquefaciens*. These findings may lead to further evaluation of utilizing *Bacillus amyloliquefaciens* as feed supplementation for improvement of animal productivity and reduction of phosphorous emission to the environment.

ACKNOWLEDGEMENT

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KEYWORD : Bacillus sp, Isolation, Phytase-like activity, pigs

Table 1: Phytase hydrolyzing activity and other enzymes activities of *Bacillus* sp. isolated from pig gastrointestinal tract

Isolates	Phytase	Amylase	Cellulase	Protease
B. amyloliquefaciens	+++	yes	yes	yes
B. subtilis	++	yes	yes	yes
B. megaterium	++	yes	yes	yes
B. aryabhattai	++	yes	yes	yes
B. cereus	-/+	yes	yes	yes
B. pumilus	-/+	no	no	yes

-/+: no or minimum activity; +: weak activity; ++: moderate activity and +++: strong activity.

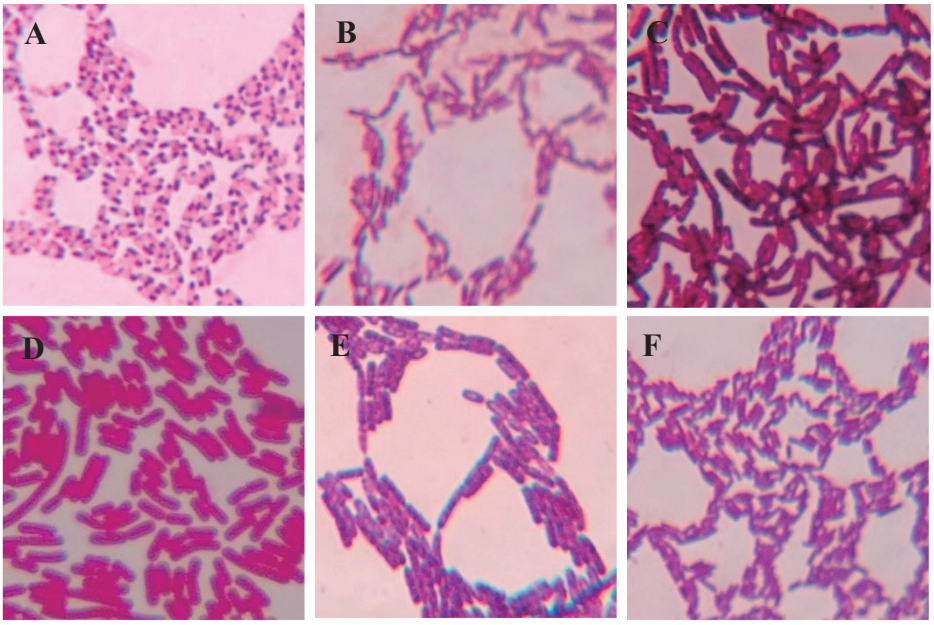

Figure 1 Cell morphology of *Bacillus* sp. isolated from pig gastrointestinal tract

Bacterial cell morphology was characterized by Gram stain technique and observed under microscope with total magnification point 1000x. A: *Bacillus amyloliquefaciens*; B: *Bacillus subtilis*; C: *Bacillus megaterium*; D: *Bacillus aryabhattai*; E: *Bacillus cereus*; F: *Bacillus pumilus*.

REFERENCES

Cui, C., Shen, C.J., Jia, G., and Wang, K.N. (2013). Effect of dietary Bacillus subtilis on proportion of Bacteroidetes and Firmicutes in swine intestine and lipid metabolism Genetics and Molecular Research, 12: 1766-1776.

Harland, B.F., and Morris, E.R. (1995). Phytate: A good or a bad food component. Nutrition Research, 15: 733-754.

Hung, L.T., Thanh, N.T., Pham, M.A., and Browdy, C.L. (2014). A comparison of the effect of dietary fungal phytase and dicalcium phosphate supplementation on growth performances, feed and phosphorus utilization of tra catfish juveniles (*Pangasianodon hypophthalmus Sauvage*, 1878). Aquaculture Nutrition, 21: 10–17.

Israr, B., Frazier, R.A., and Gordon, M.H. (2013). Effects of phytate and minerals on the bioavailability of oxalate from food. Food Chemistry, 141: 1690-1693.

Khan, M., Nakkeeran, E., Umesh-Kumar, S. (2013). Potential Application of Pectinase in Developing Functional Foods. Annual Review of Food Science and Technology, 4: 21-34.

Lei, X.G., Weaver, J.D., Mullaney, E., Ullah, A.H., and Azain, M.J. (2013). Phytase, a New Life for an "Old" Enzyme. Annual Review of Animal Biosciences, 1: 283-309.

McClure, S.J. (2008). How minerals may influence the development and expression of immunity to endoparasites in livestock. Parasite Immunology, 30: 89-100.

Nath, S., Chowdhury, S., and Dora, K.C. (2015). Application of *Bacillus* sp. as a biopreservative for food preservation. International Journal of Engineering Research and Applications, 5: 85-95.

Ravindran, V., Selle, P.H., and Bryden, W.L. (1999). Effects of phytase supplementation, individually and in combination, with glycanase on the nutritive value of wheat and barley. Poultry Science, 78: 1588–1595.

Selle, P.H., and Ravindran, V. (2007). Microbial phytase in poultry nutrition. Animal Feed Science and Technology, 135: 1-41.

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., and Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function? Journal of Physiology and Biochemistry, 56: 283-294.

Zyla, K., Gogol, D., Koreleski, J., Swiatkiewicz, S., and Ledoux, D.R. (1999). Simultaneous application of phytase and xylanase to broiler feeds based on wheat: feeding experiment with growing broilers. Journal of the Science of Food and Agriculture, 79: 1841–1848.

O-27-1 Dose-comparison study of Lactococcus lactis producing heme oxygenase-1 for treatment of dextran sulfate sodium-induced acute colitis in mice

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OBJECTIVE

Over the last two decades, recombinant technologies in lactic acid bacteria (LAB) have undergone rapid development, facilitating the production by LAB of diverse proteins of interest (Wyszyńska et al., 2015). Additionally, many researchers have proposed that genetically modified strains of LAB (gmLAB) could be valuable as live vectors for the delivery of biomedical proteins, including (auto-)antigens, allergens, and cytokines, to the host mucosal surface. This work has suggested that the mucosal delivery of such proteins using gmLAB could serve as an attractive strategy for maintenance and improvement of animal and human health via prevention and therapy of various disorders such as infectious diseases, inflammatory diseases of the gastrointestinal tract, allergies, and autoimmune diseases (Cano-Garrido et al., 2015).

In a previous study, we reported construction of a genetically modified strain of *Lactococcus lactis* (designated NZ-HO) that secreted recombinant mouse heme oxygenase-1 (HO-1) as an extracellular protein (Shigemori et al., 2015). HO-1 is a rate-limiting enzyme that catabolizes the conversion of heme to biliverdin, free iron, and carbon monoxide. Physiologically, native HO-1 production in humans is induced by various stimuli (including oxidative stress and inflammatory stimuli), and exerts anti-inflammatory and cell protective effects through various mechanisms (Abraham and Kappas, 2008). Therefore, we investigated the potential anti-inflammatory effects of NZ-HO in mice exhibiting dextran sulfate sodium (DSS) -induced acute colitis. Daily oral treatment with NZ-HO significantly improved symptoms of acute colitis in mice as compared with a vector control strain (NZ-VC) (Shigemori et al., 2015). However, the effective dose of NZ-HO for alleviation of colitis was not elucidated. Therefore, in the present study, we compared the efficacy of a range of oral doses of NZ-HO in mice with DSS-induced acute colitis to assess the correlation between improvement in colitis and dosage of NZ-HO.

METHODOLOGY

Bacterial strains and growth condition: *L. lactis* NZ9000 (NZ9000) harboring an empty expression vector pNZ8148#2:SEC (Shigemori et al., 2014) or a mouse HO-1 expression vector pNZ8148#2:SEC-HO-1 were constructed as described in our previous study, where the two strains were designated as NZ-VC and NZ-HO, respectively (Shigemori et al., 2015). These recombinant NZ9000 strains were grown as static cultures at 30℃ in GM17cm. This medium consisted of M17 broth (Becton Dickinson and Company, Sparks, MD, USA) supplemented with 0.5% glucose and 10 μg/mL chloramphenicol.

Preparation of bacterial inocula: A 47.5-mL aliquot of fresh GM17cm broth was inoculated with 2.5 mL of an overnight culture of a recombinant NZ9000 strain the resulting culture was incubated at 30℃ until the OD_{600} reached 0.4. To induce expression of the recombinant gene, nisin (MoBiTec GmbH, Gottingen, Germany) was added to the culture medium to a concentration of 1.25 ng/mL, and the culture was further incubated for another 3 h. Bacterial cells were harvested by centrifugation at 3,000 $\times g$ and 4℃ for 20 min, and then washed twice with ice-cold phosphate-buffered saline (saline). The resulting cells were re-suspended in saline at 2.5×10^{10}, 2.5×10^{8}, or 2.5×10^{6} CFU/mL. The bacterial suspensions were immediately administerd to mice (200 μL/mouse).

Induction, treatment, and assessment of acute colitis in mice: Twenty-four female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan) at 7 weeks of age. Throughout the study, mice were housed under conventional conditions, and provided with *ad libitum* access to a standard diet (MF, Oriental Yeast Co., LTD., Tokyo, Japan) and (as described below) to sterile water without or with 3% DSS (MW = 36,000-50,000 Da MP Biomedicals, LLC, Solon, OH, USA). After 2 weeks of acclimation to these conditions (using pure water), mice (20 ± 2 g each) were randomly allocated into four groups of 6 animals each. Acute colitis was induced in all mice by by shifting the animals to drinking water containing 3% DSS for 6 consecutive days (day 0-5). Animals were shifted back to pure water on day 5. We monitored drinking volumes throughout the experimental period and confirmed that all groups consumed similar volumes (4 ± 2 mL/mouse/day data not shown). From day -2 to day 5 (*i.e.*,

starting 2 days before the shift to DSS water and continuing through the last day on DSS water), mice of the respective groups were administered with a once-daily oral dose of 200 μ L of saline or of one of the bacterial inocula (corresponding to 5×10^5, 5×10^7, or 5×10^9 CFU of NZ-HO/animal/day). To assess the severity of colitis, macroscopic symptoms (*i.e.*, body weight loss, fecal bleeding, and stool consistency) were monitored daily in all mice after the initiation of DSS exposure, and these parameters were used to generate a Disease Activity Index (DAI) score (maximum score = 12) as described previously (Shigemori et al., 2015). On day 8, mice were euthanized, and the colon was extirpated and its length was measured. The experimental procedure was conducted in accordance with the Regulations for Animal Experimentation of Shinshu University.

Statistical analysis: Statistical analyses were performed using a statistical software package (ystat2004.xls, Igakutosho Shuppan, Tokyo, Japan). Bonferroni's method was used to determine the statistical differences among the groups. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Dysregulation of intestinal inflammation can be caused by a variety of events, including the breakdown of epithelial barrier function, uncontrolled replication of bacteria or viruses, excess immune activation, and disruption of homeostatic balance the resulting inflammation can have significant negative impacts on health in both clinical and veterinary settings (Jiminez et al., 2015). Several laboratories (Steidler et al., 2000 Braat et al., 2006 Hanson et al., 2014) have proposed the treatment (in either humans and animals) of intestinal inflammatory disorders using gmLAB for the production and localized delivery (to the intestine) of proteins with anti-inflammatory properties. These proposals are based on the demonstrated effectiveness, safety, and adaptability of LAB in animals the easy noninvasive oral administration of the microbes and the low production costs of these agents (Cano-Garrido et al., 2015). In fact, we have previously demonstrated that oral delivery of mouse HO-1 using *L. lactis* alleviates DSS-induced acute colitis in mice, and the corresponding strain (NZ-HO) was proposed as new therapeutic agent for the treatment of inflammatory insults to the intestine (Shigemori et al., 2015).

In the present study, different oral doses of NZ-HO were tested in mice with DSS-induced acute colitis. The DAI scores of the saline-treated group increased in a time-dependent manner after DSS exposure, reached a mean of 8.67 on day 8 (Fig. 1A). The DAI score reflects the seriousness of macroscopic symptoms (*i.e.*, body weight loss, fecal bleeding, and stool consistency), and the maximum score is 12. Therefore, the observed score indicated that the saline group developed severe colitis by day 8. There was no significant difference in mean day-8 DAI score between groups treated with saline and 5×10^5 CFU NZ-HO (Fig. 1A). On the other hand, the mean day-8 DAI scores of groups treated with 5×10^7 and 5×10^9 CFU NZ-HO (6.33 and 4.50, respectively) were significantly lower than that of the saline group (Fig. 1A). Consistent with the decreased DAI scores, tissue injury-associated colon shortening was significantly improved in groups treated with 5×10^7 and 5×10^9 CFU NZ-HO compared with that in the saline-treated group (Fig. 1B). Taken together, these results demonstrate that daily oral administration with 5×10^7 or 5×10^9 CFU NZ-HO provided beneficial effects in treatment for DSS-induced acute colitis, whereas daily oral administration with 5×10^5 CFU NZ-HO did not. The results of the present study are consistent with dose dependence of NZ-HO efficacy.

In our previous study, we demonstrated that NZ-HO inhibits massive infiltration of inflammatory cells into the colonic mucosa in mice with colitis (Shigemori et al., 2015). In addition, we showed that NZ-HO suppresses the pro-inflammatory cytokines IL-1 α and IL-6 while inducing the anti-inflammatory cytokine IL-10 (Shigemori et al., 2015). Although the anti-inflammatory properties of HO-1 mediate various physiological pathways, a positive feedback circuit consisting of HO-1 and IL-10 in monocytes and macrophages is a critical mechanism in HO-1-mediated anti-inflammatory effects (Lee and Chau, 2002). Therefore, the beneficial effects of NZ-HO for colitis may involve the HO-1/IL-10 pathway, a model supported by the results of the present study.

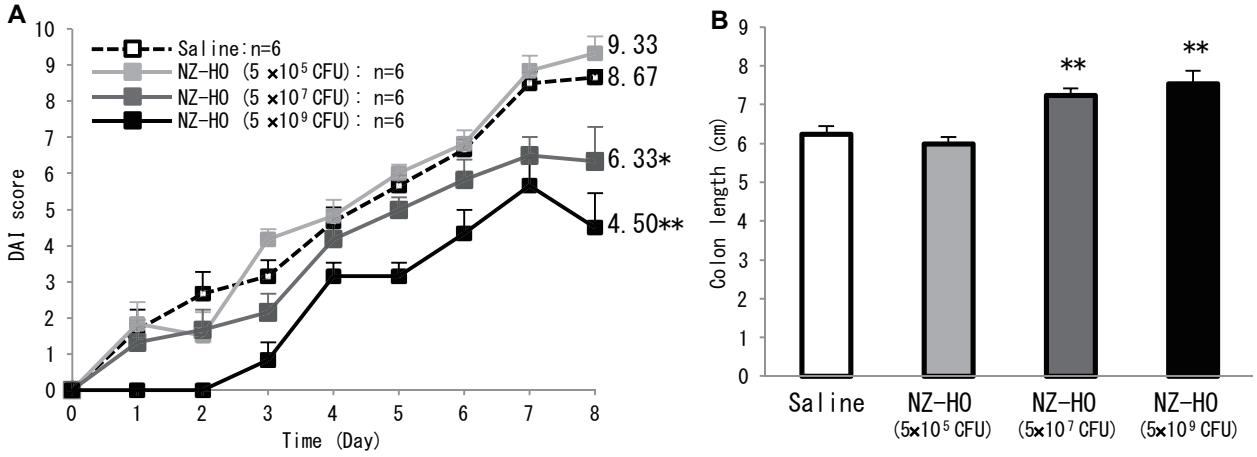
CONCLUSION

In conclusion, this study clearly demonstrated that NZ-HO has beneficial effects for the treatment of DSS-induced acute colitis in mice. Efficacy required a minimum dose of 5×10^7 CFU/day and exhibited dose dependence. These results are important in the development of NZ-HO as a mucosal therapeutic agent for treating intestinal inflammatory diseases in human and animals.

FIGURE LEGEND

Fig. 1. The chronological change in the DAI score of mice in each group (A). On day 8, the colon lengths of mice in each group were measured (B). Data are presented as the mean + standard error ($n = 6$). $^*P < 0.05$, $^{**}P < 0.01$ vs.

saline group.



KEYWORD：Acute colitis, gmLAB, Heme oxygenase-1, Mice, Mucosal delivery

REFERENCES

Abraham NG., Kappas A. 2008. Pharmacological and clinical aspects of heme oxygenase. Pharmacol Rev. 60(1):79-127.

Braat H., Rottiers P., Hommes DW., Huyghebaert N., Remaut E., Remon JP., van Deventer SJ., Neirynck S., Peppelenbosch MP., Steidler L. 2006. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol. 4(6):754-9.

Cano-Garrido O., Seras-Franzoso J., Garcia-Fruitós E. 2015. Lactic acid bacteria: reviewing the potential of a promising delivery live vector for biomedical purposes. Microb Cell Fact. 14:137.

Hanson ML., Hixon JA., Li W., Felber BK., Anver MR., Stewart CA., Janelsins BM., Datta SK., Shen W., McLean MH., Durum SK. 2014. Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice. Gastroenterology. 146(1):210-221 e13.

Jiminez JA., Uwiera TC., Douglas Inglis G., Uwiera RR. 2015. Animal models to study acute and chronic intestinal inflammation in mammals. Gut Pathog. 7:29.

Lee TS., Chau LY. 2002. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med. 8(3):240-246.

Shigemori S., Oshiro K., Wang P., Yamamoto Y., Wang Y., Sato T., Uyeno Y., Shimosato T. 2014. Generation of dipeptidyl peptidase-IV-inhibiting peptides from β -lactoglobulin secreted by *Lactococcus lactis. Biomed* Res Int. 2014:393598.

Shigemori S., Watanabe T., Kudoh K., Ihara M., Nigar S., Yamamoto Y., Suda Y., Sato T., Kitazawa H., Shimosato T. 2015. Oral delivery of *Lactococcus lactis* that secretes bioactive heme oxygenase-1 alleviates development of acute colitis in mice. Microb Cell Fact. 14(1):189.

Steidler L., Hans W., Schotte L., Neirynck S., Obermeier F., Falk W., Fiers W., Remaut E. 2000. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science. 289(5483):1352-1355.

Wyszyńska A., Kobierecka P., Bardowski J., Jagusztyn-Krynicka EK. 2015. Lactic acid bacteria--20 years exploring their potential as live vectors for mucosal vaccination. Appl Microbiol Biotechnol. 99(7):2967-2977.

O-27-2 An immunostimulatory oligodeoxynucleotide from lactic acid bacteria promotes anaphylaxis to ovalbumin in mice

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OBJECTIVE

Anaphylaxis is an acute, systemic allergic reaction (severe type I hypersensitivity) that occurs in humans and other mammals (Sicherer & Leung, 2014). In particular, food anaphylaxis can be induced by peanut, buckwheat (BW), milk, egg, and wheat allergens, among many others. Anaphylactic mouse models can be established by allergen sensitization with several low-dose subcutaneous and intraperitoneal (i.p.) injections of an allergen. Anaphylactic model mice have been developed by many research groups using ovalbumin (OVA) or peanuts as allergens (Kulis et al., 2013 Batista et al., 2014 Tordesillas et al., 2014 Tsukamoto et al., 2014). When immunity is established, allergen challenge can be performed using a large quantity of the allergen via intravenous (i.v.) injection to induce anaphylaxis. However, the i.v. challenge requires a complex procedure, and the processing time per challenge is long. We previously developed an anaphylactic mouse model using BW antigen and a strong immunostimulatory CpG oligodeoxynucleotide from the *lac Z* gene of *Streptococcus thermophilus* ATCC19258 (MsST) using only i.p. injections (Yamamoto et al., 2016). Although the mice of this established model show severe symptoms, the underlying mechanisms remain unknown due to the lack of a BW-specific antibody, and because there is very limited data on BW-induced anaphylaxis available in the literature. Therefore, we investigated the effects of MsST on anaphylactic shock in mice administered only i.p. injections of OVA, for which much data has accumulated from past research.

METHODOLOGY

Materials : Endotoxin-free phosphorothioate-bound oligodeoxynucleotide MsST (5'-CAGGACGTTGTATCACTGAA-3') was synthesized and desalted by Integrated DNA Technologies, Inc. (Coralville, IA, USA). Prior to use, the MsST was reconstituted in endotoxin-free water and passed through a 0.22-μm pore microfilter (Nihon Millipore K.K., Tokyo, Japan). Albumin from chicken egg white (OVA, grade V Sigma, St. Louis, MO) was used as an antigen.

Protocol for inducing anaphylactic shock : Pathogen-free female BALB/c mice (4 weeks of age) were purchased from Japan SLC (Shizuoka, Japan) and kept under temperature- and light-controlled conditions. Mice were given a standard diet of Labo MR Breeder (Nihon Nosan Co., Kanagawa, Japan) and sterile water *ad libitum*. After a preliminary period of 1 week, the BALB/c mice (5 weeks of age) were i.p. sensitized with 100 μg of OVA and aluminum hydroxide gel as an adjuvant (OVA to adjuvant ratio of 1:20) with or without 100 μg of MsST. After 2 weeks, the mice were challenged by i.p. injection with 1 mg of OVA and 1.3 mg of alum gel. The severity of the anaphylactic symptoms was assessed 30 min after the final challenge. The behaviors of the mice were recorded with a video camera for 1 min (from 0 to 20 s: normal condition 20 to 40 s: while beating the cage 40 to 60 s: while touching the mice with a glass rod). The challenges were assessed based on the video data using anaphylactic symptom scores graded on a scale from 1 to 4 (Table 1). In addition, body temperature was measured with a rectal thermal probe at baseline and 30 min after the OVA challenge. All experimental procedures were conducted in accordance with the Regulations for Animal Experimentation of Shinshu University, and the animal protocol was approved by the Committee for Animal Experiments of Shinshu University. Based on national regulations and guidelines, all experimental procedures were reviewed by the Committee for Animal Experiments, and final approval was obtained from the president of Shinshu University.

Statistical analysis : Statistical analyses were performed using a statistical software package (Igakutosho Shuppan, Tokyo, Japan). All data were analyzed by one-way analysis of variance with the post-hoc Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$. All data are expressed as the mean ± standard error (SE).

RESULTS AND DISCUSSION

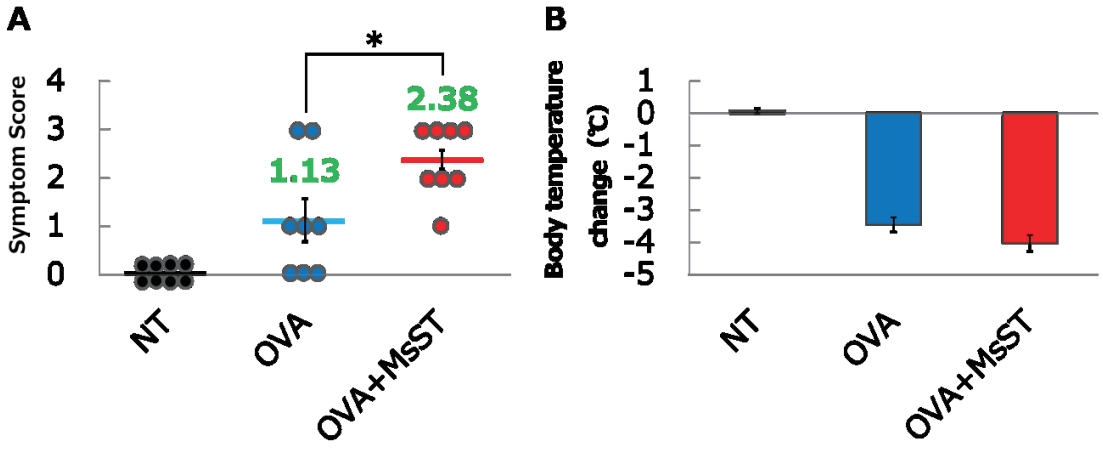
We successfully developed an OVA anaphylactic shock model using MsST, which promoted OVA-induced anaphylactic shock. The anaphylactic symptom scores were significantly higher in the MsST+ mice than in the MsST- mice (Fig. 1A). The body temperature tended to decrease after OVA challenge in the MsST+ mice (Fig. 1B).
A well-known Th1-type adjuvant is CpG oligodeoxynucleotide, which is a toll-like receptor 9 (TLR9) agonist (Kreig, 2006). The use of a Th1-directing adjuvant may be useful in both preventing and treating allergy by inducing high levels of antigen-specific IgG (Kulis et al., 2013). In fact, it was reported that CpG oligodeoxynucleotides inhibited allergic reactions, including pollinosis and asthma, in mice (Broide et al., 1998 Magone et al., 2000). In addition, inhibitory oligodeoxynucleotides, such as TLR9 inhibitor, have been shown to suppress allergic reactions to OVA (Ito et al., 2013). In contrast, other studies have demonstrated that CpG oligodeoxynucleotides aggravated allergic diseases, including BW-induced anaphylaxis and atopic dermatitis (Yamamoto et al., 2016 Wang et al., 2015). Although the mechanism behind this BW anaphylactic model appears to be related to an overexpression of interferon gamma, the details remain unknown. There is currently no specific antibody to BW antigen that can be used to study the mechanism behind BW allergy, and this lack represents a large obstacle to our research. In contrast, there is a specific antibody to OVA, and there have been numerous reports of the use of OVA allergy models. Therefore, this OVA anaphylactic model aggravated by MsST may be useful for clarifying the mechanisms underlying allergies induced by TLR9 ligands. In this way, this simple model may contribute to the further development of anaphylaxis research.

FIGURE LEGEND

Table 1. Anaphylactic symptom scores

Score	Status
0	No symptoms
1	Moving, scratching, rubbing of face and snout
2	Crouching, open eyes, moving in response to tapping of the cage with a glass rod
3	Weak cyanosis, no moving in response to tapping of the cage with a glass rod, moving in response to tapping of the body with a glass rod
4	Severe cyanosis, no moving in response to tapping of the cage with a glass rod

Fig. 1. (A) Anaphylactic symptom scores and (B) Body temperature change of non-treated (NT) mice and mice sensitized with ovalbumin (OVA) and MsST, and induced to experience anaphylaxis. Data are shown as the mean ± standard error for each animal group n = 5 to 8 mice/group. *P < 0.05 for the indicated group(s).



KEYWORD：anaphylactic shock, ovalbumin, oligodeoxynucleotide, Streptococcus thermophilus, adjuvant

REFERENCES

Yamamoto, Y., S. Shigemori, S. Nigar, K. Oshiro, Y. Wang, T. Sato and T. Shimosato. 2016. Development of a simple

IgE-independent anaphylactic model using buckwheat antigen and B-type CpG oligodeoxynucleotide from *Streptococcus thermophiles*. Animal Science Journal 87, 710-717.

Sicherer, S. H and D.Y. Leung. 2014. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2013. Journal of Allergy and Clinical Immunology 133, 324–334.

Kulis, M., B. Gorentla, A. W. Burks, X. P. Zhong. 2013. Type B CpG oligodeoxynucleotides induce Th1 responses to peanut antigens: modulation of sensitization and utility in a truncated immunotherapy regimen in mice. Molecular Nutrition Food Research 57, 906–915.

Batista, N. V., R. V. Pereira, M. L. Noviello, L. P. Dourado, D. A. Perez, G. Foureaux, A. J. Ferreira, A. V. Ferreira, D. C. Cara. 2014. Prolonged ingestion of ovalbumin diet by sensitized mice improves the metabolic consequences induced by experimental food allergy. Clinical and Experimental Immunology 178, 416–427.

Tordesillas, L., R. Goswami, S. Benede, G. Grishina, D. Dunkin, K. M. Jarvinen, S. J. Maleki, H. A. Sampson, M. C. Berin. 2014. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. The Journal of Clinical Investigation 124, 4965–4975.

Tsukamoto, K., K. Yamamoto, T. Makino. 2014. Counteractive effect of Paeonia lactiflora root constituent mudanpioside E against suppressive effect of Shoseiryuto-extract on passive cutaneous anaphylaxis reaction in mice. Journal of Ethnopharmacology 153, 884–889.

Kreig, A. M. 2006. Therapeutic potential of Toll-like receptor 9 activation. Nature Reviews Drug Discoverry 5, 471-484.

Broide, D., J. Schwarze, H. Tighe, T. Gifford, M. D. Nguyen, S. Malek, U. J. Van, O. E. Martin, E. W. Gelfand, E. Raz. 1998. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. The Journal of Immunology 161, 12, 7054-62.

Magone, M. T., C. C. Chan, L. Beck, S. M. Whitcup, E. Raz. 2000. Systemic or mucosal administration of immunostimulatory DNA inhibits early and late phases of murine allergic conjunctivitis. European Journal of Immunology 7, 1841-50.

Ito, Y., S. Shigemori, T. Sato, T. Shimazu, K. Hatano, H. Otani, H. Kitazawa, T. Shimosato. 2013. Class Ⅰ / Ⅱ hybrid inhibitory oligodeoxynucleotide exerts Th1 and Th2 double immune suppression. FEBS Open Bio 3, 41-45

Wang, Y., Y. Yamamoto, S. Shigemori, T. Watanabe, K. Oshiro, X. Wang, P. Wang, T. Sato, S. Yonekura, S. Tanaka, H. Kitazawa, T. Shimosato. 2015. Inhibitory / Suppressive Oligodeoxynucleotide Nanocapsules as Simple Oral Delivery Devices for Preventing Atopic Dermatitis in Mice. Molecular Therapy 23, 2, 297-309.

O-27-4

Sustainability Tools for Small Dairy Farmers in Thailand

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INTRODUCTION

Dairy cattle in the tropics faces unfavorable environments and produced less milk as compare to cows in temperate environments. Majority of dairy farmers in SE Asia maintains between 75% to 95% Holstein Friesian (HF) blood through upgrading breeding system. In Thailand more than 90% of 300,000 dairy cow population maintained 87.5% HF blood and yields an average of 20 kg, 15 kg, and 10 kg for early, mid, and late lactation periods under small farm conditions. An average milk yield of dairy cow in Thailand is 4,000 kg per 300 day lactation period (DLD, 2015). The upper 10% of cows from medium to large farms yield an average of 6,500 kg from 30 kg, 23 kg, and 18 kg early, mid, and late lactations, accordingly. Majority of cow housing is low roof set and no fan and sprinkle with low feed and feeding method. It is known that feed and feeding are important factors affecting milk yield and cow conditions. Many researchers and extension officers introduced many alternative feed and feeding methods to increase milk yield and quality of small dairy farmers (Cheva-Isarakul, 1990 Choophen et al, 2005). Rice straw is the main roughage for dairy cattle whereas crop wastes from agro-industry were supplemented at a minimum quantity (Tumwasorn, 2010). It was reported that 2% and 3.5% of dry matter was consumed by cows raised in small and large farms which exhibited significant milk yield (Kraiprom et al, 2014 Maneerat, 2014). In Thailand partial mixed ration (PMR) , and total mixed fiber (TMF) were introduced in 2008 and 2013. TMR was introduced to dairy farmers in 2015 and now being expanded to larger dairy cooperatives. However, due to limited knowledge in making TMR and its higher cost, farmers are reluctant to use and it need more time for farmers' adoption. It was also observed that less than 5% of farms give sprinkle and fan to cows and small farmers have no access to this technology. This study is intended to evaluate performance of cows exposing to different feed and feeding under different production systems.

MATERIALS AND METHODS

This study used farming system research and extension (FSR/E) model in two dairy cooperatives in eastern Thailand (Ratchaburi and Prajuab Kirikha provinces). Five small dairy farms in each province each of less than 5 milking cows per farm were randomly selected. Due to small number of observation in each farm, the before and after treatment were employed through RBD design with covariate variable on initial milk yield imposed to adjust milk yield at different stage of lactation. Three treatments were imposed : (1) normal feeding/no sprinkle and fan (2) TMR feeding/no sprinkle and fan (3) TMR feeding/with sprinkle and fan. All barns were insulated under roof to reduce heat. TMR was made and ensilaged in double layer bag and called fermented TMR. The total number of 50 cows were allotted in each treatment for a period of 30 days during Summer (April 2015). Sprinkle and fan were applied with thermostat control (every 3 hour for 45 minutes each) starting from 10 am to 4 pm. The proximate analysis of TMR used in this experiment were carried out from the Nutrition laboratory of the Department of Animal Science, Kasetsart University as shown in Table 1. Milk yield and quality, rectal temperature, in barn temperature and outside barn temperature were measured approximately 2 meters from the floor including cost benefit analysis in each system were analyzed through computer package (SAS, 1990). The result of farmers' acceptance and adoption in new feed and management system were analyzed and report.

RESULTS AND DISCUSSIONS

Temperature and moisture

The temperature during the day outside the barn was found to be 3 degree C more than the temperature inside the barn. Inside the barn temperature without sprinkle and fan varied from 27.2 degree C in the morning a 6 am (range between 26.0 to 30.0 degree C) and increased to 39.8 degree C in the afternoon at 1 pm (range between 39.0 to 40.9 degree C). The rectal temperature of cow before sprinkled and fan at 10 am averaged 38.6 degree C (range between 37.8 to 39.7 degree C) and increased to 39.8 degree C (range between 39.0 to 40.9 degree C) in the afternoon at 1 pm. After being sprinkle and fan the cow body temperature averaged 38.6 degree C at 10 am (range between 36.0 to 39.9 degree C) and increased to 38.8 degree C (range between 38.8 to 39.0 degree C) at 1 pm. The monthly average of inside temperature, temperature before imposing sprinkle and fan and after giving

sprinkle and fan were reported in Table 2. It was seen that rectal temperature of cows after being cooled with sprinkle and fan reduced 1 degree C at all times ($p<0.0001$). Temperature inside the barn and rectal temperature before cooling at 1 am was not significantly different while other pairs were significantly different. The moisture contents in the morning averaged 60% and increased to 80% in the afternoon during 1 month period of the study.

Feed Intake

Cows receiving normal feeding with no sprinkle and fan consumed an average of 2.2% of dry matter as compare to 3% and 3.5% in treatment 2 and 3, respectively. Details of feed intake were shown in Table 3. Farmers in cooperative 1 in treatment 1 gave feed to cows at an average of 19.6 kg/h/d as compare to 18.5 kg/h/d in Cooperative 2 with an average of 2.05% of dry matter of cow body weight (450 kg). When TMR was given to cows, the dry matter intake was increased to 2.45% in treatment 2 and increased to 2.9% in treatment 3.

Milk Yield and Composition

Since there was variation in stage of lactation, this study calculated mid-lactation parameters between 60 to 180 days of milking. Cows in the first treatment yielded an average of 12.5 kg/h/d while the cows in treatment 2 and 3 yielded an average milk yield of 15.4 and 17.8 kg/h/d, respectively. An average of 4.2% fat, 3.47% protein, 4.78% lactose, 12.73% total solid and 9.25% solid not fat were obtained after TMR with sprinkle ad fan were applied. Cows with normal feeding and management yielded an average of 4% fat, 3.35% protein, 4.5% lactose, 12.5% total solid and 9.1% solid not fat. The somatic cell count of cows receiving TMR with sprinkle and fan averaged 296,000 cell/ml as compared to 560,000 cell/ml of the control treatment. Table 4 showed milk yield and composition of milk obtained from cows in the three treatments from weekly average.

Cost and Benefit

Details of feed cost per kg of milk were shown in Table 5. The traditional feed cost per day in treatment 1 averaged 135 Baht and the milk yield for mid lactating cows averaged 12.5 kg/h/d. Feed cost per kg of milk in the first treatment was 10.8 Baht/kg. When TMR was fed, the milk yield was increased to 15.5 kg/h/d and feed cost increased to 150 Baht/h/d which yielded feed cost to be 9.67 Baht/kg of milk. Additional sprinkle and fan with TMR feeding pushed milk yield up to 18 kg/h/d and the cost of feeding and management increased to165 Baht/h/d and yielded 9.16 Baht feed cost per 1 kg milk. The realized gross profit obtained from treatment 1, 2, and 3 in Baht/cow/month were 3,000. Bht, 3,960. Bht, and 4,700. Bht, respectively.

Farmers' Participation

It is difficult to change farmers' attitude especially towards new innovation to be implemented under small farm conditions. Farmers' participation in using TMR and cooling device. Variation in performance is usually found since farmers did not follow direction and guidance in feeding TMR. It was observed that high milk yield was obtained from the farm that totally use TMR and not supplement other feed while feeding. If more locally available feed sources were supplemented, more variation in performance would be obtained. Due to small income and shortage of revolving fund, it is difficult to implement the TMR feed and cooling device to small dairy farmers. It is therefore the national policy to stimulate farmers to use TMR and cooling device to increase farm income and sustain number of small dairy farmers which is the majority of dairy farmers in Thailand.

CONCLUSIONS AND RECOMMENDATIONS

Fermented TMR is a new technology in Thailand and extended to dairy farmer few years ago. Farming system research and extension (FSR/E) was implemented to test farmers' participation and adoption of new technology. Even though it was found that farm income increase significantly towards TMR and TMR plus cooling device, farmers were reluctant to adopt the new production system due to limited revolving fund and large amount of debt. To sustain small dairy farmers in developing countries, the government needed to support these small farmers through some means such as public and private participation through TMR and cooling device. The new feeding and management techniques imposed to small dairy farmer revealed non-significant income as compare to large farm production system.

KEYWORD : dairy cattle, small farmer, sustainability, sprinkle and fan, TMR

Table 1. Proximate analysis of TMR used in the experiment

Content	Percent
Moisture	55.50
Crude Protein	18.82
Fat	1.35
Fiber	23.61
Ash	6.56
AIA	3.03
NDF	64.33
ADF	30.47
ADL	3.34
Calcium	0.95
Phosphorus	0.16
NaCl	0.18
Gross Energy cal/g	4,092.34

Table 2. Mean and (standard deviation) of temperature inside the barn, rectal temperature of cows before and after being sprinkled and fanned during summer month in degree celsius*

Time	Outside Barn	Inside Barn	Before Cooling	After Cooling	P value
6:00 AM	30.1 (1.55)[a]	27.23 (1.21)[b]	38.59 (0.45)[c]	-	<0.0001
10:00-10:45 AM	40.5 (1.83)[a]	37.03 (1.58)[b]	39.47 (0.48)[c]	38.60 (0.62)[d]	<0.0001
1:00-1:45 PM	42.8 (1.66)[a]	39.83 (1.23)[b]	39.86 (0.49)[b]	38.85 (0.50)[d]	<0.0001
4:00-4:45 PM	39.5 (1.72)[a]	36.90 (1.33)[b]	39.54 (0.70)[c]	38.48 (0.40)[d]	<0.0001

* Different superscripts mean significant at less than at least 0.05 probability level.

Table 3. Mean Feed intake and (standard deviation) in all treatments during summer month

Content	Treatment 1	Treatment 2	Treatment 3
Cooperative 1:			
Number of cow, head	10	10	10
Rice straw 90%DM, kg/h/d	2.1 (0.3)		
Pine Apple Waste 20%DM, kg/h/d	5.2 (1.0)		
Sweet Corn Waste Silage 25%DM, kg/h/d	5.3 (0.8)		
Fresh cut and carry grass 20%DM, kg/h/d	2.0 (0.2)		
TMR 18%CP/45%DM, kg/h/d	-	24.0 (2.3)	28.0 (2.5)
Concentrate 18%CP/90%DM, kg/h/d	5.0 (0.9)		
Total Feed Intake, kg/h/d	19.6 (2.5)	24.0 (2.3)	28.0 (2.5)
Dry matter feed intake, %Body weight	2.0 (0.6)	2.4 (0.3)	2.80 (0.5)
Cooperative 2:			
Number of cow, head	10	10	10
Rice straw 90%DM, kg/h/d	1.5 (0.2)		
TOFU waste 40%DM, kg/h/d	5.0 (1.2)		
Sweet Corn Waste Silage 25%DM, kg/h/d	5.0 (1.5)		
Fresh cut and carry grass 20%DM, kg/h/d	2.0 (0.5)		
TMR 18%CP/40%DM, kg/h/d	-	25.0 (2.5)	30.0 (2.4)
Concentrate 18%CP/90%DM, kg/h/d	5.0 (0.8)		
Total Feed Intake, kg/h/d	18.5 (0.5)	25.0 (2.5)	30.0 (2.4)
Dry matter feed intake, %Body weight	2.1 (0.5)	2.5 (0.4)	3.0 (0.4)

Table 4. Milk yield and composition (Standard deviation) of cows in 3 treatments

Content	Control (Normal feeding without Cooling Device)	With TMR without Cooling Device	With TMR with Cooling Device	P value
Milk Yield (kg)	12.50 (0.8)	15.45 (0.7)	17.85 (0.8)	<0.05
Fat (%)	3.37 (0.5)	3.99 (0.4)	4.20 (0.4)	>0.05
Protein (%)	3.22 (0.4)	3.38 (0.4)	3.47 (0.5)	>0.05
Lactose (%)	4.88 (0.5)	4.79 (0.5)	4.78 (0.5)	>0.05
Solid Not Fat (%)	8.82 (0.6)	8.90 (0.5)	9.25 (0.5)	>0.05
Total Solid (%)	12.18 (0.7)	12.87 (0.6)	12.73 (0.6)	>0.05
SCC (cell per ml)	560,000 (9,500)	289,750 (5,500)	296,000 (4,500)	<0.05
Body Score	2.8 (0.3)	3.0(0.2)	3.5 (0.1)	<0.05

Table 5. Cost and benefit from feeding TMR with cooling device, TMR without cooling device, and traditional feeding of small dairy farmers in Eastern Thailand in Baht/cow/day

Content	Control	TMR	TMR + Cooling	P value
Expense Baht (SD)	131 (18)	154 (20)	165 (18)	<0.05
Income Baht (SD)	231 (16)	286 (23)	324 (20)	<0.05
Gross Margin Baht (SD)	100 (13)	132 (15)	159 (13)	<0.05
Incremental Gross Margin Baht (SD)	0	32 (5)	59 (6)	<0.05

REFERENCES

Tumwasorn, S. 2010. Partial Mixed Ration (PMR) as New Feed Resource from Agro-Industry and Alternative Energy By-Products. Invited Speaker. Proceedings of the 14th AAAP Animal Science Congress, Pingtung, Taiwan. Vol. 3: 439-444.

Kraiprom, T., S. Parasanpanich, P. Kongmum, S. Sivichai, and S. Tumwasorn. 2014. Effect of Total Mixed Fiber (TMF) as Roughage Source on Rumen Fermentation in Lactating Dairy Cows. Journal of Life Sciences. Vol. 8. No 3. Pp. 262-265.

Maneerat, W. 2014. The Use of Agro-Industrial By-Product as Roughage Source in Dairy Cow in Southern Thailand. Ph.D. Dissertation. Department of Animal Science, Kasetsart University, Bangkok 10900, Thailand.

Tumwasorn, S. 2015. Appropriate Genetic and Feed for Dairy Cattle. Invited Article. Khon aen Agriculture Journal. Vol. 43. No. 4. Pp. 591-596.

Boonkum, W., I. Misztal, M. Duangjinda, V. Pattarajinda, S. Tumwasorn, and J. Sanpote. 2010. Genetic Effects of Heat Stress on Milk Yield of Thai Holstein Crossbreds. J. Dairy Scie. 94: 487-492.

Dairy Cattle Statistics. 2015. Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand

Cheva-Isarakul, B. 1990. Non-conventional Feed for Ruminants in Thailand. ASPAC/FFTC Ext Bull. No. 307. Food & Fertilizer Technology Center, Taipei.

Choophen, P., S. Sruamsiri, P. Jitramanoand, K.T. Rangabhet. 2005. The Use of Pineapple Waste as Cattle Feed: Physical Characteristic and Nutritive Value of Silage from Pineapple Waste. Pp. 174-179. In Proceedings of the 6th Annual Conference. Jaejo University, Chiang Mai, Thailand.

O-27-8

Performance and profit of feeding expired milk powder for pre weaning kids of Etawah Crossed Bred in a farmer group

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ABSTRACT

The aim of this study was to evaluate performance and economic profit of keeping Etawah crossbred kids during the preweaning period using expired cow milk powder as a substitution for fresh goat mother's milk. The study was conducted in a farmer group in Turi,

Sleman, and Yogyakarta. Ten pre-weaning goats age 14 days were used in this study. The kids were divided into groups of Control (fed fresh goat mother's milk) and Treatment (fed expired cow milk powder) and fed for 3 weeks. Body weight, average daily gain, and milk consumption were collected. Data were statistically analyzed using the Independent Sample T-test. The results showed no significant effect of feeding expired cow milk powder on body weight in the first, second, or third week of treatment (Control = 8.00 0.00 kg, 9.68 0.41 kg, and 11.32 0.08 kg, respectively Treatment = 7.96 0.08 kg, 9.70 0.67 kg, and 11.45 0.05 kg, respectively). Average daily gain was 148 13.56 g/day (Control) and 169.6 10.04 g/day (Treatment). The utilisation of expired cow milk powder had a significant effect on feed efficiency (Control = 17.3 and Treatment = 23.7) and feed convertion ratio (Control = 14.9 and Treatment = 5.1). Estimated profit by feeding expired cow milk powder as a substitution for fresh goat mother's milk was Rp 37,875/kid/day. We conclude that feeding expired cow milk powder did not have significant effect of body weight gain over fresh goat mother's milk of pre weaned kids, but gave a better feed efficiency and economic profit for farmers and could therefore be suggested as a substitute for fresh goat mother's milk.

INTRODUCTION

Breed goat jamnapari or Etawah is the most popular breed goat as produced milk in india and east-south asia. Etawah goat is big, long ear and current from arround gangga river, jumna and chambel in India. This goat also in Etawah district from Uttar Pradesh province (Davendra and Burns, 1994). Etawah crossbred current from crossing between native Indonesia goat with India native goat is between Kacang bred and Etawah bred. Etawah crossbred can produce milk ranges from 1.5 to 3.5 liters per head per day (Sutama, 1994). According Sodiq and Abidin (2008), production milk Etawah crossbred is quite low, between 0.5 to 0.9 liters per cow per day. According Atabany (2002), stated that the production of milk goats ranges from 1 to 3 kg per head per day, depending on lactation, temperature and humidity, fed, maintenance and management. Etawah crossbred capable producing as much milk from 0.4 to 2.2 litter/head/day with long lactation period is very diverse 92 to 256 days with average of 156 days. An 90 days total milk production during lactation goats ranges from 26 to 74 kg per head with the average 45 kg with the average 45kg/head.

Expired cow milk powder is milk that are not used or no longer consumed by humans. Expired cow milk powder often found in the remains of processing factories that utilize milk as the main raw material. Expired cow milk powder can be an alternative to be added to the commercial feed. Expired cow milk powder is the remnants of milk powder attached to the means of production or usually also outdate milk so that nutrient levels are not much different from the milk that is not expired (Irianto, 2011).

Components of expired powder milk is a nutrient rejects macro and micro nutrients. Macro nutrients include protein, fat and lactose. The content of macro nutrients average milk powder per 100 grams of expired is 25.8 grams protein, 0.9 grams fat, 4.6 grams of lactose, calcium 1.17 grams (Poedjiadi, 2006). Levels of micro-nutrients in milk powder is very comprehensive rejects, such as vitamins, minerals and amino acids. Vitamins contained in milk fat are vitamin A, D, E, K, whereas soluble vitamins in milk are vitamin B complex, vitamin C, vitamin A and vitamin D. Soluble vitamins in milk are important is vitamin B1, B2, nicotinic acid and pantothenic acid. The minerals contained in milk powder are calcium, magnesium, phosphorus, vitamin K, vitamin C and vitamin B6 (Poedjiadi, 2006).

The aim of this study was to evaluate performance and economic profit of keeping Etawah crossbred kids during the preweaning period using expired cow milk powder as a substitution for fresh goat mother's milk.

MATERIALS AND METHODS

Material

The material used in this study were 10 kid goats aged 14 days, which is derived from the fifth mother, goat mother's milk (control) and expired cow milk powder by PT Bhakti Karya Mandiri (treatment). The tools used in this research is a type of digital scales KINLEE EBLS - 20 capacity of 20 kg for the weighing of the kid. Drink bottles to give milk to the kids and a measuring cup to measure the expired powder milk. Proximate analysis test device in the form of water content, protein content (Kjeldahl method) and fat content test (Babcock methods).

Data Collection

This study was conducted in November 2015 until February 2016, at the "Peternakan Sukorejo 1", Turi, Turgo, Sleman, Yogyakarta. Five pregnant goats were waited to gave a birth. After birth, multiple births kid separated from their mother and placed in individual cages. Kid weighed. The first day until the seventh day, the kids were given colostrum ad libitum with a bottle.

Start at day 9, 10 kid goats are separated into two groups. 5 kids were given goat mother's milk (control) and 5 kids were fed expired cow milk powder as goat mothr's milk replacer. Milk was given two times a day in the morning (08:00 a.m.) and afternoon (4:00 p.m.). Milk was given using a bottle. If the bottle is brought near the mouth and the kid did not give a response indicated the kid is already full. Then the feeding was stopped. Milk that remained in the bottle each day was measured and recorded.

Body weight of kid was measured once a week. The recording was made until the fifth week, because after the fifth week of the kid already consume forage. Goat kid's weight (kg) weighed on digital scales with a capacity of 20 kg. Average daily weight gain was obtained by subtracting the weight of age last weighing birth weight divided by the length of raising.

Milk Quality Analyze

Milk samples to test the quality of milk is goat's milk and milk samples were taken randomly. Milk quality observed fat content, protein content and total solid. Analysis of milk quality in accordance with the instructions of AOAC (2005) that the milk to be measured by evaporation of the content of the milk at a temperature of 105ºC to obtain total water content. Amounted total milk solid measured with formula(Total solid = 100 % - water content). Protein assay with the Kjeldahl method. Babcok method used for determine fat content.

RESULTS AND DISCUSSIONS

Performance

Observations of goat body weight by comparing data from the goats are in treatment with the control, it can result in the following

Table 1. Average body weight of kid goats

Performance

Table 1. Average body weight of kid goats

Data collection	Body weight (kg)	
	Control	Treatment
Initial body weight	7.60±0.31	7.13±0.01
1st week	8.00±0.00	7.96±0.08
2nd week	9.68±0.41	9.70±0.67
3rd week	11.32±0.08	11.45±0.05

Initial Weight

Based on table 1, the initial weight of pre-weaning Etawah crossbred that will be milked by the mother was 7.60 0.31kg and 7.60 0.31 that will consume expired cow milk powder. When compared with reseach Chaniago and Hastono (2001) kids goat aged 1 month has weight 5.78 0.83 kg for male and 5.42 0.54 kg for female. The weight of early kids goats were used in this study had a higher weight than the literature even though the kids were only 14 days. That is because the kid goats were used in this study has a good genetic.

Weaning weight

The mean weight of the third week for kid goats who consume goats mother's milk is 11.32 0.08 kg. As for the kids who consumed expired cow milk powder is 11.45 0.05 kg. After the third week of data observation or 6 weeks old kid goat, kid goats already at weaning. According Sulaksana and Farizal (2010) results were obtained goat weaning weight was 9.72 ± 1.94 kg. According Triwulaningsih (1986) reported weaning weight in goats in Bogor were 9.95 ± 3.60 kg. The results showed that the weight of the kid weaning goats fed by the goat mother's milk and expired cow milk powder has a very well weaning weight.

Table 2. Average weight gain of kid goats

Weaning weight

Table 2. Average weight gain of kid goats

Data Collection	Weight gain (kg)	
	Treatment	Control
1st week	0.38±0,31	0.86±0.08
2nd week	1.60±0,42	1.74±0.73
3rd week	1.64±0,43	1.66±0.74

Weight gain

Daily weight gain of goat kids who consume goat mother's milk (control) was 148 13.56 g/day, while goat kids who consume expired cow milk powder (treatment) was 169.6 10.04 grams/day. Statistical results showed that the replacement of goat mother's milk with milk expired cow milk powder showed no real difference to the daily weight gain.

According Kostaman and Sutama (2005) the average growth of kid from buck Boer crossbred goat and doe Etawah crossbred is 116.40 49.95 grams/day, while the kid from the buck Etawah crossbred and doe Etawah crossbred is 105.29 28.36 grams/day. The results of the study when compared with the literature showing daily weight gain kid goats both control and treatment had a very good weight gain.

Table 3. Proximate analyze result

Feed

Table 3. Proximate analyze result

Variable	Goat mother's milk	Expired cow powder milk
Water content	83.99	4.27
Ash content	0.84	6.81
Total solid	16.01	95.83
Fat	4.31	12.54
Protein	3.87	24.23

Table 4. Milk consumption and efficiency

Table 4. Milk consumption and efficiency

Variable	Control	Treatment
Milk Consumtion (ml/day)	850	733.3
Feed Effeciency (%)	17.32	23.72
Feed Convertion Ratio	14.87	5.11

The average consumption of goat mother's milk is 850 ml/day and average milk consumption of expired cow milk powder is 733.33 ml/day. Expired cow milk powder consumption is lower than the goat mother's milk

consumption due to Proximate analyze results indicate that goat mother's milk has ash content, total solid, fat, protein, and organic matter content of the milk that is lower than expired cow milk powder. So kids who consume goat mother's milk need to consume more than the kid goats fed expired to meet nutrient needs.

Feed efficiency is an attempt to optimize the use of feed. Through the efforts of the efficiency of production cost can be reduced without reducing livestock production (Setiawan and Farm, 2011). Feed efficiency for goat mother's milk is 17.32 %. While expired cow milk powder feed efficiency is 23.72 %. In comparison, expired cow milk powder has better feed efficiency. Feed Conversion Ratio (FCR) is the ratio between the amount of feed consumed divided by weight gain. The smaller the value of FCR, the more efficient use of feed (Guntoro, 2012). Feed Conversion Ratio (FCR) goat mother's milk is 14,87. While FCR expired cow milk powder is 5,11. In comparison, expired cow milk powder had better FCR than goat mother's milk.

Economice analyze

Tabel 5. Economical analyze

Variable	Control	Treatment
Outcome	-	2125
Income	-	40000
Total	-	37875

Replacing goat mother's milk with expired cow milk powder will affect to the income of farmers. The cost to purchase expired cow milk powder per day is 8000 rupiah. But with the replacement of goat mother's milk with expired cow milk powder, the farmer can sell goat mother's milk at a price of Rp 20000 per liter. Etawah crossbred milk production ranges from 1.5 to 3.5 liters per goat per day (Sutama, 1994). If the estimated goats produce milk 2 l/day, the farmer can sell goat mother's milk as much as 2 l with revenues amounting to Rp 40000. Profit margin obtained by the revenue minus the cost of capital for the purchase of Tabel 5. Economical analyze expired cow milk powder, farmers then make a profit of about Rp 37875 per day for each goat.

CONCLUSION

Feeding expired cow milk powder has same body weight gain over fresh goat mother's milk of pre weaned kids, but gave a better feed efficiency and economic profit for farmers and could therefore be suggested as a substitute for fresh goat mother's milk.

KEYWORD : Expired milk, Pre-weaning performance, Economic profit, Etawah crossed bred

REFERENCES

AOAC. 2005. Official Method of Analysis The Association of Official Analytical Chemists. 18th ed. Maryland; AOAC International. Wiliam Harrwitz (ed). United States of America.

Atabany, A. 2002. Strategi pemberian pakan induk kambing perah sedang laktasi dari sudut neraca energi. Makalah Pengantar Falsafah Sains (PPS702) Program Pascasarjana Institut Pertanian Bogor.

Chaniago, T. D., and Hastono. 2001. Pre-weaning growth of Etawah crossed kid fed with replacement milk. Seminar Nasional Teknologi Peternakan dan Veteriner. 241-246

Davendra, C., and M. Burns. 1994. Produksi Kambing di Daerah Tropis. Institut Teknologi Bandung. Bandung.

Guntoro, S. 2012. Membuat Pakan Ternak dari Limbah Pertanian. Agromedia Pustaka. Jakarta.

Irianto, A. 2011. Pengaruh pemberian yoghurt susu afkir yang diperkaya nata de coco dalam mengendalikan kolesterol darah tikus putih. Universitas Jendral Soedirman. Purwokerto.

Kostaman, T. and I-K Sutama. 2005. Laju pertumbuhan kambing anak hasil persilangan antar kambing Boer dengan Peranakan Etawah pada periode pra-sapih. JITV 10:106-112.

Poedjiadi, A. 2006. Kajian Pustaka Susu Skim. UPI. Bandung.

Setiawan, B. S. and M. T. Farm. 2011. Beternak Domba dan Kambing. Agromedia Pustaka. Jakarta.

Sodiq, A. and Z. Abidin. 2008. Meningkatkan Produksi Susu Kambing Peranakan Ettawa. Agromedia Pustaka. Jakarta

Sulaksana, I. and Farizal. 2010. Pertumbuhan anak kambing Peranakan Etawah (PE) sampai umur 6 bulan di pedesaan. Jurnal Penelitian Universitas Jambi seri sains. 12 (2) : 25-28.

Sutama, I.K., 1994. Kinerja reproduktivitas sekitar pubertas dan beranak pertama kambing Peranakan Ettawa (PE). Ilmu dan Peternakan. Balitnak Ciawi. Bogor.

Triwulaningsih, E.J. 1986. Beberapa parameter genetik sifat kuantitatif kambing Peranakan Etawah. Tesis. Pascasarjana. Institut Pertanian Bogor. Bogor.

O-27-9

Farming scale impact on ration and dairy cow fs performances under traditional farm management in major producing province of Indonesia

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INTRODUCTION

Several factors limits dairy farming scale in Indonesia such as decreasing land and family worker availabilities as well as increasing concentrate price. Dairy cattle population, the major milk contributor in Indonesia (Indonesian Ministry of Agriculture, 2016) distributed mainly (99%) in Java island which is only 6.77% from the total Indonesian area (BPS, 2016). The cows distributed mainly in fertile highland with high agricultural to non-agricultural land conversion rate. According to National Dairy Survey (2012) in average dairy farmer cultivated 0.44 ha land to supply 62.7% forage required by 6.07 AU cows they kept. The remaining were supplied from other sources such as gathering natural grass from side road, under plantation area, or open field. Some farmer purchase agriculture by product which were seasonally available. Natural grass and plant by-product contributions determined sustainability of dairy farming especially in drought season (Despal et al. 2014).

Increasing human population pressure on the scarce resources have negative effects (Geertz 1963) such as reducing farmer capacity to provide forage for their cattle. Although the pressure on farmland could be relaxed through labor movement to urban sectors (Liu and Yamauchi 2014), however, it led to less family worker available and increase labor cost for agriculture sector in rural area especially worker for gathering natural grass. The lack of forage availability were overcome by dairy farmer by increasing concentrate supplement utilization in ration. The strategy increased farming cost and reduced farmer income. On larger holding farm however, human population pressure could be maintain through induction of technology and institutional innovation (Hayami and Ruttan 1985) as well as intensification in agricultural production (Boserup 1981).

The study was aimed at comparing impact of farming scale on dairy farmer capacity to provide feed and nutrition to their cattle and their impact to the cow's performances and farmer income in the four major dairy farm area.

METHODS

The study have been conducted in 4 major dairy cattle population provinces in Indonesia, namely West Java, DI Yogyakarta, Central Java and East Java. The study used survey methods with interview, direct observation and measurement as well as laboratory analyses. The amount of 129 farmers have been interviewed (43 in Wes Java, 29 in Central Java, 29 in DI Yogyakarta and 28 in East Java) and 415 lactating cows (145 in Wes Java, 105 in Central Java, 43 in DI Yogyakarta and 122 in East Java) have been observed to get information on cattle ownership, type and the amount of feed offered, milk production and farm income. The observations were aimed at confronting data from interview. Laboratory analyses were conducted to determine nutrient contents of feeds used and milk compositions.

Interviews were conducted by 4 trained enumerators with guidance of a questioner. The amount of feeds offered were measured gravimetrically, while milk productions were measured volumetrically. Cows' body weights were estimated using Schoorl's formula. Body conditions were scored according to five scales Penn State University (2004) procedure. Proximate analyses followed Naumann and Bassler (1997) procedures, while Ca and P sample preparation followed Reitz *et al*. (1987) procedure. Determination of Ca sample concentration followed AOAC (2003) procedure and P sample concentration determined using Taussky & Shorr (1953).

The study used imbalance randomized design. Collected data were analyzed using ANOVA procedure. Correlation between parameters have been analysis prior to regression.

RESULTS AND DISCUSSIONS

Farming scale, milk production, and farmer income are shown in Table 1 and nutrients offered are shown in Table 2. The table did not show any significant different between parameters observed due to large variation. The results showed that traditional dairy farming in Indonesia vary greatly in scale, capacity to provide nutrients, cow's performances and incomes. In average, traditional dairy farmer in Indonesia kept 6.61 AU with 76.33 percentage of lactating cow, where East Java province tent to be higher than other provinces. National Average

milk production was 13.81 L/d, higher than dairy cattle in tropic reported by Pérez et al. (2009). West Java Province tent to have better cow's performances than any other area in Indonesia. In Average, dairy farmer in Indonesia earned Rp 3.37 mio income per month, slightly lower than average national GDP income (Rp 3.8 mio) which showed that dairy farmer had lower exchange value and if the condition occurs in the long run, it will lead to disincentive of young generation to work in this field.

The amount of nutrient offered related to the feed resources availability especially forage which influenced by land occupation. In average, farmer in Indonesia provide macro nutrients (TDN and CP) above their cow's requirement, but not for micro nutrients (Ca and P). In DIY province, farmer offered less nutrients than their cow's required. The forage availability and nutrients offered in the different area explained cattle movement which frequently occurred since calf until lactating. Rearing mostly held in Central Java which can provided better nutrients surplus than any other provinces. Close to the calving period, the cows is transported to West or East Java which had advantage from their market value.

In term of nutrient procurements, higher holding farmer (East and Central Java provinces) were more resilient to the human population pressure by inducing technology such as utilization higher percentage of concentrate supplement and intensification in dairy farming production system (Liu and Yamauchi 2014). Labor shortages which were a serious issue in agriculture (Otsuko et al. 2013) were overcome through utilization of machinery such as chopper, hand tractor and milking machine. While West Java province, maintain nutrients supply by reducing farming scale.

Regression analysis on relationship between scale (X) and milk production (Y) showed a quadratic response Y = -0.5336X2 + 18.653X -17.883 with scale to achieve maximum production at 17.47 AU. There was no accurate estimation of nutrient availability can be produced from farm scale. With the current national scale (6.61 AU), farmer in Indonesia still have capacity to provide sufficient nutrient for their cows and increase their production level as well as income.

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KEYWORD : dairy cows, farming scale, income, nutrient sufficiency, performance

Table 1. Farming Scale, Milk Production, and Farmer Income

Parameters	National	Province			
		West Java	Central Java	DIY	East Java
Farming Scale					
Total cattle (AU)	6.61±5.30	4.98±2.70	7.10±5.48	2.88±1.42	8.95±5.30
Lactating cow (AU)	5.05±3.77	4.40±2.63	4.83±3.46	2.25±1.89	6.91±4.83
Lactating cow (%)	76.33±22.47	88.44±13.96	68.04±30.28	78.26±27.73	77.16±17.11
Milk Production (L/d)					
Total	68.40±55.59	64.17±33.04	60.08±43.89	25.50±17.25	96.91±78.75
Average	13.81±3.34	15.40±3.03	12.99±2.61	12.38±1.84	13.78±4.46
Income (Rp 000)					
Farm	3377±3211	3017±2000	3154±2467	1905±2467	4504±4276
Per lactating cow	643±300	695±342	642±279	627±416	604±278

Table 2. Nutrient Sufficiency

Parameters		Provinces				
		National	West Java	Central Java	DIY	East Java
Offered (kg)	TDN	8.70±3.06	9.84±2.71	8.56±2.27	6.50±1.42	8.61±4.17
	CP	1.82±0.74	2.02±0.54	1.89±0.77	1.36±0.31	1.71±0.95
	Ca	0.05±0.03	0.05±0.02	0.04±0.01	0.04±0.02	0.07±0.05
	P	0.03±0.02	0.04±0.01	0.03±0.01	0.02±0.01	0.03±0.02
Requirement (kg)	TDN	7.48	8.20	6.77	7.25	7.39
	CP	1.57	1.71	1.45	1.44	1.55
	Ca	0.06	0.07	0.05	0.06	0.06
	P	0.04	0.04	0.03	0.04	0.04
Balanced (kg)	TDN	1.22±3.00	1.64±2.72	1.79±2.27	-0.75±1.42	1.23±4.17
	CP	0.27±0.73	0.31±0.54	0.44±0.77	-0.08±0.31	0.17±0.95
	Ca	-0.01±0.03	-0.02±0.02	-0.01±0.01	-0.02±0.02	0.01±0.03
	P	-0.01±0.02	-0.01±0.01	-0.01±0.01	-0.01±0.01	-0.01±0.02

Note: TDN (Total Digestible Nutrient), CP (Crude Protein), Ca (Calcium), P (Phosphor)

REFERENCES

[AOAC] Associaton of Official Analitycal Chemist. 2003. *Official Method of Analysis of The Association of Official Analytical of Chemist*. Virginia (US): Association of Official Analytical Chemist Pr.

Boserup, E., 1981. Population and Technological Change: A Study of Long-Term Change. University of Chicago Press, Chicago.

BPS, 2016). Luas Daerah dan Jumlah Pulau Menurut Provinsi, 2002-2014. https://www.bps.go.id/ linkTabelStatis/ view/id/1366

Despal, Malyadi, J., Destianingsih, Y., Lestari, A., Hartono, H., and Abdullah, L. 2014. Natural Grass and Plant Residue Qualities and Values to Support Lactating Cows Requirement on Forage at Indonesian Small Scale Enterprise and Traditional Dairy Farming. Proceeding of International Workshop on Tropical Bio-resources for Sustainable Development, Bogor 13 – 15 Agustus 2014

Geertz, C., 1963. Agricultural Involution: The Process of Ecological Change in Indonesia. Association of Asian Studies Monographs and Papers No. 11. Berkeley, CA, US, University of California Press.

Hayami, Y., Ruttan, V.W., 1985. Agricultural Development: An International Perspective, second ed. Johns Hopkins University Press, Baltimore.

Indonesian Ministry of Agriculture, 2016. Basis Data Pertanian. https://aplikasi.pertanian.go.id/ bdsp/newkom.asp

Liu Y and Yamauchi F. 2014. Population density, migration, and the returns to human capital and land: Insights from Indonesia. Food Policy 48 (2014) 182–193

National Dairy Survey, 2012. Evaluasi Kinerja Pembangunan Sapi Perah sebagai Dasar Perencanaan Pembangunan Berkelanjutan Menuju Penguatan Kontribusi Domestik terhadap Ketahanan Pangan Nasional. Kerjasama Fakultas Peternakan IPB dengan Direktorat Jenderal Peternakan dan Kesehatan Hewan.

Naumann, C., Bassler, R. 1997. VDLUFA-Methodenbuch Band III, Die chemische Untersuchung von Futtermitteln. 3rd ed. VDLUFA-Verlag. Darmstadt, Germany.

Otsuka, K., Liu, Y., Yamauchi, F., 2013. Factor Endowments, Wage Growth, and Changing Food Self-Sufficiency: Evidence from Country-Level Panel Data. Am. J. Agric. Econom. 95, 1252–1258.

Penn State University. 2004. *Begginer's Guide to Body Condition Scoring*: a tool for dairy herd management. Revised Edition. Washington DC (US): National Academy Pr.

Pérez CA, Vera JK, Garnsworthy PC. 2009. Effects of bypass fat on energy balance, milk production and reproduction in grazing crossbred cows in the tropics. Livestock Science 121 64–71

Reitz LL, Smith WH, Plumlee MP. 1987. Animal Science Department. West Lafayette : Purdue Univ Pr.

Taussky HH, Shorr E. 1953. A micro colorimetric method for the determination of inorganic phosphorus. J Biol Chem (202) :675-685.

O-28-2

Dietary Fat Preference And Dependence In Weaning Piglet And Their Performance

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OBJECTIVE

The objective of this experiment was to evaluate the interplay of dietary lard and feeding regime as determinants of fat utilization and interdependence from sow milk to solid food of newly weaned piglets. 184 piglets, 8 piglets per group, were randomly allocated to two behavior observations and one feeding experiment for evaluating dietary fat dependence and preference of piglet under sudden weaning process. In the experiments, lard was first used to simulate the oleic acid content in sows' milk fat. The fixed combination and free choice combination of lard containing ratio were used to compare performance differences between treatments.

METHODOLOGY

Experimental Design and Animals

Experiment 1: Soon after weaning, 72 piglets were purchased from commercial farm to form 9 experimental groups and were randomly allocated to three lipid source treatments. After two weeks adjustment, piglets were offered a series of double choices between a common reference diet (6% CF) and the diet with the additional 3% of soya oil, coconut oil or lard under evaluation. The reference diet contained soybean meal, 3% lard, and corn, which were considered as the feedstuffs of reference for the protein, fat, and carbohydrate sources respectively. Preference is expressed as percentage of the tested diet to total daily feed intake for continuously 3 days' observation.

Experiment 2: Soon after weaning, 64 piglets were select from 10 litters to form 8 experimental groups. 4 barrows and 4 females were randomly picked for each group. Lard was used as a substrate in a 6% crude fat content reference diet with 6% and 3% being the content ratio to 12% and 9% crude fat. The piglets were weaned at 8:00 without any feed and were introduced to the behavior observation pen at 14:00 for one hour observation then move back to original regrouping pen for ad libitum of reference diet. The following two days, feeds were withdrawing from 8:00 to 14:00 then again move to behavior observation pen at 14:00. The observation pen has 3 round troughs in different locations. Day 1, trough No. 1 full in 6%, trough No. 2 full in 9% and trough No. 3 full in 12% crude fat experimental diet. Day 2, 6% rotated to trough No. 3, 9% rotated to tough No. 1 and 12% rotated to tough No. 2. Day 3, 6% rotated to trough No. 2, 9% rotated to tough No. 3 and 12% rotated to tough No. 1.

Experiment 3: Soon after weaning, 48 piglets were purchased from commercial farm to form 3 experimental groups and were randomly allocated to three feeding method treatments. Lard was first used to simulate the oleic acid content in sows' milk fat. A 4-week growth performance of a simple free choice combination feeding (6% vs 9%) group and two fixed combination groups (6% vs 6% and 9% vs 9% non-free choice) were conducted and compared. The feeding regimes were simultaneously lays aside two lipid sources in two individual troughs to keep for 4 weeks. 4 barrows and 4 females were randomly picked for each group and were introduced to experimental groups without train the piglets' experience at weaning.

Data collection

All behavior observations began at 14:00 in the afternoon and lasted for 1 hour. One-minute interval scanning was used for recording the general behavior, with 3 observers recording at same time. Other observations, for which it was necessary to know more details about sequence and duration, were recorded more carefully by scanning combined with continuous observation. The time spent in the behaviors was analyzed from the recording sheet, which had the time scale on it. Behavior definitions were Standing, Lying, Eating, Drinking, Defecating, Moving, Rooting, Dog-sit and Chain chewing.

Eating frequency and preference calculations in Exp. 1 was the same as Solà-Oriol et al, (2009). Exp. 2 was modified from that. In each observing pen, eating appearance from the 3 feeders was recorded for individual pig. Preference was measured as time appearance at different feeder over total time spent eating expressed as percentage of total eating time. The following mathematical equation was used: % Preference = ((time spent in

specific feeder) / ((time spent in feeder 1) + (time spent in feeder 2) + (time spent in feeder 3))) × 100
The body weight of each piglet was measured at day 1, 8, 15, 22 and 29. The total amount of feed consumption was also measured weekly for each group and was adjusted at day1, 8, 15 and 22 by using the equation: feed offered per day per trough (g) = 120*(AV. body weight) $^{0.75}$*12 piglets. The leftover feed was removed, weighed and was not given again to the experimental groups.

Statistical analysis

The statistical procedure was used with SPSS (IBM SPSS, 2013) to perform the analysis. One-way ANOVA was used to compare the characteristics of piglets' weekly body weight. The Levene's test was used before a comparison of means. Comparison between treatments was done by calculating standard error of the difference between two means (S.D.) and least significant difference (LSD). When Levene's test was significant, the data was tested again by Brown-Forsythe test and Tamhane's T2 test was selected for posteriori comparisons. The treatment effects on feed intake, body weight gain and feed conversion ratio were analyzed by simple one factor and one factor with covariates. The performances of the first two weeks and last two weeks were used as the covariate for analyzing final performance accordingly. For investigation of the effects of treatment, the pen was considered the experimental unit for the growth performance data, and the pig was the experimental unit. An α level of 0.05 was considered statistically significant.

RESULTS

The preference values of piglets with 14 days adjustment

Table 1 shows the preference values for diets containing additional 3% coconut oil, additional 3% soybean oil or additional 3% lard offered in a double-choice feeding setup compared with a 6% crude fat reference diet. Piglets preferred additional 3% coconut oil and additional 3% lard offered in a double-choice feeding setup compared with a 6% crude fat reference diet. However, they avoided addition 3% soybean oil diet.

The preference values of piglets without adjustment

Table 2 shows the average of the time spent for each behavior in 1 hour to compare the differences between different day postweaning. There were not significant differences between days for most of the observed behavior patterns. Piglets spent the longest time eating (p=0.014) but the least time for rooting (p=0.028) at the second day postweaning. They spent one third of time lying and one fifth of time eating.
When comparing effects of lipid content ratios and their eating frequencies and preference, factorial analysis of variance with mixed design was used to analyze the differences. The lipid content ratios (6%, 9% and 12%) were dependent variables factor of which different days were used as the covariate for analyzing. Table 3 showed that piglets spent an average of 5.9 minutes eating 9% crude fat content feed and also prefer (45.43%) this feed to the other two ratios.

The effects of lipid sources (dependence) and providing way (preference) on piglet performance

Table 4 shows the average of body weight at different weeks, comparing the differences between lipid sources. As can be seen, piglets were selected from the same pool with similar initial body weights. Piglets that were fed with 9% vs 12% free choice feeding had the heaviest body weights at 3rd (14.24 kg, P=0.045) and 4th (17.43kg, 0.007) week of age. The piglets that were fed with 12% fixed combination had the lightest body weights at both 3rd and 4th week of age.
The average daily feed intake, body weight gain and FCR are shown in Table 5. There were no differences in average daily feed intake (p=0.104), body weight gain (p=0.398) and FCR (p=0.704) between lipid contain ratio and feeding method during 4weeks post-weaning stages. The performances of the first two weeks and last two weeks were used as the covariate for analyzing final performance accordingly. There were no covariate effects from the first two weeks' performances. However, last two weeks' performances had covariate effects on final performances Piglets that were fed with 6% vs 9% free choice feeding had the heaviest body weights at 3rd and 4th week of age. The also converted more feed to body weight gain throughout the experiment (p=0.030), but not the other two performances (p=0.110 and p=0.448).

Discussions

Weaning piglets have difficulties initiating dry feed intake. Pluske et al. (1997) reported that it takes up to 3

weeks for piglets to reestablish pre-weaning levels of energy intake, during which their gut integrity needs to be established. Sow's milk fat contains high levels of 33.3% to 37.0% palmitic acid and 37.5% to 33.0% oleic acid (Douglas et. al., 2001 Darragh and Moughan, 1998). The oleic acid component of lard is similar to that of sow's milk fat (NRC, 2012). Piglets that were fed with 6% vs 9% free choice feeding had the heaviest body weights at 3rd (14.24 kg, P=0.045) and 4th (17.43kg, 0.007) week of age. It is therefore seen that piglets still have the dependence on high oleic acid content milk like feeds, even without any training at weaning.

Even though there was no significant feeding method between average daily feed intake and average daily gain for different treatments, some facts can be observed concerning feed conversion ratio. Piglets that were fed with 6% vs 9% free choice feeding had the heaviest body weights at 3rd and 4th week of age. The also converted more feed to body weight gain throughout the experiment (p=0.030). Free choice combinations shows better feed conversion ratio, this may imply that free choice combination is a better way to increase learn-chance under sudden event change at weaning. Previous results from our group reported that in an artificial rearing condition, piglets offered creep feed were observed to wean themselves before cessation of milk availability, and the timing of this self-weaning depended on the nursing frequency. The piglets nursed at one hour intervals weaned themselves between day 22 and day 29, those nursed at 3 h intervals weaned themselves between day 15 and day 22, whilst those nursed at 6 h intervals weaned themselves between day 8 and day 15. The piglets which were nursed at 6 h intervals had the highest total dry matter intake in weeks 3 and 4 when fed with milk, creep feed and water but not when fed milk only (Weng et. al., 2009).

CONCLUSIONS

In sudden weaning process, piglets still has the dependence on high oleic acid content milk like feeds at weaning. The free choice combination of lipids is a better way to increase learn-chance under sudden event change at weaning.

KEYWORD : Weaning piglet, Fat preference, Fat dependence, Performance

Table 1 Preference values for diets containing 3 selected fat sources offered in a double-choice feeding

	Coconut oil	Soybean oil	Lard	SD	*P*-value
Preference values (%)	52.6^{b}	38.8^{c}	54.8^{a}	0.348	0.000
(SE)	1.31	2.03	1.89		

SD : standard error of the difference between two means; ^{a,b} : Means with the same letter are not significantly different

3 selected fat sources were used as substrates in a 6% crude fat content reference diet with 3% being the content ratio to 9% crude fat.

Table 2 Piglet behaviors observed from different days post-weaning

	Day 1	Day 2	Day 3	SD	*P*-value
Eating(minutes)	11.8^{b}	14.3^{a}	12.9^{ab}	0.328	0.014
Drinking(minutes)	2.3	2.2	3.0	0.218	0.311
Standing(minutes)	13.1	9.8	11.9	0.832	0.268
Rooting(minutes)	2.4^{a}	0.6^{b}	2.9^{a}	0.356	0.028*
Lying(minutes)	20.3	23.4	19.9	0.733	0.109
Moving(minutes)	6.3	5.5	6.0	0.629	0.876
Chain chewing(minutes)	1.9	2.0	1.2	0.508	0.783
Elimination(minutes)	1.3	1.9	1.8	0.206	0.479
Dog-sit(minutes)	0.6	0.4	0.6	0.102	0.629

SD : standard error of the difference between two means; ^{a,b} : Means with the same letter are not significantly different

* : Levene's test was significant, the data was tested again by Brown-Forsythe test and Tamhane's T2 test was selected for posteriori comparisons

Table 3 Preference values for 3 containing levels offered in a free-choice setup for untrained post-weaning piglets

	Reference (6%)[x]	9%[y]	12%[z]	SD	*P*-value
Eating frequency (minute)	3.49[b]	5.90[a]	3.56[b]	0.342	0.000
(SE)	2.773	3.086	2.823		
Preference value (%)	26.78[b]	45.43[a]	27.90[a]	1.321	0.000
(SE)	17.854	21.046	19.067		

SD：standard error of the difference between two means; [a,b]：Means with the same letter are not significantly different
[x]：Reference diet with 6% crude fat containing; [y]：Additional 3% lard was used as substrates in a 6% crude fat reference diet
[z]：Additional 6% lard was used as substrates in a 6% crude fat reference diet

Table 4 Effects of lipid containing ratio and feeding method on piglet growth performance at nursery stage

	6%[x] vs 6%[x]	9%[y] vs 9%[y]	6%[x] vs 9%[y]	SD	*P*-value
Initial Av. Body weight(kg)	7.93	7.90	7.99	0.133	0.979
1st week Av. Body weight(kg)	8.51	8.31	9.11	0.175	0.156
2nd week Av. Body weight(kg)	10.40	10.47	10.95	0.211	0.405
3rd week Av. Body weight(kg)	13.02[ab]	12.91[b]	14.24[a]	0.266	0.049
4th week Av. Body weight(kg)	16.21[b]	15.74[b]	17.43[a]	0.319	0.007

SD：standard error of the difference between two means; [a,b]：Means with the same letter are not significantly different
[x]：Reference diet with 6% crude fat containing; [y]：Additional 3% lard was used as substrates in a 6% crude fat reference diet

Table 5 Effects of lipid containing ratio and feeding method on piglet growth performance at nursery stage

	6%[x] vs 6%[x]	9%[y] vs 9%[y]	6%[x] vs 9%[y]	SD	*P*-value	R^2
	One-way ANOVA					
Av. Daily feed intake(kg)	0.469	0.478	0.532	0.009	0.104	0.678
Av. Daily gain(kg)	0.297	0.286	0.346	0.016	0.398	0.369
Feed/Gain	1.600	1.672	1.550	0.055	0.704	0.161
	One-way MANOVA with covariate of first two week performance					
Av. Daily feed intake(kg)	0.469	0.478	0.532	0.011	0.204	0.681
Av. Daily gain(kg)	0.297	0.286	0.346	0.018	0.756	0.413
Feed/Gain	1.600	1.672	1.550	0.036	0.368	0.731
	One-way MANOVA with covariate of last two week performance					
Av. Daily feed intake(kg)	0.469	0.478	0.532	0.005	0.110	0.926
Av. Daily gain(kg)	0.297	0.286	0.346	0.005	0.448	0.955
Feed/Gain	1.600[b]	1.672[a]	1.550[b]	0.011	0.030	0.976

SD：standard error of the difference between two means; [a,b]：Means with the same letter are not significantly different
[x]：Reference diet with 6% crude fat containing; [y]：Additional 3% lard was used as substrates in a 6% crude fat reference diet

REFERENCES

Darragh A.J. and P.J. Moughan. The Composition of Colostrum and Milk. In: M.W.A. Verstegen, P.J. Moughan and J.W. Schrama. 1998. The Lactating Sow. Wageningen Pers. ISBN 90-74134-43-2.

Douglas G. Burrin. Nutrient Requirements and Metabolism. In: Wilson G. Pond and Harry J. Mersmann. 2001. Biology of the Domestic Pig. Comstock Publishing Associates. Cornell University Press. Ithaca and London. ISBN 0-8014-3468-8.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. pp.366-367.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livest. Prod. Sci. 51:215-236.

Solà-Oriol, D., E. Roura and D. Torrallardona. 2009. Feed preference in pigs: Effect of cereal sources at different inclusion rates. J. Anim. Sci. 87:562-570.

Weng, R. C., S. A. Edwards and L. C. Hsia. 2009. Effect of Nursing Frequency and Creep Feed Provision on the Milk and Feed Intake and Performance of Zero Day Weaned Piglets Reared on a Dummy Sow. Asian-Aust. J. Anim. Sci. Vol. 22, No. 11 : 1540-1546.

O-28-4

Effects of Feeding Methods in Summer Season on Reproductive Performance of Lactating Sows

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Objective

Feeding is the most important aspect of swine production that contributes for their performance and health status. In the normal course, pig receives dry feed and receives water by a nipple in the feeder. Sows drink more water and eat less in summer. The amount of water in the feeder is more than normal in hot season due to the excessive touch of water nipples. Therefore, it can disturb the feeding and decrease the average feed intake in sows. The present experiment was designed to study the effect of automatic feeders and sows with different backfat thickness on the performance, body weight change, litter performance, blood metabolites and milk quality in sows.

Methodology

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Republic of Korea.

A total of 64 crossbred sows (Yorkshire × Landrace) were allotted to one of four treatments. All sows used in the present study were artificially inseminated 2 times after onset of estrus, and pregnancy was detected and confirmed at d 30 post breeding using a Pharvision B-mode ultrasound machine (AV 2100V Ambisea Tech. Corp). During gestation, all sows were housed in individual gestation stalls (2.20 × 0.65 m) with fully slatted concrete flooring. Two different feeding methods and two level of backfat thickness.

Sow backfat thickness at 10th rib, 6.5 cm from one side of the backbone was measured at d 107 of gestation, after farrowing (d 1 of lactation), and at weaning (d 21 of lactation) by using an ei-medical imaging ultrasound (Loveland, CO). Changes in backfat thickness of sows during lactation were measured by calculating the difference between backfat thicknesses at d 1 of lactation and backfat thickness at weaning. Standard litter traits like number born and born alive, body weight (kg) at birth, and weaning, growth rate (kg/d), and average daily gain (g/piglets) were recorded. Feed intake (kg/d) of each sows and weaning-to-estrus interval (d) were also recorded.

Commercial kits (Fujifilm Corp., Saitama, Japan) were used for analysis of plasma metabolites (Triglyceride, glucose, blood urea nitrogen and creatinine) using an automated chemistry analyzer (Fuji Dri-chem 3500i, Fujifilm Corp.).

Colostrum and milk were collected (30 ml) just after the birth of the first piglet and 10 days after farrowing respectively. The samples were analyzed for its total solid, protein, fat, lactose composition. Nutritional composition (total solid, protein, fat and lactose content) was estimated by Milko-Scan 133B (Type 10911) within 24 hours.

Data generated in the present experiment was analyzed as a 2 × 2 factorial arrangement in a completely randomized design. Sow was considered the experimental unit. The main effects of housing type, backfat thickness at 107 d of gestation, and their interaction were determined by mixed procedure of SAS statistical program (SAS Inst., Inc., Cary, NC). P-values ≤ 0.05 were considered statistically significant. The data were tested for main effects of feeding types, back fat, and their possible interaction.

Results

The body weight, backfat thickness, daily feed intake and weaning to estrus interval of sows are shown in Table 2. There was no free feeding time × backfat thickness interaction for any of the measured variables. Free feeding time and backfat thickness had significant effects ($p<0.05$) on sows body weight changes during gestation day109 to weaning. Sows body weight changes were greater with ≥ 20 mm backfat thickness than 0.05) during summer season.

The effect of free feeding time and backfat thickness on litter size and piglet performance of sows is shown in

Table 3. There was no free feeding time × backfat thickness interaction for any of the measured variables. Backfat thickness had significant effects ($p<0.05$) on initial litter weight and piglets weaned. Free feeding time and backfat thickness had no effects ($p>0.05$) on litter size, survivability, average daily gain and total weight gain. However, total weight gain of piglets tended to be higher with ≥ 20 mm backfat thickness ($p=0.054$) during summer season.
The effects of free feeding time and backfat thickness on blood metabolites are presented in Table 4. There were no significant effects ($p>0.05$) and interaction between free feeding time and backfat thickness on blood urea nitrogen, glucose, triglyceride and creatinine level of weaning and post farrowing sows during summer season. However, triglyceride of weaning sows tended to be lower with ≥ 20 mm backfat thickness ($p=0.081$).
Colostrum and milk composition of sows are presented in Table 5. There was no free feeding time × backfat thickness interaction for any of the measured variables. Free feeding time and backfat thickness had no significant effects ($p>0.05$) on colostrum and milk composition of sows during summer season.

Conclusion

In conclusion, there were no significant interaction effects between feeding methods and back-fat thickness in all parameters. Feeding methods did not change the performance of sows however sows in FFT group had higher feed intake and lower losing weight during the lactation period. Sows with thicker back-fat improved the number of weaned piglets.

KEYWORD : Feeding method, lactating sows, back-fat

Table 1. Formula and chemical composition of gestation and lactation sow diets (as -fed basis)

Item	Gestation	Lactation
Ingredients, %		
Corn	41.50	41.00
Wheat	12.00	10.00
Wheat bran	4.00	-
Palm kennel meal	4.00	2.00
DDGS	12.00	8.00
Rapeseed meal	3.00	-
Soybean meal(Local)	6.95	28.71
Coconut meal	4.00	-
Corn gluten feed	2.00	-
Animal fat	5.21	4.03
Molasses	2.00	3.00
$_{L}$-Lysine·HCl(78%)	0.08	0.14
$_{DL}$-Methionine(88%)	-	0.04
Choline chloride(50%)	0.06	0.06
Limestone	1.47	1.38
MDCP	0.85	0.85
Salt	0.55	0.50
Vitamin premix[1]	0.20	0.16
Mineral premix[2]	0.10	0.10
Phytase	0.03	0.03
Total	100.00	100.00
Calculated composition, %		
ME, kcal/kg	3,250	3350
CP	14.50	20.10
Ca	0.75	0.75
Av. P	0.32	0.32
Lys	0.65	1.15
Met+Cys	0.56	0.72

Table 2. Effects of free feeding time (FFT) and backfat thickness on backfat thickness changes, feed intake and weaning to estrus interval in sows during summer season

Item	Control		FFT		SEM[1]	p-value[2]		
Backfat thickness, mm	<20 (n=14)	≥20 (n=14)	<20 (n=13)	≥20 (n=15)		F	B	F×B
Parity	3.43	3.86	3.31	3.80	0.26	0.810	0.216	0.931
Sow body weight, kg								
Gestation, d109	228.86	234.80	220.48	234.19	4.81	0.511	0.153	0.569
Weaning	207.70	211.63	203.46	213.35	4.18	0.833	0.250	0.618
Change, -	21.16	23.17	17.02	20.84	0.86	0.007	0.015	0.440
Sow Backfat thickness, mm								
Gestation, d109	19.71	22.21	19.54	22.07	0.30	0.538	<0.001	0.957
Weanling	15.93	17.36	16.38	17.93	0.23	0.050	<0.001	0.816
Change, -	3.79	4.86	3.15	4.13	0.22	0.022	0.001	0.873
Daily feed intake, kg/d	4.81	5.22	5.21	5.71	0.11	0.006	0.004	0.758
Weaning to estrus interval, d	5.07	4.86	4.85	4.67	0.13	0.274	0.300	0.927

[1]Standard error of means.

[2]F : free feeding time, B : backfat thickness, F × B : free feeding time × backfat thickness.

Table 3. Effects of free feeding time (FFT) and backfat thickness on litter size and piglet performance in sows during summer season

Item	Control		FFT		SEM[1]	p-value[2]		
Backfat thickness, mm	<20 (n=14)	≥20 (n=14)	<20 (n=13)	≥20 (n=15)		F	B	F×B
Litter size								
Initial litter size	10.21	10.36	10.23	10.40	0.25	0.933	0.659	0.970
Piglets weaned	10.07	10.14	10.08	10.20	0.24	0.929	0.781	0.941
Survivability, %	98.33	98.11	98.64	98.18	0.71	0.852	0.742	0.910
Litter weight, kg								
Initial litter	13.12	13.89	13.16	13.90	0.23	0.961	0.020	0.958
Initial litter, kg/pig	1.30	1.35	1.32	1.34	0.03	0.910	0.491	0.728
Piglets weaned	69.80	72.73	71.49	74.91	1.01	0.180	0.030	0.865
Piglets weaned, kg/pig	7.10	7.22	7.28	7.35	0.19	0.566	0.725	0.921
Total weight gain	56.67	58.84	58.34	61.02	0.86	0.125	0.054	0.834
Average daily gain, g/pig	230.78	233.83	237.73	239.38	6.22	0.490	0.795	0.938

[1]Standard error of means.

[2]F : free feeding time, B : backfat thickness, F × B : free feeding time × backfat thickness.

Table 4. Effects of free feeding time (FFT) and backfat thickness on blood metabolites of lactating sows during summer season

Item	Control		FFT		SEM[1]	p-value[2]		
Backfat thickness, mm	<20	≥20	<20	≥20		F	B	F×B
Post farrowing, mg/dl								
Blood urea nitrogen	17.51	17.46	17.39	17.58	0.65	0.994	0.940	0.900
Glucose	91.21	92.32	91.73	92.64	1.21	0.809	0.565	0.954
Triglyceride	57.56	56.53	57.24	56.94	0.62	0.959	0.456	0.685
Creatinine	2.09	2.07	2.10	2.08	0.10	0.946	0.887	0.998
Weanling, mg/dl								
Blood urea nitrogen	18.38	18.58	18.52	18.78	0.71	0.873	0.824	0.978
Glucose	91.95	92.32	93.14	93.48	1.58	0.601	0.887	0.996
Triglyceride	27.76	26.71	28.05	26.48	0.53	0.963	0.081	0.726
Creatinine	1.75	1.64	1.69	1.67	0.09	0.935	0.799	0.914

[1]Standard error of means.

[2]F : free feeding time, B : backfat thickness, F × B : free feeding time × backfat thickness.

Table 5. Effects of free feeding time (FFT) and backfat thickness on colostrum and milk composition of lactating sows during summer season

Item	Control		FFT		SEM[1]	p-value[2]		
Backfat thickness, mm	<20	≥20	<20	≥20		F	B	F×B
Colostrum, %								
Total solid	25.34	24.97	24.53	24.45	0.40	0.253	0.703	0.807
Protein	15.37	15.53	15.70	15.92	0.27	0.368	0.618	0.940
Fat	5.09	5.12	5.29	5.28	0.11	0.296	0.962	0.908
Lactose	3.28	3.30	3.32	3.35	0.06	0.586	0.776	0.960
Milk, %								
Total solid	19.67	19.76	20.09	20.27	0.39	0.414	0.814	0.940
Protein	5.45	5.56	5.81	5.92	0.16	0.136	0.655	0.990
Fat	6.97	7.04	7.23	7.33	0.17	0.290	0.746	0.959
Lactose	5.48	5.54	5.86	5.90	0.17	0.129	0.840	0.991

[1]Standard error of means.

[2]F : free feeding time, B : backfat thickness, F × B : free feeding time × backfat thickness.

REFERENCES

National Research Council. 1998. Nutrient Requirements of Swine, 10th edn. National Academy Press, Washington, DC.

O-28-6

Efficacy of mycosorbents to ameliorate the adverse effects of naturally mycotoxin contaminated corn in Cherry Valley ducks

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ABSTRACT

This study was conducted to evaluate the efficacy of mycosorbents to ameliorate the adverse effects of mycotoxins contaminated corn in Cherry Valley ducks. A total of 144, seven-day-old Cherry Valley ducks were randomly assigned to 4 treatments: 1) basal diet 2) mycotoxin contaminated corn diet (MCD) 3) MCD + 0.2% hydrated sodium calcium alumino silicate (HSCAS) and 4) MCD + 0.2% multi-mycosorbents (bentonite, clinoptiolite, yeast cell wall, and organic acids) (MM). There were 3 replications of 12 birds each treatment. Feed and water were provided *ad libitum* throughout the 42-d study period. At d 42, blood samples from 6 ducks per treatment were collected to determine hematological and serum biochemical parameters. Body weight was measured for calculation of growth performance. The results showed that ducks fed MCD had lower growth performance, poorer hematological and serum biochemical parameter values ($P<0.05$). The supplementation of 0.2% HSCAS and 0.2% MM to the MCD improved BWG, ADG, FCR, CVBW, SVR, and EPEF ($P<0.05$), PCV, Hb, Ca, P, and reduced activities of AST, ALT, ALP and LDH in serum when compared with MCD ($P<0.05$). In conclusion, feeding mycotoxin contaminated diet caused adverse effects on growth performance, hematology and serum biochemistry parameters, and supplementation of 0.2% HSCAS or 0.2% multi-mycosorbent to the mycotoxin contaminated diet ameliorated the adverse effects of mycotoxins in Cherry Valley ducks. However, 0.2% multi-mycosorbent showed better improvement than 0.2% HSCAS.

INTRODUCTION

Mycotoxins are toxic secondary metabolite produced from fungi that have adverse effects in both animals and humans. The toxicity leads to illnesses and economic losses of animal industries (Zain, 2011). Corn is the main source of energy in poultry diets in Thailand. However, producers have some limitation to its use because corn may be easily contaminated with molds and mycotoxins. There are often more than one mycotoxin found in a contaminated feed ingredients of animal feeds. Their synergistic interaction may increase the adverse effects in animals (Schatzmayr and Streit, 2013). The most common mycotoxin found in corn is aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), and ochratoxin A (OTA). They may cause deleterious effects on hematological, biochemical, immune responses and growth performance of animals (Gowda et al., 2008). Aflatoxins are a major problem in poultry production and ducks are highly susceptible to AF more than chickens (Khajarern et al., 2003 Bintvihok, 2011). The most potent dietary approach to preventing the negative effects of mycotoxins in poultry is to use mycosorbents (Surai and Dvorska, 2005). Various mycosorbent products are available on the commercial markets, such as single ingredients of clay, bentonite, zeolite, hydrated sodium calcium aluminosilicates (HSCAS), or combination of mycosorbents with enzyme or yeast-derived or both. However, there are no clear evidence of the efficacy of these products and their function is not well understood. Thus, the objective of this study was to compare the efficiency of two types of mycosorbents, HSCAS and multi-mycosorbents (MM), to ameliorate the adverse effects of mycotoxins contaminated corn on growth performance, hematology, and serum biochemistry in Cherry Valley ducks.

MATERIALS AND METHODS

Animals, Experimental design and Management

A total of 144, seven-day-old mixed-sex Cherry Valley ducks was included in the experiment. The ducks were weighed and randomly assigned to 4 treatments with 3 replications. Treatments were 1) basal diet, control group (fed corn-soybean meal) 2) mycotoxins-contaminated corn diet (MCD) 3) MCD + 0.2% hydrated sodium calcium alumino silicate (HSCAS) (ALCA Co., LTD. Bangkok, Thailand) and 4) MCD + 0.2% multi-mycosorbents (MM) (mixture of bentonite, clinoptiolite, yeast cell wall, and organic acids) (Nutrex Achterstenhoek, Lille, Belgium). The experimental diets were formulated based on the NRC (1994) recommendations. The ducks were reared under

open house system and were provided with feed and water *ad libitum*.

Analysis of mycotoxins

The concentrations of natural mycotoxins contaminated in corn were determined for the levels of AF (B1, B2) by HPLC (In house method TE-CH-025 based on AOAC, 2005) and determined the FUM levels by liquid chromatography with tandem mass spectrometry (In-house method by LC-MS/MS). In this experiment, only AFB_1 exceeded the upper limit of US Food and Drug Administration (FDA) at 20 μg/kg in corn-containing animal feed ingredient, while other mycotoxins did not exceed the upper limits according to Yang et al. (2014).

Growth performance determination

The ducks were examined and mortality rate was recorded. The ducks were weighed at 42 days of age, and feed intake was recorded for calculation of body weight gain (BWG), average daily weight gain (ADG), average daily feed intake (ADFI), feed consumption ratio (FCR), coefficient of variation of body weight (CVBW), survival rate (SVR), and European productive efficiency factors (EPEF).

Hematological and serum biochemical analysis

At 42 days of age, 4 mL of blood samples from 6 birds (2 birds per replication) of each treatment were taken from the wing vein for analysis of blood hematology, i.e. pack cell volume (PCV) and hemoglobin (Hb) concentration, and serum biochemistry, i.e. alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca), and phosphorus (P) using Roche/Hitachi cobas c501 automatic analyzer.

Statistical analysis

All data were analyzed using ANOVA for completely randomized designs. Differences among treatments were examined by Duncan's multiple range tests.

RESULTS

Growth performance

Growth performance indicators are summarized in Table 1. At 42 d, the ducks fed MCD had significantly lower BWG, ADG, ADFI, SVR ($P<0.05$) and poorer FCR, CVBW, and EPEF ($P<0.05$) when compared to control and other treatment groups. Supplementation with 0.2% HSCAS and 0.2% MM in MCD significantly increased BWG, ADG, ADFI, SVR ($P<0.05$) and improved FCR, CVBW, EPEF ($P<0.05$) of ducks when compared with the MCD. The FCR of ducks in 0.2% MM supplementation group was better than that of 0.2% HSCAS supplementation group.

Hematology and Serum biochemistry

Hematological and serum biochemical parameters are presented in Table 2. At 42 d, the ducks fed MCD had significantly lower PCV and Hb concentration ($P<0.05$) when compared to the other treatments. In the present study, supplementation with 0.2% HSCAS and 0.2% MM in MCD significantly increased PCV and Hb concentration ($P<0.05$) of ducks when compared with the MCD. However, both of the mycosorbents could not completely ameliorate the adverse effects of MCD on PCV and Hb concentration to the same level as control group.

For serum biochemistry, the ducks fed MCD had significantly lower Ca and P levels ($P<0.05$), and significantly increased levels of ALT, AST, ALP and LDH ($P<0.05$) when compared to control group and other treatments. In the present study, supplementation with 0.2% HSCAS and 0.2% MM in MCD had significantly increased the levels of Ca and P ($P<0.05$) of ducks when compared with the MCD. However, both of the mycosorbents were unable to completely neutralize the adverse effects of MCD on Ca level since it was still lower than Ca of the control group.

DISCUSSION

This study showed that mycotoxins contaminated corn in diets could have adverse effects on growth performance (decreased BWG, ADG, ADFI, EPEF, SVR and increased FCR, CVBW) of Cherry Valley ducks ($P<0.05$). Similar findings were observed by Han et al. (2008) who reported that AFB_1 (20 and 40 μg/kg) contaminated rice in diets resulted in decreased BWG, ADFI and increased FCR of Cherry Valley ducks. According to Wan et al. (2013), animals received natural mycotoxins contaminated diets showed linearly decreased ADG and uniformity. Moreover, increased mortality of ducks was observed.

Blood hematology and serum biochemistry parameters can be used to evaluate health status, diagnosis of liver

injury, impaired liver function, and nutritional deficiencies of animals (Quist et al., 2000). In this study, MCD showed the advert effects by decreasing the level of PCV and Hb concentrations ($P<0.05$) when compared with the control diet. Similarly, Khajarern et al. (2003) founded that AFB_1 (30, 60 and 120 μg/kg) contaminated diets decreased PCV, and AFB_1 (60 and 120 μg/kg) decreased Hb concentrations in Cherry Valley ducks. Li et al. (2012) reported that Cherry Valley ducks fed AFB_1 (98.73 and 103.61 μg/kg) contaminated diet had decreased Hb and mean corpuscular Hb concentrations.

In this study, MCD alone caused increased AST, ALT, ALP, and LDH activity and decreased levels of Ca and P ($P<0.05$) when compared with the control diet. According to Khajarern et al. (2003), AFB_1 (60 and 120 μg/kg) contaminated diets decreased levels of Ca and P, but ducks fed AFB_1 30 μg/kg contaminated diet showed no different levels of Ca and P when compared with the control. Similarly, Han et al. (2008) reported that AFB_1 (20 and 40 μg/kg) contaminated rice increased activities of ALT and AST but showed no effects on Ca and P of Cherry Valley ducks. These reports agreed with Chen et al. (2014) who found that feeding AFB_1 (110 to 210 μg/kg) was associated with increased ALP and AST activity and reduction of Ca and P in Pekin ducks. A dose-response relationship was also detected.

In summary, supplementation with 0.2% HSCAS or 0.2% multi-mycosorbents can ameliorate the adverse effects of mycotoxins in Cherry Valley ducks.

CONCLUSIONS

Feeding mycotoxin contaminated diet caused negative effects on growth performance, hematology and serum biochemistry parameters of Cherry Valley ducks. Supplementation with 0.2% HSCAS or 0.2% multi-mycosorbents is able to ameliorate the adverse effects of mycotoxins in ducks. However, 0.2% multi-mycosorbents showed better results than 0.2% HSCAS for alleviation of the adverse effects of mycotoxins contamination in diet.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Khon Kaen University, Thailand for the financial support of this study.

KEYWORD : Corn, Mycotoxin, Mycosorbent, Ameliorate, Duck

Table 1. Formula and chemical composition of gestation and lactation sow diets (as -fed basis)

Item	Gestation	Lactation
Ingredients, %		
Corn	41.50	41.00
Wheat	12.00	10.00
Wheat bran	4.00	-
Palm kennel meal	4.00	2.00
DDGS	12.00	8.00
Rapeseed meal	3.00	-
Soybean meal(Local)	6.95	28.71
Coconut meal	4.00	-
Corn gluten feed	2.00	-
Animal fat	5.21	4.03

Table 2. Effects of free feeding time (FFT) and backfat thickness on backfat thickness changes, feed intake and weaning to estrus inte in sows during summer season

Item	Control		FFT		SEM[1]	p-value[2]		
Backfat thickness, mm	<20 (n=14)	≥20 (n=14)	<20 (n=13)	≥20 (n=15)		F	B	F×
Parity	3.43	3.86	3.31	3.80	0.26	0.810	0.216	0.9
Sow body weight, kg								
Gestation, d109	228.86	234.80	220.48	234.19	4.81	0.511	0.153	0.56
Weaning	207.70	211.63	203.46	213.35	4.18	0.833	0.250	0.61
Change, -	21.16	23.17	17.02	20.84	0.86	0.007	0.015	0.44
Sow Backfat thickness, mm								
Gestation, d109	19.71	22.21	19.54	22.07	0.30	0.538	<0.001	0.95
Weanling	15.93	17.36	16.38	17.93	0.23	0.050	<0.001	0.81
Change, -	3.79	4.86	3.15	4.13	0.22	0.022	0.001	0.87
Daily feed intake, kg/d	4.81	5.22	5.21	5.71	0.11	0.006	0.004	0.75
Weaning to estrus interval, d	5.07	4.86	4.85	4.67	0.13	0.274	0.300	0.92

[1]Standard error of means.

[2]F : free feeding time, B : backfat thickness, F × B : free feeding time × backfat thickness.

REFERENCES

Bintvihok, A. 2011. Controlling aflatoxin danger to duck and duck meat. Worlds Poult. Sci. J. 17:18–20.

Chen, X., N. Horn, P. F. Cotter, and T. J. Applegate. 2014. Growth, serum biochemistry, complement activity, and liver gene expression responses of pekin ducklings to graded levels of cultured aflatoxin B1. Poult. Sci. 93:2028–2036.

Gowda, N. K. S., D. R. Ledoux, G. E. Rottinghaus, A. J. Bermudez, and Y. C. Chen. 2008. Efficacy of turmeric (*Curcuma longa*), Containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci. 87:1125–1130.

Han, X.-Y., Q.-C. Huang, W.-F. Li, J.-F. Jiang, and Z.-R. Xu. 2008. Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livest. Sci. 119:216–220.

Khajarern, J. M., S. Khajarern, T. H. Moon, and J. H. Lee. 2003. Effects of dietary supplementation of fermented chitin-chitosan (FERMKIT) on toxicity of mycotoxin in ducks. Asian-Australas. J. Anim. Sci. 16:706–713.

Li, Y., Y. H. Liu, Z. B. Yang, X. L. Wan, and F. Chi. 2012. The efficacy of clay enterosorbent to ameliorate the toxicity of aflatoxin B1 from contaminated corn (Zea mays) on hematology, serum biochemistry, and oxidative stress in ducklings. J. Appl. Poult. Res. 21:806–815.

Quist, C. F., D. I. Bounous, J. V. Kilburn, V. F. Nettles, and R. D. Wyatt. 2000. The effect of dietary aflatoxin on wild turkey poults. J. Wildl. Dis. 36:436–444.

Schatzmayr, G., and E. Streit. 2013. Global occurrence of mycotoxins in the food and feed chain: facts and figures. World Mycotoxin J. Available from: http://www.wageningenacademic.com/doi/abs/10.3920/WMJ2013.1572

Surai, P. F. and J. E. Dvorska. 2005. Effects of mycotoxins on antioxidant status and immunity. The mycotoxin blue book. D. Diaz, ed. Nottingham Univ. Press, UK. pp. 93-137.

Wan, X. L., Z. B. Yang, W. R. Yang, S. Z. Jiang, G. G. Zhang, S. L. Johnston, and F. Chi. 2013. Toxicity of increasing aflatoxin B1 concentrations from contaminated corn with or without clay adsorbent supplementation in ducklings. Poult. Sci. 92:1244–1253.

Zain, M. E. 2011. Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc. 15:129–144.

O-28-8

EFFECT OF SURGICAL AND IMMUNOLOGICAL CASTRATION ON COLLAGEN SOLUBILITY AND SHEAR FORCE VALUE IN PORK

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The objectives of this study were to determine collagen solubility and shear force value from surgical (SC) and immunological castration (IMC) pork and to compare the studied traits between this pork. There were 15 pigs from each surgical method raised with 12, 14, and 16% protein commercial pig ration during the age of 10 to 24 weeks old. Surgical castration was performed at the age of one week old. The pigs in immunological castration group were vaccinated with IMPROVAC at the age of 16 and 20 weeks old. Fresh *Longissimus dorsi* (LD) muscle samples between the 13th and 14th ribs were taken and processed to determine collagen solubility and shear force value. The results revealed that the soluble collagen and percentage of collagen solubility of surgical and immunological castration pork were 0.45 and 0.52 mg/g, 10.63 and 12.29%, respectively. The soluble collagen and percentage of collage solubility of immunological castration pork were higher than those from surgical castration pork ($p < 0.05$). However, the castration methods had no effect on pork shear force value ($p > 0.05$). The pork shear force value of SC and IMC were 4.14 and 4.15 kg, respectively.

Introduction

The objective of doing castration of male pig is to prevent the accumulation of skatole and androstenone in the fatty tissue or control of boar taint. Due to the animal welfare concerns, the immunological castration has been used as an alternative method for surgical castration in pigs. Many research reported that both surgical and immunological castration have affected growth performance, carcass, and also meat quality (Skrlep et al., 2012). In Thailand farmers prefer to do surgical castration in male pigs, however more information about the effect of the immunological castration on pork quality should be reported because it would be important information to support the used of the immunological castration as an alternative method for surgical castration in pigs in Thailand. The objectives of this study were to determine collagen solubility and shear force value from surgical (SC) and immunological castration (IMC) pork and to compare the studied traits between this pork.

Methodology

There were 15 three way crossbred pigs [Duroc*(Large White*Landrace)] from each surgical method raised with 12, 14, and 16% protein commercial pig ration during the age of 10 to 24 weeks old. Surgical castration was performed at the age of one week old. The pigs in immunological castration group were vaccinated with IMPROVAC at the age of 16 and 20 weeks old. Fresh *Longissimus dorsi* (LD) muscle samples between the 13th and 14th ribs were taken and processed to determine collagen solubility and shear force value. Collagen solubility was measured by using Hill (1966) method. The shear force measurement was obtained by using a Warner-Bratzler meat shear machine.

Data Analysis

The independent t-test was used to compare the collagen solubility and shear force value of pork from surgical and immunological castration methods.

Results and Discussions

The results of this study were summarized in Table 1 and 2. The insoluble, soluble collagen, total collagen, and percentage of collagen solubility of surgical and immunological castration pork were 3.73 and 3.87, 0.45 and 0.52 mg/g, 4.31 and 4.28, and 10.63 and 12.29 %, respectively. The soluble collagen and percentage of collagen solubility of immunological castration pork were higher than those from surgical castration pork ($p < 0.05$). However, the castration methods had no effect on pork shear force value ($p > 0.05$). The pork shear force value of SC and IMC were 4.15 and 4.14 kg, respectively.

This study showed that there were no differences of total collagen and shear force value of pork from SC and

IMC pigs. These results correspond with the finding of Font-i-Furnols et al. (2012) who reported that IMC did not affect fat and meat quality but reduced the skatole and andrestenone levels. And Boler et al. (2011) reported that there were no differences between IMC and SC pork for shear force value, ultimate pH, color score, or drip loss. However, this study revealed that the soluble collagen and percentage of collagen solubility of immunological castration (IMC) pork were higher than those from surgical castration (SC) pork ($p < 0.05$). Dikeman (2007) reported that the immunocastration affected growth performance, carcass composition, meat quality, and preventing boar taint. D'Souza et al. (1999) showed the tenderness of pork loin steaks from immunocastration was more tender than from boar and barrow. Seideman (1986) revealed that there was the negative correlation between percentage collagen solubility and tenderness. Voutila (2009) said that the more soluble collagen the more tenderness of meat. In this study IMC pork should be more tenderness than SC pork but the shear force value of IMC and SC pork were no differences. So, more information on the relationship between soluble collagen and the pork tenderness is required.

Conclusions

According to the results of this study it could be concluded that immunological castration affects collagen solubility which may involve in the pork tenderness but the shear force value did not confirm so more study on the relationship between collagen solubility and the pork tenderness should be performed.

Acknowledgement

The authors acknowledge the financial support from the Zoetis Thailand and Faculty of Industrial Education, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

KEYWORD : Surgical castration, Immunological castration, Collagen solubility, Shear force value, Improvac

Table 1. The means and standard deviation of insoluble, soluble, and total collagen (mg/g), and collagen solubility (%) from surgical (SC) and immunological castration (IMC) pork.

Traits	Castration method		Sig.
	SC	IMC	
Insoluble Collagen (mg/g)	3.73 ± 0.47	3.87 ± 0.64	0.509
Soluble Collagen (mg/g)	0.45 ± 0.05	**0.52 ± 0.06**	0.010
Total Collagen(mg/g)	4.31 ± 0.46	4.28 ± 0.53	0.902
Collagen solubility (%)	10.63 ± 1.04	**12.29 ± 1.39**	0.003

Table 2. The means and standard deviation of shear force value (kg) from surgical (SC) and immunological castration (IMC) pork.

Trait	Castration method		Sig.
	SC	IMC	
Shear force value (kg)	4.15 ± 0.58	4.14 ± 0.66	0.962

Table 2. The means and standard deviation of shear force value (kg) from surgical (SC) and immunological castration (IMC) pork.

Trait	Castration method		Sig.
	SC	IMC	
Shear force value (kg)	4.15 ± 0.58	4.14 ± 0.66	0.962

REFERENCES

Boler, D.D., Killefer, J., Meeuwse, D.M., King, V.L., McKeith, F.K., and Dilger, A.C. (2011). Effects of harvest time post-second injection on carcass cutting yields and bacon characteristics of immunologically castrated male pigs. Available on line at www.journalofanimalscience.org

Dikeman, M. E. 2007. Effects of metabolic modifiers on carcass traits and meat quality. Meat Science 77 (2007) 121-135. Avialable online at www.sciencedirect.com

D'Souza. D. N., Hagan.C. R., Hooper, J. A., Nicholls, R. R., and Mullan, B. P. 1999. Influence of genotype and sex on pork eating quality: a consumer taste panel assessment. In P. D. Cranwell (Ed), Manipulating pig production VII (pp. 177). Werribee: Australasian Pig Science Association

Font-i-Furnols, M., Gispert, M., Soler, J., Diaz, M., Garcia-Regueiro, J.A., Diaz, I., and Pearce, M.C. 2012. Effect of vaccination against gonadotrophin-releasing factor on growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured ham production. Meat Science 91 (2012) 148-154

Hill, F. 1966. The solubility of intramuscular collagen in meat animalsof various ages. Journal of Food Science 31: 161-166

Skrlep, M., N. Batorex, M. Bonneau, M. Prevolnix, V. Kubale, and M. Candex-Potokar. 2012. Effect of immunolcastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass, and meat quality. Czech J. Anim. Sci., 57, 2012(6): 290-299

Seideman, S.C. 1986. Methods of expressing collagen characteristics and their relationship to meat tenderness and muscle fiber types. Journal of Food Science. pages 273–276, March 1986. Article first published online: 25 Aug. 2006. Available online at http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1986.tb11107.x/abstract

Voutila, L. 2009. Properties of intramuscular connective tissue in pork and poultry with reference to weakening of structure. Doctoral dissertation (article-based). University of Helsinki, Faculty of Agriculture and Forestry, Department of Food Technology, meat technology. Available online at https://helda.helsinki.fi/handle/10138/20800

O-28-9

A New Development System for Automated Measurement and Recording pig weight

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Objective

In Taiwan, staffs of pig industry still use traditional way to measure pig weight by manually driving pigs to the weighing platform. The advantage of it is that only some workers and weighing scales are needed. However, it takes a lot of time to weigh every pig in a large pig farm. Additionally, driving pigs usually scares them. Then pigs will become so emotional that they defecate, urinate and even step on each other. As a result, the hygiene of the pig house gets worse, raising risks of infections or diseases.

A new technique of real time pig weight measurement not only shows the economic value of pigs but also helps to predict physiological status of pigs if combined with other physiological data of pigs. The data of pig weight can be further combined to artificial intelligence to upgrade automated breeding technology. Automated breeding technology has been developed recently. Chen et al. (2013,2014,2015) developed a system for dairy milking process based on wireless sensors and cloud database technology. Arias et al. (2004) designed an image interpretation software which can automatically measure the weight of dairy cattle by evaluating different parts of cattle body. Ferdous et al. (2011) proposed an image analysis technology to reduce the error of pig weight measurement committed by different postural changes. Tscharke et al.(2011) reviewed currently available technologies to measure the weight of livestock and discussed their limitations.

In this study, radiofrequency identification (RFID) is used to identify pigs when they come to water tank to drink. Then a weighing scale under the water tank is built to measure pig weight. When a pig drinks water, it will be identified and its weight will be transferred to a computer to compute, judge and record. Because every pig drinks water several times in a day, data of pig weights will become big data. This big data can be used to analyze and predict the physiological status of pigs.

Methodology

Figure 1 shows the flowchart to get pig weight in this study. Pigs go to the water platform to drink water when they feel thirsty. A tag which is put on their ear will receive a signal from the RFID reader and then resend a signal back to the reader when pigs come close enough to the water platform. The tag is unique to each pig, so it is easy to identify each pig. In order to measure their weights when they drink water, a weight measure system is designed within the red area as shown in Figure 2. Then the pig identity and weight will be sent by a wireless signal transmitter to internet cloud to compute, judge and record. Users can use a cell phone or a computer to know the weight of each pig real time. The flowchart of this automatic identification and weighing system is defined in Figure 3.

(1) Radiofrequency Identification system (RFID) and signal transmission

This is a non-contact automatic identification system which uses radio waves to transmit identity information. The basic RFID system consists of three parts: a set of tags, a reader and an antenna. The tag is made up by coupling components, chips, and an internal antenna used to communicate with RF antenna. The function of the reader is to read or even to record the information from the tag. The antenna transmits RF signals between the tag and the reader.

There are many types of tags. They can be divided into different types according to the characteristics of power source, radiofrequency and access mode. The power source can be passive, semi-passive, and active. The radiofrequency can be low, high, ultra-high frequency, and microwave. The access mode can be read-only, write once, readable and rewritable. In this study, a read-only tag which uses passive power source and ultra-high

radiofrequency is used.

(2) Weighing system and signal transmission
Where the weighing system will be located is shown in Figure 2. The system should fit to the red area. The data of pig weight measured by the weighing system will be transformed into the voltage signal (0-5V) and then sent to the transmitter module by a transmission line. The transmitter module will send the signal to a cloudy server, which can record the data of pig weight transformed from voltage signal according to identities. The specification of weighing system in this study is shown in Table 1.

(3) Signal transmitter, cloudy computing, data record and received module
The pig identity signal from RFID and the weight signal are transmitted separately to a transfer module by two transmission lines. The transfer module will send the signals to the cloudy server by wireless technology (SIM card of 3G or Wi-Fi). The cloudy server can then compute the data. In the end, users can receive and monitor the data by 3G cell phone or computers which have internet network function. The signal transmission module used in this study is shown in Figure 4.

Results and conclusion

The key point of this system is to record the pig weight corresponding to a specific pig identity. However, it might happen that several pigs drink water at the same time. Then this system records one pig weight with several pig identities. Among the weight data with different numbers of pigs, some strategies should be developed to screen and classify the data. In addition, the size of the pig may exceed the area of weighing system as the pig grows up. Therefore, some solutions should be found to adjust the weighing area according to the pig size. After solving these two problems, we will start a four-week experiment continuously measuring weights of five pigs. The goal of this system is to reach more than 90% accuracy in pig identification and weight error less than 0.5Kg.
This automatic weighing system will bring lots of benefits. Firstly, it will meet the economic need for pig farmers. In addition, the big data of pig weight can be further used to analyze the relations to the pig disease, feeding strategies, breeding environment and efficiency optimization. Finally, introducing automatic measuring and analysis system to pig industry in Taiwan is an irreversible trend because of a low birth rate in humans. Further extensions and applications of this system are predictable.

KEYWORD：Pig Weight, Intelligence Machine, Pig Industry

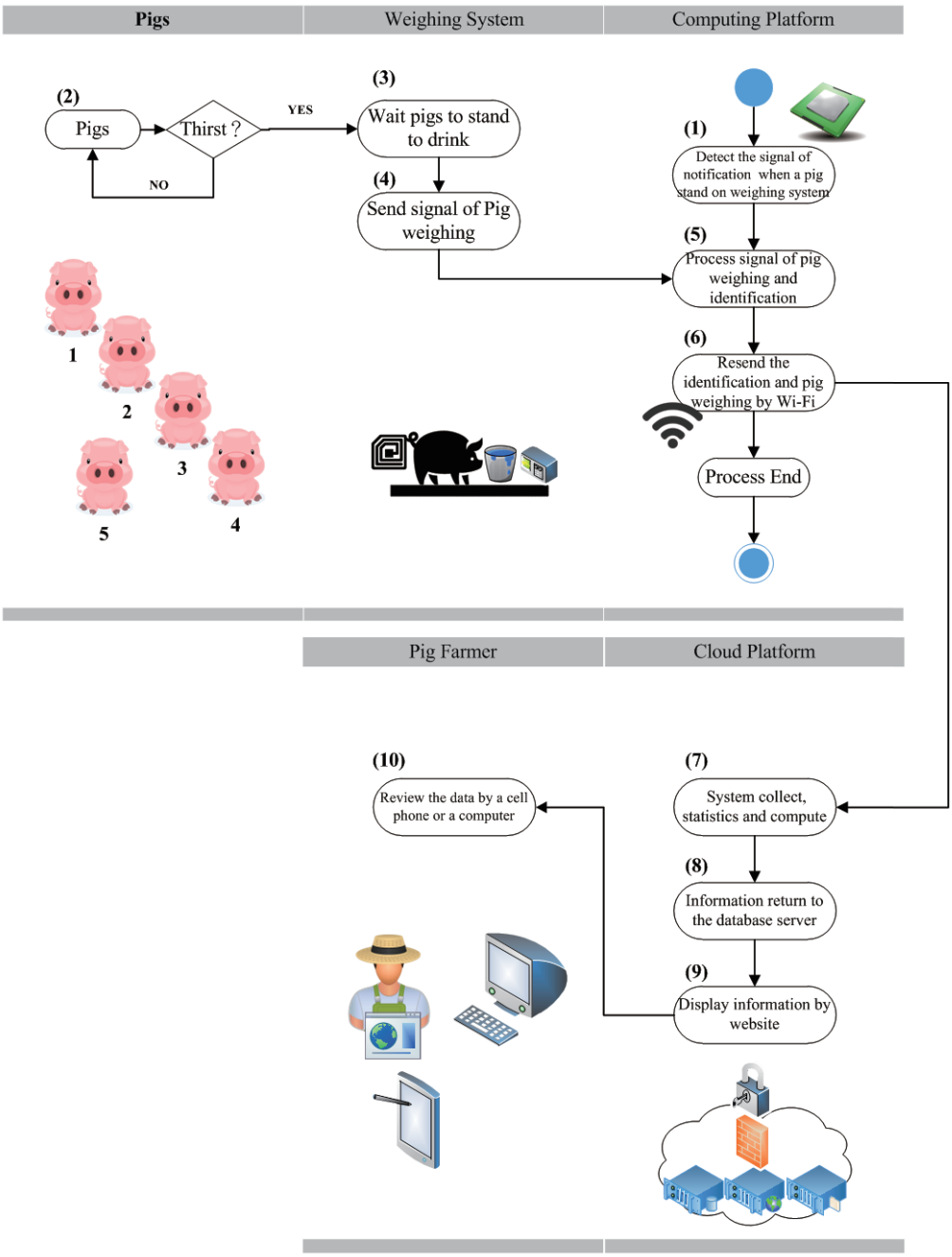

Fig. 1 The flowchart of getting pig weight in this study

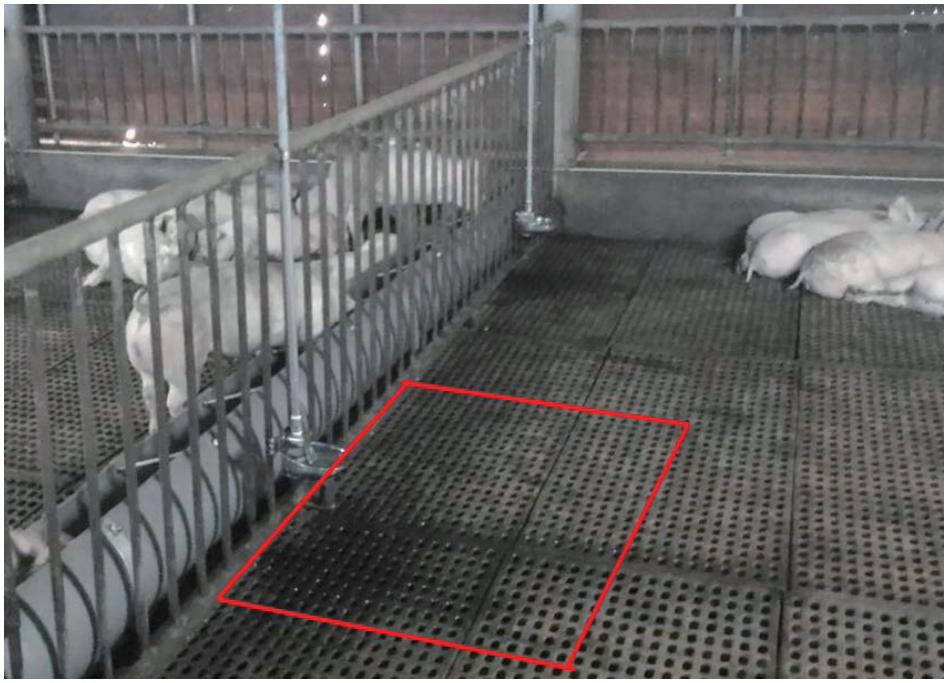

Fig. 2 The area to develop a weight detection system to pigs

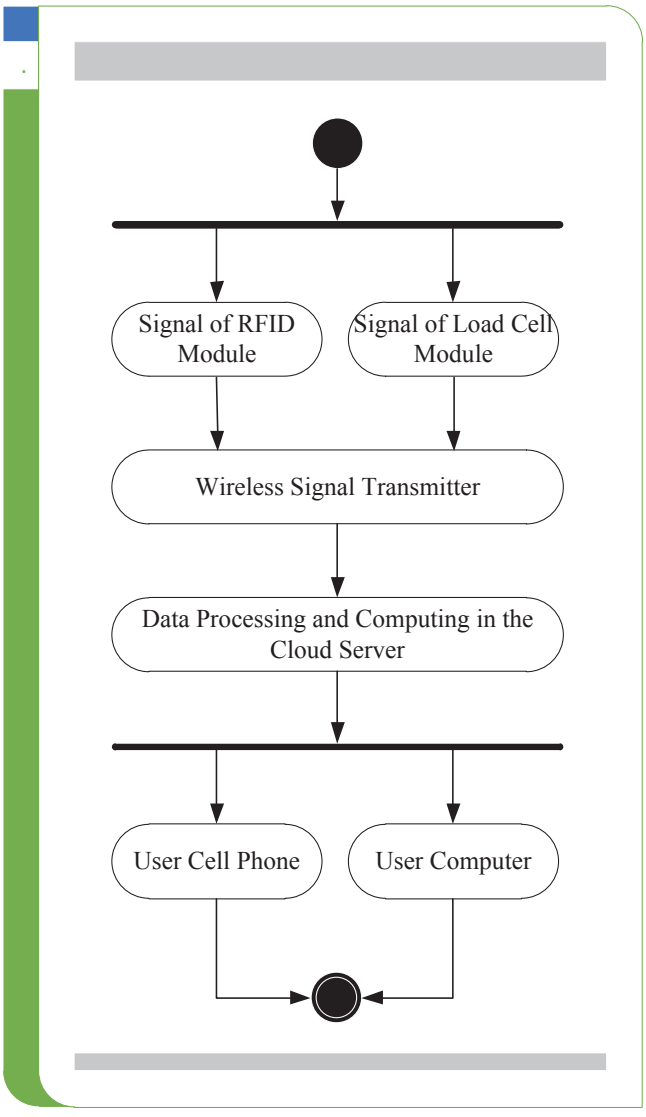

Fig. 3 The flowchart of automatic identification and weighing system

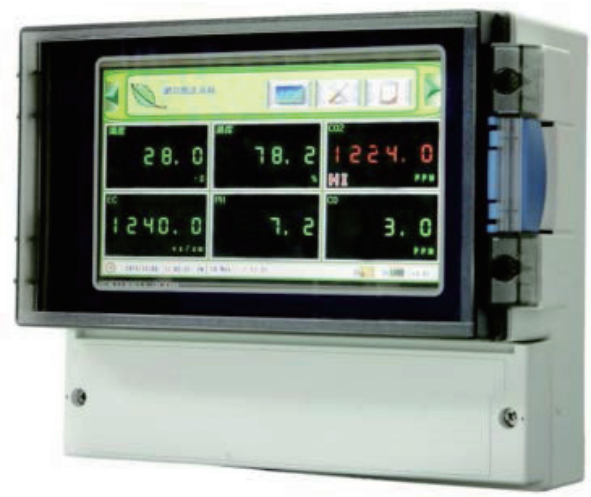

Fig. 4 The modules of signal transmitter, cloudy computing and record in this study

Table 1 The specification of weight platform in this study

Description	Specification
Dimension	1340 x 570 x 680(mm)
Capacity	300 Kg (Division：0.1 Kg)
Load Cell	250kg x 4Sets
Weighting indictor	0-5V

REFERENCES

Arias, Néstor Alonso, Molina, Maria Leonor and Gualdron, Oscar, 2004, "Estimate of the weight in bovine livestock using digital image processing and neural network," Proceedings of the SPIE, vol.5622,pp.224-228.

Chen, Chien-Hsing; Chen, Ming-Chih; Chiu, Yi-Wen; Siang, Chong-Yu and Yang, Jung-Sheng, 2013, Quarter Milking Management System for Dairy Cow using Wireless Sensor Technologies, Advanced Science Letters, Vol.19,No11,pp.3368-3373(6).

Chen Chien-Hsing and Lin Hung-Ru, 2015, Estrus Detection for Dairy Cow Using ZigBee-Based Sensor Networks, Intenational Jounal of Information and Electronics Engineering, Vbl.5,No.4.

Ferdous, W.M., Tscharke, M.Saunders, C., Lee, S.H. and Banhazi, T.M,. 2011, "Digital image processing methods for the identification of pigs posture during weight estimation," In: 5thEuropean Conference on Precision Livestock Famm ing, Prague.

Chen Ming-Chih, Chen Chien-Hsing, and Siang Chong-Yu, 2014, Design of Information System for Milking Dairy Cattle and Detection of Mastitis, Mathematical Problems in Engineering, Vol.2014.

Tscharke, M. and Banhazi, T.M., 2011, "Review of methods to detemm ine weight, size and composition of livestock from images," In: SEAg: Diverse Challenges, 1nnovative Solutions, Gold Coast, Australia.

O-29-2

Genetic Diversity of Ongole Crossbred Cattle in Kebumen based on MC4R Gene

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NTRODUCTION

The Ongole crossbred cattle was formed as a result of grading up of Java and Sumba Ongole cattle that comes from Madras, India in 1930 (Hardjosubroto, 1994). The Ongole crossbred cattle was known as meat and draught cattle. The characteristics of Ongole crossbreed cattle are white-gray color on body, with a large head, neck and knees were dark. The body size of this cattle is relatively short, large humped and has a wattle at the neck to stomach. The potential of Ongole crossbred cattle as one of the genetic resources of livestock and protein sources should be increased, including through the selection. A growth trait such as body weight is an economic trait which became one of the considerations as selection criteria in selecting a cattle. The predominance of Ongole crossbred cattle in Kebumen proved by ministry decision letter of 47/Kpts/SR.120/1/2015. Ongole crossbred cattle also has larger body size than Indonesian National Standard of Ongole cattle. One of the associations of Ongole crossbred cattle breeder in Central Java was called ASPOKEB (Association of Ongole crossbreed cattle breeder in Kebumen)

The *Melanocortin-4-receptor* (MC4R) gene is known as an important candidate gene for the growth trait. It was involved in regulation of feeding behavior and body weight in animals. The melanocortinergic system has been detected in association with body weight in the agouti mouse (Chen et al., 2004). The MC4R response to leptin signaling in association between food intake and body weight (Marsh et al., 1999). Neuropeptide Y (NPY) signal in the central nervous system parallel with MC4R signal (Hahn et al. 1998). The previous study proved that several mutations in MC4R were related with obesity in humans (Vaisse et al. 1998 Yeo et al.1998A SNP of C1069G of MC4R was significantly associated with live weight (LW), carcass weight (CW), backfat thickness (BF), and Marbling score (MS) in Qinchuan cattle (Liu et al, 2010). Therefore, the objective of this study was to determine genetic diversity of Ongole crossbred cattle in Kebumen based on MC4R gene**.**

MATERIALS AND METHODS

Animals and data collection

Sixty Ongole crossbred cattle in Kebumen were used for this study. The animals derived from the district of Klirong consist Tanggulangin, Pandan Lor, Kedungsari, Gebangsari and Tambak Progaten village. The ± 3 ml of blood samples were collected for genomic DNA isolation using gSYNC™DNA Exstraction Kit (Genetika Science).

Polymerase Chain Reaction (PCR)

The primer sequences according to Seong *et al*. (2012) for PCR amplification and the restriction enzyme for PCR-RFLP are shown in Table 1.

Polymerase chain reaction (PCR) was performed in 20 µl volumes, each containing 2 µl DNA product,
30 µl 10xbuffer, 30.4 µl dNTP, 3.8 µl Taq DNA polymerase, 15,2 µl of each primer and 239,4 µl Double Destilated Water (DDW). PCR conditions were 5 min at 94˚C for pre-denaturation and 35 cycles of 30 s at 94˚C for denaturation, 30 s at 58˚C for annealing, 30 s at 72˚C for extension, and 10 min at 72˚C for final extension using a Parkin Elmer Thermal Cycler PCR system. The PCR products were visualized by 1.5% standard agarose gels stained with ethidium bromide.

PCR-RFLP and Genotyping Determination

The PCR products were sequenced by PT Genetics Science Indonesia with the same primers for PCR. The sequences result were analyzed with the BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA). The SNP 1133 C>G was confirmed based on the electrophoregram results. The SNP 1133 C>G was used for genotyping by the PCR-restriction fragment length polymorphism (PCR-RFLP) method with *HpyCH4*IV restriction enzyme. The restriction enzyme digestion was performed in 20 µl reaction volumes with approximately 15 µl of PCR products, 2 µl 10xbuffer, 0,5 µl restrisction enzim and 2,8 µl DDW . The digested products were run

on 4% agarose gels.

Statistical Analysis

Allele frequencies were calculated with the following method :

C = (2 CC + CG)/2n

G = (2 GG + CG)/2n

Genotype frequencies were calculated with the following method :

$CC = n \times C^2$

$GG = n \times G^2$

$CG = 2n \times C \times G$

Where C and G were allele frequency of observed sample in population CC, GG, CG were animal genotype and n was number of observed samples.

Pearson's Chi-square test was used to verify the Hardy-Weinberg equilibrium status for the allele and genotype frequencies. The following mathematical model was:

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where, X^2 is Chi-square test value, Oi is observed frequency, Ei is expected frequency, n is the number of possible outcomes of each event.

RESULTS AND DISCUSSION

The SNP 1133 C>G of MC4R gene was initially detected by direct sequencing using PCR product pool by PT Genetics Science Indonesia (Fig.1a). The SNP was used for genotyping by using PCR-RFLP methods with *HpyCH4IV* restriction enzyme. Animals having homozygote CC were defined when the fragments size being recognized at 493, while homozygote GG was 173 and 320 bp. The heterozygote CG existed by PCR-RFLP method at the same position of the homologous chromosome with 173, 320 and 493 bp of fragments size (Fig. 1b). As the results, most of animals in this study have heterozygote (CG) genotype followed by GG and CC genotype. The allele and genotype frequencies are shown at Table 2.

The result of PCR-RFLP indicated C and G alleles (Table 2). The frequencies G allele was greater than C alelle. The highest genotype frequencies have been detected in CG heterozygote animals followed by GG and CC homozygote animals. The results of Pearson 's Chi-square test indicated that the genotypes of the cattle were not deviated (P>0,05) from the Hardy-Weinberg equilibrium (HWE). Base on The Hardy-Weinberg equilibrium on the cattle population of this study gives the sense that the frequency of allele and genotype in the population will be constant from one generation to the next generation as long as there are no confounding factors, selection, mutations, migration, and marriage between individuals in these populations randomly (Warwick *et al.,* 1990).

CONCLUSIONS

the SNP 1133 C>G of MC4R gene may have been able to determine the genetic diversity of Ongole crossbreed cattle in Kebumen. Our suggest the 1133 C>G of MC4R gene may associate with growth traits and can be used as a marker for selecting the Ongole crossbred cattle having desire traits.

KEYWORD : Genetic diversity, Kebumen Ongole crossbreed cattle, MC4R

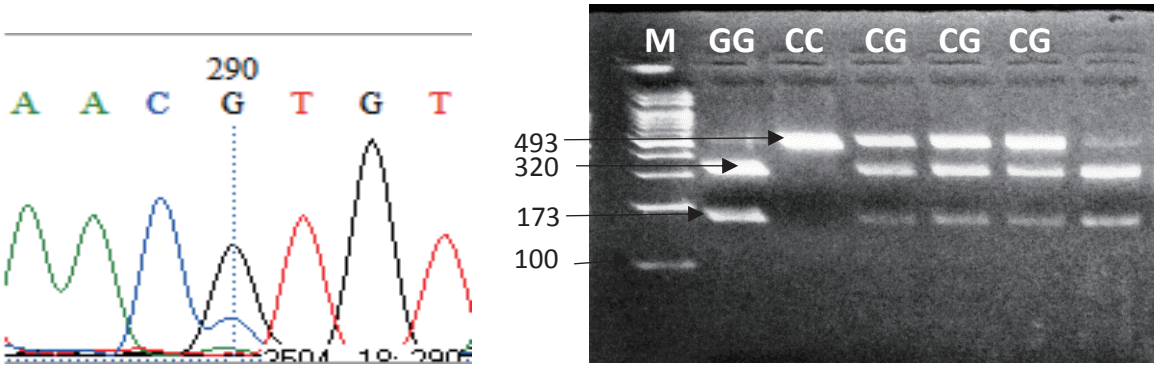

Figure 1 (a). Electrophoregram result for the identified SNP g.1133C>G in MC4R gene, (b) PCR-RFLP patterns of SNP 1133C>G (digested with *HpyCH4*IV)

Table 1. Primers for PCR amplification and restriction enzyme information for genotyping of MC4R gene

GenBank	Primer	PCR product size	Restriction enzyme
EU366350.1	F: 5'-GTCGGGCGTCTTGTTCATC-3' R : 5'-GCTTGTGTTTAGCATCGCGT-3'	493	*HpyCH4*IV

Table 2. The allele and genotype frequencies of Ongole crossbred cattle based on MC4R gene with PCR-RFLP methods using SNP 1133 C>G

SNP	Alelle Frequency		Genotype Frequency		
	C	G	CC	GG	CG
1133 C>G	0.41	0.59	0.15	0.33	0.52

REFERENCES

Chen, M., A. Wang, J. Fu, and N. Li. 2004. Different allele frequencies of MC 4R gene variants in Chinese pig Breeds. Tierz., Dummerstorf. 5:463-468.

Hardjosubroto, W. 1994. Aplikasi Pemuliabiakan Ternak di Lapangan. Grasindo, Jakarta.

Liu H, Tian W, Zan L, Wang H, Cui H. 2010. Mutations of MC4R gene and its association with economic traits in Qinchuan cattle. Mol Biol Rep. 37:535–540.

Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA. 1999. Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. Nat Genet. 21:119–122.

Hahn TM, Breininger JF, Baskin DG, Schwartz MW. 1998. Coexpression of Agrp and NPY in fasting activated hypothalamic neurons. Nat Neu-rosci 1:271–27.

Vaisse C, Clement K, Guy-Grand B, Froguel P 1998A frameshift mu-tation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113–114.

Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG. 1998. A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20:111–112.

Warwick, E.J., J.M. Astuti, dan W. Hardjosubroto. 1990. Pemuliaan Ternak. Universitas Gadjah Mada Press. Yogyakarta, Indonesia.

O-29-3

Genetic Diversity and Association between Reproductive Traits and Four Microsatellites in Thai Locally Adapted Cattle

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INTRODUCTION

Microsatellite markers was particularly useful because of the wide variability, randomly distributed throughout the genome of Mendelian inheritance (Tautz 1993) and influence the possibility of gene regulation (Schroth et al, 1992 Comings 1998). Microsatellite markers tend to be concentrated in transcription initiation regions, with the virtual absence of these sequences at the intergene positions and pseudogenes. Sequence rich in purines and pyrimidines, (CA)n, such as microsatellites, are formed Z-DNA under physiological conditions (Comings, 1998). These facts suggested the potential role of microsatellites in gene regulation. By these considerations, the association between a microsatellite and a given polygenic phenotype would be not dependent on a specific allele but on a repeat size threshold.

In a study on Thai locally adapted cattle (Thai Native, Crossbreed Brahman, and Crossbreed Charoles) population involving individuals with different degrees of fertility, Maungkhiow and Pothkom (2011) observed differences in the age at first calving (AFC). Average AFC of 75% Brahman, 50% Brahman, 50% Charoles, and Thai Native are 41.88 +0.83, 40.82+0.41, 40.88+0.93, 39.20+0.41, and 38.21+0.54 months respectively ($P<0.01$).

The studies conducted on various species have demonstrated that estrogen plays an important role on follicular development, including follicle stimulation and maturation (Goldenberg *et al.* 1972) and the increased expression of follicle-stimulating hormone and luteinizing hormone receptors by granulosa cells (Richards *et al.* 1976 Richards *et al.* 1979). In cattle, the mature follicle will present levels of estrogen higher than unmatture follicle (Evans and Fortune 1997). Evidence also suggested that insulin-like growth factor (IGF) system consisting of IGF-I, -II, IGF receptors, and IGF- binding protein (IGFBP) was important for the effect of nutrition on postpartum anestrus. (Monget, 1997, Martin et al, 2000). So estrogen receptors and IGF-I were considered a good candidate of possible associations between molecular and reproductive efficiency.

The present study analyzed genetic diversity of reproductive traits and the possible association between genetic markers and reproductive efficiency through four microsatellite loci as the estrogen receptor genes.

MATERIALS AND METHODS

Animal

The association with reproductive efficiency was tested in a sample of 300 individuals obtained from Thai locally adapted beef cattle (75 Thai indigenous cattle (TC), 75 Thai Brahman cattle(TB), 75 Tak cattle(TA) (5/8 Charoles x 3/8 Brahman), and 75 Kabinburi cattle(KB) (1/2 Simental x 1/2 Brahman)). They were collected data for reproductive traits according to the average of their calving interval and age at first calf. These populations had been raised in Livestock Research and Breeding Center of Department of Livestock Development as *ex situ* conservation in vivo.

Microsatellites

Four microsatellites (MM12E6 chromosome 9, BL1071, HEL5 chromosome 13, and AFZ1 chromosome 21) were investigated. The MM12E6 was the same of the estrogen receptor gene (ESR1). The BL107 and HEL5 were closed to IGF-IR gene. The AFZ1 was closed to IGF1-R locus (Barendse *et al.* 1997 http://www.ncbi.nlm.nih.govhttp://www. marc.usda.gov http://www.inra.fr).

Genomic DNA was extracted from Blood samples according to the method of Mohammadi and Saberivand (2009). Polymerase chain reaction (PCR) was performed in a total volume of 25 μ l containing 200 ng of genomic DNA, 3 mM of $MgCl_2$, 5mM of each dNTP, 2.5 μM of each primer and 1 unit of *Taq* polymerase (Vivantis, USA). The PCR cycle was accomplished in the step of denaturation for 30s at 94°C, primer annealing for 45s at specific annealing temperature, and an extension for 45s at 72°C and repeated 34 times finally post extension for 5 min at 72°C for

1 cycle. The products were separated on a 12% denaturing polyacrylamide gel (Sigma, USA). Allele visualization was achieved by silver staining according to manufacture's standard protocol (Promega, USA). The amplification product sizes at each locus were estimated using molecular weight ladder (DNA Ladder, ØX*Hin*fI).

Analysis

Genetic diversity of reproductive traits within and between populations were analyzed by POPGENE v.1.32 software package (Yeh *et al*., 1999). Hardy-Weinberg equilibrium at each locus was determined and genetics distances among the populations were calculated (Nei, 1972). UPGMA method was used to construct phylogenetic tree.

Association analyses between genotype classes of genetic markers and reproductive performance were determined by one-way analysis of variance, considering the genotype classes of the alleles as independent variables and reproductive records measurements as dependent variables.

RESULTS AND DISCUSSION

Genetic diversity of reproductive traits

The observed alleles and the frequencies of genotypes were shown in Table 2. Total heterozygosity was similar for the four systems, varying from 0.52 to 0.70. The 4 markers were in linkage disequilibrium (p = 0.00). All amplified loci were polymorphic, showing percentage of polymorphic loci (PPL) as 100%. This high PPL value indicated the usefulness of these markers in population studies.

The estimated parameters pertaining to genetic polymorphism in 4 breeds, observed and effective number of alleles, observed and expected heterozygosity were presented in Table 1. A total of 14 alleles were detected across the 4 loci related to reproductive traits with an average of 2.01, 2.36, 2.30, and 2.98 alleles per locus (mean number of alleles in TI, TB, TA, and KB cattle, respectively. The number of observed alleles ranged from 3 at loci MM12E6, BL1071, AFZ1 to the highest of 5 alleles at locus HEL5. In KB cattle, there were highest observation alleles, 3 alleles for MM12E6, BL1071, AFZ1 and 5 alleles for HEL5. There were differences in locus BL1071 of TA cattle (2 alleles) compared with other breeds (3 alleles). It also showed differences in locus HEL5. KB cattle had highest alleles in locus HEL5 (5 alleles), 4 alleles in TB and TI, 3 alleles in TA cattle. The observed number of alleles demonstrated that almost all the microsatellite loci utilized in the present study were sufficiently polymorphic. The increase of number of alleles at different loci, there was increase in mean genetic diversity in population and supported by Moioli *et al.* (2001).

The Nei's genetic distance matrix (Table 2) showed that TI cattle was the most divergent from the others. This result was expected since TI was a *Bos indicus* while TA and KB were synthetic breed from *Bos tuarus and Bos indicus*. However, TB was *Bos indicus*, but it was closed to KB and TA. Because of the selection program for beef cattle. The TI breed was Thai indigenous that had the good reprocuctive traits. So by these reasons, TI breed was the most divergent from other breeds. The closest related breeds were TB and KB, because KB is developed from Simental and Brahman, and selected by the selecting program of beef cattle for growth performance. The KB breed were closed to TB than TA because KB breed had 50% Brahman and TA breed had 37.5% Brahman in breeding plan. This result showed a very distinct clustering between 1) TB, KB, TA and 2) TI, because the cluster 1 breed group were developed from Brahman and they were in selection program of beef cattle for major goals of growth performance. While TI breed was Thai indigenous animal genetic resources that had good reproductive traits, and they were in conservation program for base genetics to establish new breed of dairy and beef cattle for food security.

Association between reproductive traits and four microsatellites

In Table 3, locus MM12E6 associated with reproductive traits. The genotype AA gave the best AFC (32.89 months), and the best CI (423.66 days). The best genotype in locus BL1071 is BC associated with AFC of 34.12 months and genotype BB for 474.13 days for CI respectively. The homozygous genotype of the biggest size alleles of locus HEL5, EE give the best AFC and CI. The heterozygous genotype AB and homozygous genotype BB give the best AFC, 33.39 months, and CI 457.27 days respectively.

This study examined the possible association between reproduction selected loci depends on the role of estrogen and IGF-I in the control of regulations of folliculogenesis and initiation of postpartum cyclicity in beef cattle. The findings marker HEL5 and AFZ1 related to the gene synteny IGF-IR on the performance of the reproductive system. The IGF system was composed of IGF-I, -II, IGF-IR and IGFBP that had been shown to be an important component of the mechanisms that coordinate functions of ovarian follicular different species including cattle (Spricer and Echternkamp 1995). The alterations in the concentration pattern of expression of components of IGF

and mechanism follicular may suggest the influence that control the system that in control of cyclicity reinitiation in cow. The IGF-IR mRNA had been detected in theca interstitial and granulosa cells, with its levels increasing during the development of dominant follicles, suggesting its role in follicular development.

The approach involving the establishment of allele classes for microsatellite loci proposed by Comings (1998), instead of considering the association with each allele, permitted the inclusion of a larger number of individuals per genotype, thus increasing the reliability of association tests. Another aspect to be considered was the higher mutation rate observed for microsatellites compared to other sequences, as well as the type of mutation (stepwise) frequently observed in these repetitive DNA regions (Schlötterer, 1998). In view of these considerations, it was reasonable to suppose that genotypes of these systems that only differ in a small number of base pairs exerted a similar effect on Quantitative Trait Locus (QTL). The results of the present study, using this approach permitted the detection of highly significant associations, validating the use of this method and representing a pioneered on the marker-assisted selection (MAS) in domestic animals.

According to Moody, et al (1996), the HEL5 locus was in linkage equilibrium with IGF-IR, and also with AFZ1 locus. These data suggested that these microsatellites might be acting on the IGF-IR regulation or that their different allele classes were linked to alternative forms of this gene.

The associations observed in this study indicate the possible utilization of favorable genotypes of 4 microsatellite in this population to increase reproductive efficiency. The population in this study was Thai locally adapted beef cattle consisted of *Bos indicus* (TI and TB), and synthetic breed with *Bos tuarus* (KB and TA). Additionally, further investigations, using screening approaches such as evaluation of previously described polymorphic markers in IGFI-R locus (Moody *et al.*, 1996), were needed to understand possible modulatory effects involved in reproductive efficiency.

CONCLUSION

These 4 Thai locally adapted cattle had a very distinct clustering between 1) TB, KB, TA and 2) TI in reproductive characteristics because the cluster 1 breed group were in selection program of beef cattle for major goals of growth performance. While TI breed is Thai indigenous animal genetic resources that had good reproductive traits, and they were in conservation program for base genetics to establish new breed of dairy and beef cattle for food security.

The genotype of A allele and AA genotype of MM12E6 locus gave low calving interval. By the results of the present study, we can use this approach of MM12E6 markers to represent a pioneering step towards marker-assisted selection in beef cattle.

KEYWORD : microsatellite, estrogen receptor, reproductive

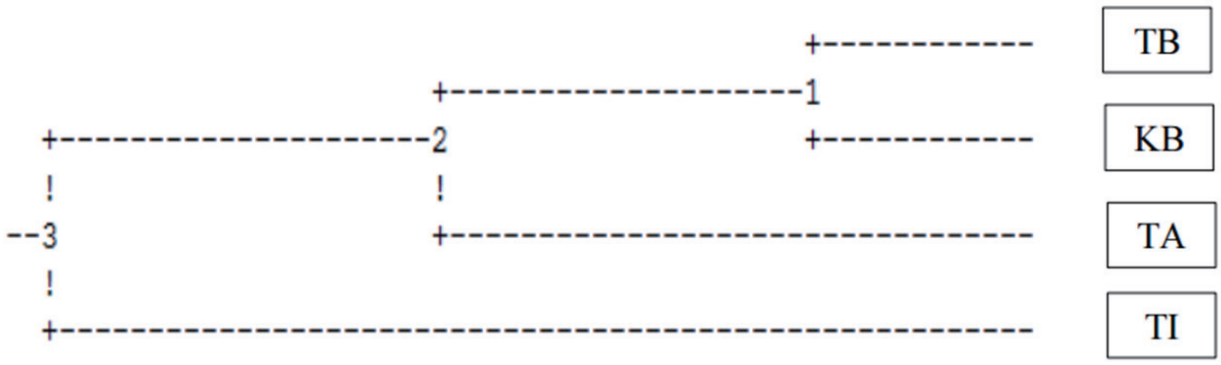

Figure 1 UPGMA dendrogram based on genetic distance showing the genetic relationships among the four populations.

Table 1 Allele sizes and genotype frequencies of the individuals classified as homozygous and heterozygous.

Locus	Total alleles	Size	Genotype frequency							
			AA	AB	AC	BB	BC	CC		
MM12E6	3	108-146	0.20	0.14	0.14	0.11	0.25	0.17		
			AA	AB	AC	BB	BC	CC		
BL1071	3	150-218	0.06	0.13	0.08	0.43	0.29	0.02		
			AC	AD	BC	BD	BE	CC	CD	CE
HEL5	5	125-226	0.01	0.01	0.12	0.13	0.02	0.08	0.17	0.09
			DD	DE	EE					
			0.10	0.11	0.16					
			AA	AB	AC	BB	BC	CC		
AFZ1	3	100-139	0.06	0.13	0.08	0.43	0.29	0.02		

Table 2 Nei's genetic distance matrix obtained from allele frequencies of 9 loci in four cattle breeds

Breed	TI	TB	TA	KB
TI	****			
TB	0.5112	****		
TA	0.7334	0.2227	****	
KB	0.5937	0.0652	0.0932	****

P<0.01)

Table 3 Results of association analyses between age at first calving, calving interval and microsatellite genotype classes homozygous and heterozygous.

Locus	Genotype	AFC±sd* (month)	CI±sd* (day)
MM12E6	AA	32.89^{a} ±3.04	423.66^{a} ±73.09
	AB	35.03^{ab} ±3.63	494.91^{ab} ±80.80
	AC	35.82^{b} ±3.19	516.91^{b} ±67.92
	BB	33.46^{a} ±2.31	466.33^{ab} ±77.35
	BC	35.19^{b} ±2.44	525.46^{b} ±62.15
	CC	35.84^{b} ±3.35	523.64^{b} ±33.64
BL1071	AA	35.95^{ab} ±1.82	483.71^{a} ±69.31
	AB	37.13^{b} ±4.06	516.26^{ab} ±60.84
	AC	34.31^{a} ±2.24	543.74^{b} ±50.35
	BB	34.32^{a} ±3.29	474.13^{a} ±80.05
	BC	34.12^{a} ±2.54	499.43^{a} ±77.47
	CC	34.84^{a} ±0.62	523.19^{b} ±4.86
HEL5	AC	33.73^{ab} ±2.88	563.67^{c} ±87.64
	AD	36.64^{b} ±8.72	551.24^{c} ±4.11
	BB	36.46^{b} ±0.76	526.59^{c} ±49.44
	BC	36.67^{b} ±3.54	523.83^{c} ±43.38
	BD	36.30^{b} ±3.55	530.85^{c} ±53.72
	BE	35.86^{b} ±1.65	482.49^{bc} ±56.46
	CC	36.11^{b} ±1.80	533.87^{c} ±37.83
	CD	35.12^{b} ±3.25	533.63^{c} ±50.93
	CE	33.84^{ab} ±1.90	533.50^{c} ±75.75
	DD	33.42^{ab} ±1.67	458.34^{bc} ±73.52
	DE	32.46^{b} ±1.84	464.78^{bc} ±74.52
	EE	31.64^{a} ±0.56	384.81^{a} ±2.34
AFZ1	AA	35.51^{c} ±5.49	493.55^{a} ±77.23
	AB	33.09^{a} ±1.75	490.31^{a} ±71.89
	AC	36.19^{c} ±3.22	526.06^{b} ±64.02
	BB	33.59^{ab} ±2.25	457.27^{a} ±73.95
	BC	34.66^{b} ±3.24	493.56^{ab} ±80.48
	CC	35.68^{c} ±2.56	500.62^{b} ±64.20

* $P<0.05$

REFERENCES

Barendse, W., D.Vaiman, S.J.Kemp, Y.Sugimoto, S.M.Armitage, J.L.Williams, H.S.Sun, A.Eggen, M.Agaba, S.A.Aleyasin, M.Band, M.D.Bishop, J.Buitkamp, K.Byrne, F.Collins, L.Cooper, W.Coppettiers, B.Denys, R.D.Drinkwater, K.Easterday, C.Elduque, S.Ennis, G.Erhardt, L.Ferretti, N.Flavin, Q.Gao, M.Georges, R.Gurung, B.Harlizius, G.Hawkins, J.Hetzel, T.Hirano, D.Hulme, C.Jorgensen, M.Kessler, B.W.Kirkpatrick, B.Konfortov, S.Kostia, C.Kuhn, J.A.Lenstra, H.Leveziel, H.A.Lewin, B.Leyhe, L.Lil, I.M.Burriel, R.A.Mcgraw, J.R.Miller, D.E.Moody, S.S.Moore, S.Nakane, J.J.Nijman, I.Olsaker, D.Pomp, A.Rando, M.Ron, A.J.Shalom, U.Thieven, B.G.D.Urquhart, I.Vage, A.V.De Weghe, S.Varvio, R.Velmala, J.Vilkki, R.Weikard, C.Woodside, and J.E.Womack. 1997. A medium-density genetic linkage map of the bovine genome. Mamm Genome 8:21-28.

Comings, D.E. 1998. Polygenic inheritance and micro/minisatellites. Molecular Psychiatry 3:21-31.

Evans, A.C.O., and J.E.Fortune. 1997. Selection of the dominant follicle in cattle occurs in the absence of differences in the expression of messenger ribonucleic acid for gonadotropin receptors. Endocrinology 138:2963-2971.

Goldenberg, R.L., J.L.Vaitukaitis, and G.T.Ross. 1972. Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology 90:1492-1498.

Maungkhiow, J., and T.Pothkom. 2011. Reproductive Performances of Brahman crossbreds in Southern Thailand. Copy paper. 15 pp.

Moioli, B., A.Georgoudis, E.Napolitano, G.Catillo, E.Giubilei, C.Ligda and M.Hassanane, 2001. Genetic diversity between Italian, Greek and Egyptian buffalo populations. Livest. Prod. Sci., 70: 203-211.

Monget P., and G.B.Martin. 1997. Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. Hum Reprod 12 Suppl 1:33-52.

Moody, D.E., D.Pomp, and W.Barendse. 1996. Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. Mammalian Genome 7:168-169.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106:283-292.

Richards, J.S., J.J.Ireland, M.C.Rao, G.A.Bernath, A.R.Midgley, and L.E.Reichert. 1976. Ovarian follicular development in rat hormone receptor regulation by estradiol, Follicle Stimulating Hormone and Luteinizing Hormone. Endocrinology 99:1562-1570.

Richards, J.S., J.A.Jonassen, A.I.Rolfes, K.Kersey, and L.E.Reichert. 1979. Adenosine-3',5'-monophosphate, luteinizing hormone receptor, and progesterone during granulosa cell differentiation - Effects of estradiol and follicle stimulating hormone. Endocrinology 104:765-773.

Schroth, G.P., P.J.Chou, and P.S.Ho. 1992. Mapping Z-DNA in the human genome - Computer-aided mapping reveals a nonrandom distribution of potential Z-DNA forming sequences in human genes. Journal of Biological Chemistry 267:11846-11855.

Schlötterer, C. 1998. Microsatellites, In: Hoelzel, A.R. (ed), Molecular Genetic Analysis of Populations: A Practical Approach. Oxford University Press, New York, pp 237-261.

Spicer, L.J. and S.E.Encternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domestic Animal Endocrinology 12:223-245.

Tautz, D. 1993. Notes on the definition and nomenclature of tandemly repetitive DNA sequences, In: Pena, S.D.J., J.T.Chakraborty, J.T.Epplen, and A.J.Jeffreys. (eds), DNA Fingerprinting: State of the Science. Birkhäuser Verlag Basel, Switzerland, pp 21-28.

Yeh,F.C., R.C.Yang, T.B.J.Boyle, Z.H.Ye, J.X.Mao. 1999. POPGENE Ver. 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

O-29-4

ESTIMATION AND PREDICTION OF BREEDING VALUES FOR RIBEYE AREA IN BEEF CATTLE FROM LIVESTOCK COOPERATIVE FARMS

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INTRODUCTION

High quality beef in Thailand is produced from crossbreds of minimum ½ *Bos taurus* and ½ *Bos indicus*. Charolais, a famous breed for *Bos taurus*, which is used as sire, while Brahman, Thai Native, or their crosses is *Bos indicus* used as cow. Generally, artificial insemination with frozen semen both from inland born and imported is available. The beef cattle were fattening at initial weight of about 350 kg until about 600 kg of final weight. Reports concerning estimation of genetic parameters and breeding values of beef cattle in Thailand mostly were done in reproductive and production traits (Commungkhun, 1997, Chitprasan et al., 1999, Chaovapasee et al., 2008, and Jeanmas et al., 2009, Konkluea, 2010), but in carcass traits, especially in the crossbreds, it was not found. Ribeye area is one of the most important carcass traits, which is easy to measure and it can be heritable. Hence, heritability and breeding values of sires in the trait had been estimated and predicted.

MATERIALS AND METHODS

Animals:

Three hundred and sixteen crossbred males from member farms of Pon Yang Kharm Livestock cooperatives under the Armed Forces Security Command located in Sakhon Nakhon province were collected. The animals were offspring of 14 (AI) Charolais sires and 4 crossbred cow lines such as Thai Native (000), Brahman (002), 1/2Charolais x 1/2Asia (1003), and 1/4Charolais x 3/4Asia (2002). At about 2 years old, they were fattening for eight to nine months with special feed from the cooperatives and fresh grass or hay with molasses as supplement. The animals were slaughtered during March to November 2014. After slaughtering, carcass was cut into two halves and chilled at 4℃ for 7 days. On the 5th days of chilling, ribeye area (REA) at the 12th rib of the left side was measured by drawing the area on transparency. Beef plastic grid template was used to calculate the REA from the transparency in unit of cm^2.

Statistics:

Descriptive statistics were used to analyze the data distribution. Linear Mixed-Effects model based on restricted maximum likelihood (REML) method was used to estimate the breeding value. Slaughter dates, dam lines, and age of offspring (2 to 5 years old) were fixed effects, while sires and sires within dam lines were random effects. Heritability was calculated from the variance component. Breeding values of sires and sires within dam lines were predicted and ranked.

RESULTS AND DISCUSSION

Descriptive statistics for the REA of the 316 crossbreds were 129.11, 15.29, 93.00, and 175.50 cm^2 for mean, standard deviation (SD), minimum (min), and maximum (max), respectively. Jeanmas *et al*. (2014) studied the REA of 198 crossbred cattle (Thai Native x Brahman x Charolais) and reported the mean, SD, min, and max of the trait which were 84.34, 11.85, 55.50 and 120.10 cm^2, respectively. The REA from this study was larger than Jeanmas et al. (2014) due to age of the cattle, feedlot, period of fattening and management.

Table 1 shows factors of fixed effects. Slaughter dates were highly significantly influenced to the REA trait ($P<0.01$), while the significant difference was not found in the others ($P>0.05$). Intercept of the trait was estimated of 120.83 ± 14.07 cm^2.

Estimated REA and standard error of prediction due to slaughter dates shows in Figure 1. The TOP3 of estimated REA and standard error, 28.19 ± 11.39, 22.86 ± 14.21, and 19.38 ± 10.49 cm^2 showed on the slaughter dates of 10/06, 05/09, and 23/05 in the year 2014, meanwhile, the 3 TOPLESS of the trait were -23.22 ± 13.09, -20.36 ± 16.81, and -11.73 ± 11.71 cm^2, for the slaughter dates on 08/04, 29/07, and 03/06/2014, respectively.

Variance component of sire, sire within dam lines, and residual were 1.307, 15.7318, and 198.49 respectively.

Additive and phenotypic variances were 5.23 and 215.53, respectively. Therefore, the heritability (h^2) of this trait was 0.02 with the Var (A) of 538 and SE (A) of 23.19. Rios Utrera and Van Vleck (2004) reported average estimate h^2 of REA was 0.40, which meant REA was moderately inherited. However, it ranged from 0.01 to 0.97 depending on many factors. In our result, the h^2 was very low it might be due to the small number of records and pedigree information.

Estimated breeding values (EBV) of offspring from each sire and the predicted BV of sire (twice of EBV of offspring) for 14 (AI) sires shows in Table 2. From the ranking of EBV, the TOP5 bulls were c-48, c-41, c-38, c-30, and c-39 with the EBV of +0.75, +0.62, +0.49, +0.41, and +0.34, respectively. It was interesting that c-48, c-44 and c-47 were imported frozen semen, only the c-48 had the highest BV. The c-47 and c-44 were ranked as 6 and 9, respectively, which their BV were less than many bulls born in Thailand. Their BVs might be higher, if they had more opportunities to mate with more cows than in this study. The imported frozen semen were more expensive than those born in Thailand, therefore, the farmers were less interested in imported frozen semen.

Results in Table 3 show that within the Thai Native cows, the c-41 bull showed the highest BV (5.30), while in the Brahman cows, the c-48 BV was the highest (4.80). The BV of c-38 was the highest in the 1/4Charolais x 3/4Asian crossbreds, whereas the BV of CHA501(c-39) was the highest in the 1/2Charolais x 1/2Asian crossbreds. The results indicate that not only the bull, but the cow lines are also very important to produce the trait.

CONCLUSIONS

The results of the study indicate that: 1) although our h^2 was very low, but it could be improved when number of records is increased and more details of pedigree information are available. 2) Not only the bull, but the cow lines are also very important to produce the trait. 3) In order to support the Beef Cattle Strategy Plan of Thai Government, breeding values of all sires, especially, in carcass traits in Thailand, both inland born and imported frozen semen should be further studied.

ACKNOWLEDGEMENTS

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KEYWORD : Breeding values, Charolais bull, Ribeye area, Cow lines, Beef cattle

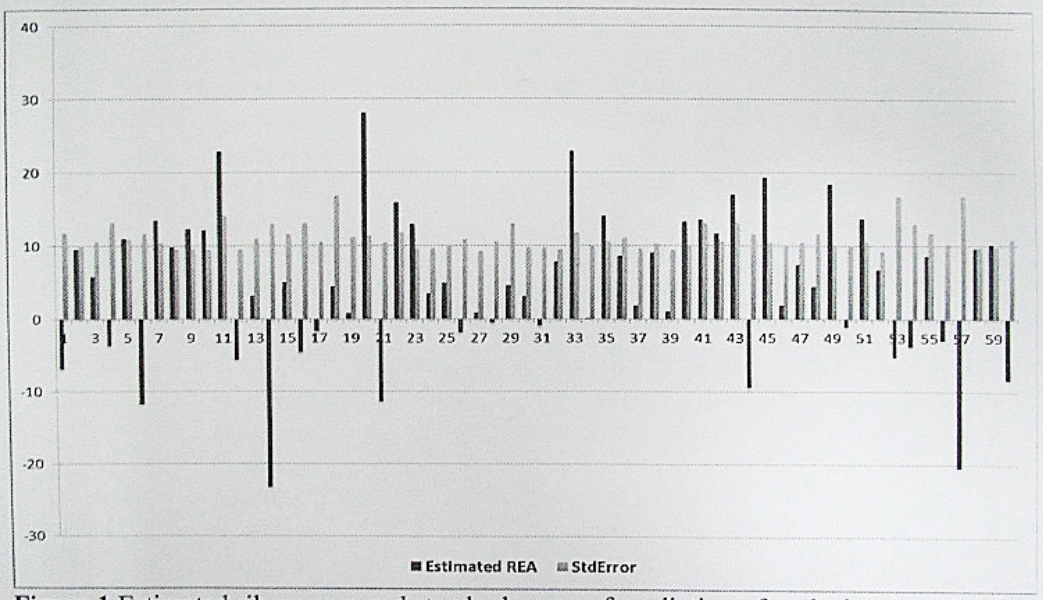

Figure 1 Estimated ribeye area and standard errors of prediction of each slaughter date.

Table 1 The F-value, P-value, numerator, and denominator degrees freedom of the fixed effects factors of the studied trait.

Effects	Numerator DF	Denominator DF	F Value	Pr > F
Slaughter dates	60	218	1.62	0.006 **
Dam lines	3	18	0.51	0.681 NS
Age of offspring	3	218	0.23	0.876 NS

** P<0.01, NS: P>0.05

Table 2 Estimated BV of offspring from each sire, standard error of prediction, predicted breeding value of sires and sire ranking

Sire name (code)	No. of mating	EBV of offspring	Std.Err Pred	BV of sire	Ranking
KLOD (149)	9	-0.20	1.13	-0.40	11
NAJA (c-30)	7	0.20	1.13	0.41	4
CHA224/8 (c-36)	17	-0.22	1.13	-0.44	12
CHA500 (c-37)	12	0.10	1.12	0.20	7
CHA7/9 (c-38)	36	0.24	1.10	0.49	3
CHA501 (c-39)	15	0.17	1.12	0.34	5
CHAPKV198E (c-40)	62	-0.46	1.09	-0.91	14
CHAPKV313E (c-41)	71	0.31	1.09	0.62	2
NECESSAIRE (c-44)	3	-0.12	1.14	-0.23	9
JACGUARD (c-47)	1	0.11	1.14	0.23	6
SAVANT (c-48)	12	0.37	1.12	0.75	1
CHA440 (c-49)	41	-0.33	1.10	-0.67	13
CHA536 (c-50)	19	-0.17	1.12	-0.33	10
MUNGKORN (x-5)	15	-0.02	1.12	-0.05	8

Table 3 Estimated BV of offspring in each sire within dam lines, standard error of prediction, breeding value of sire within dam lines, and ranking

Sire code	Dam lines	No. of mating	Estimate	Std Err Pred	Ranking
149	Thai Native	3	-1.28	3.76	9
c-30	Thai Native	4	-2.33	3.57	10
c-36	Thai Native	9	-0.81	3.31	7
c-38	Thai Native	11	0.80	3.20	4
c-39	Thai Native	30	-0.30	3.71	6
c-40	Thai Native	12	-3.13	3.21	11
c-41	Thai Native	19	5.30	2.96	1
c-44	Thai Native	1	-0.02	3.85	5
c-48	Thai Native	1	1.86	3.84	2
c-49	Thai Native	10	-1.20	3.23	8
x-5	Thai Native	13	1.12	3.16	3
149	Brahman	6	-1.13	3.43	9
c-30	Brahman	3	4.80	3.64	1
c-36	Brahman	7	-2.51	3.44	13
c-37	Brahman	10	0.61	3.20	5
c-38	Brahman	24	0.51	2.76	6
c-39	Brahman	10	1.46	3.25	3
c-40	Brahman	47	-0.62	2.49	8
c-41	Brahman	48	0.04	2.46	7
c-44	Brahman	2	-1.38	3.75	10
c-47	Brahman	1	1.37	3.83	4
c-48	Brahman	10	3.26	3.27	2
c-49	Brahman	31	-2.82	2.65	14
c-50	Brahman	18	-2.17	2.94	12
x-5	Brahman	2	-1.41	3.77	11
c-37	1/4Cha x 3/4Asian	2	0.58	3.80	2
c-38	1/4Cha x 3/4Asian	1	1.63	3.86	1
c-40	1/4Cha x 3/4Asian	1	-0.39	3.86	4
c-41	1/4Cha x 3/4Asian	2	-1.99	3.81	5
c-50	1/4Cha x 3/4Asian	1	0.18	3.86	3
c-36	1/2Cha x 1/2Asian	1	0.72	3.87	2
c-39	1/2Cha x 1/2Asian	2	0.89	3.80	1
c-40	1/2Cha x 1/2Asian	2	-1.34	3.79	4
c-41	1/2Cha x 1/2Asian	2	0.36	3.80	3

REFERENCES

Chaovapasee, K., Molee, A., and Chaiwattanasin, W. 2008. Estimation of genetic parameter and breeding value for some economic traits in Kamphaeng Saen beef cattle. Proceedings of 46th Kasetsart University Annual Conference: Animals and Veterinary Medicine. P: 99-106.

Chitprasan, P., Tumwasorn, S., and Kuha, K. 1999. Breeding value estimation of birth weight in beef cattle at Loong Chow farm in Supanburi province. The Proceeding of 37th Kasetsart University Annual Conference: Animals. P: 205-210.

Commungkhun, W. 1997. Breeding value estimation of imported beef cattle under Ang Thong and Uthai Thani village production system. M.S. thesis, Kasetsart Univ. Bangkok. 70 p. (in Thai).

Jeanmas, A., Innurak, P., and Sopannarath, P. 2009. Genetic trends for birth and weaning weights in crossbred cattle among Thai native, Brahman and Charolais. Proceedings of 47th Kasetsart University Annual Conference: Animals. P: 106-116.

Jeanmas, A., Sopannarath, P., Tumwasorn, S., and Loongyai, W. 2014. Association of SNP marker in the IGF Gene with Carcass Traits in crossbred cattle among Thai Native, Brahman and Charolais. Kasetsart J. (Nat. Sci.) 48: 605-610.

Konkluea, P. 2010. Estimation of genetic parameters and breeding values for growth and fertility traits in White Lamphun cattle of Chiang Mai University. M.S. thesis, Chiang Mai University, Chiangmai. 103 p. (in Thai).

Rios Utrera, A. and Van Vleck, L. Dale. 2004. Heritability estimates for carcass traits of cattle: a review. Genetics and Molecular Research 3(3): 380-394.

O-29-5

EFFECT OF HEAT STRESS ON PHYSIOLOGICAL INDICES AND HEAT SHOCK PROTEIN 70 EXPRESSION IN INDIGENOUS BREEDS OF GOAT IN NIGERIA

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Objective

Livestock experience a variety of stressors during the entire production cycle which adversely affect their overall productivity and health status because of neuroendocrine disruption and stress-induced immunosuppression. Environmental stressors such as heat stress are detrimental to animal agriculture especially in the tropical regions of the world.At molecular and cellular levels, temperature beyond the comfort zone reduces the rates of enzymatic reactions, diffusion, transport and induces the denaturation and disaggregation of proteins. Under thermal stress, transcriptional activation and accumulation of a set of proteins called “heat shock proteins” (HSP) is well known Research has shown that there is a strong correlation between the induction of HSPs and thermotolerance. The objective of the present study is to analyze the genetic variation in physiological responses and expression of HSP 70 in some indigenous breeds of goats in Nigeria.

Methodology

One thousand and two hundred goats representing 3 Nigerian breeds were sampled. The data set consist of 400 West African Dwarf (WAD), 401 Red Sokoto (RS) and 399 Sahel (SH) breeds. The goats originated from different flocks and were reared under the traditional extensive system, where they graze during the day on natural pasture and scavenge on kitchen and household wastes before they are brought to the market where they are left in the open.

Non contact infrared thermometer was used to measure the skin temperature via the rump of the goats. Rectal temperature was measured with the digital thermometer placed 2cm in the rectum, heart rate was measured in breaths per minute using a stethoscope placed on the 5^{th} half intercostal space while pulse rate was measure by the palpation of the femoral artery in the inner thigh. Respiratory rate was measured in breaths per minute by counting the number of flank movements per minute using a stop watch. The relationship between environmental temperature and relative humidity was used to derive temperature humidity index (THI).

Serum stored at freezing temperature were brought to room temperature and concentration of HSP 70 in serum were determined by a sandwich ELISA assay using standard protocol (USCN Life Science Inc, Wuhan, China). The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the colour change was measured spectrophometrically at a wavelength of 450nm + 10nm (USCN Life Science Inc, Wuhan, China).

Statistical Analysis

Data are presented as mean ± SD and subjected to analysis of variance, means were compared using Duncan Multiple Range Test (DMRT) test of the SAS, (2008) software.

Results

All physiological traits measure were significantly different ($P<0.05$) across the breed except skin temperature. RS goats had the highest heart rate, pulse rate and respiratory rate (107.00 ± 10.41 beats/min, 99.30 ± 10.85 beats/min and 104.09 ± 12.81 breaths/min) compared to SH goats with the lowest value (92.20 ± 11.39 beats/min, 91.13 ± 64.43 beats/min and 95.80 ± 13.96 breaths/min), rectal temperature was highest in WAD goats (38.70 ± 0.40℃) but lowest in RS goats (37.56 ± 0.67℃) Body weight was highest in RS goats (19.76 ± 1.87kg) but lowest in WAD goats (8.35 ± 1.48kg) (Table 1).

Table 1: Mean values (± SD) of physiological traits of extensively managed Nigerian Goats under heat stressed conditions

WAD
RS
SH

Heart rate (beats/min)	99.36 ± 9.75[b]	107.00 ± 10.41[a]	92.20 ± 11.39 [c]
Pulse rate (beats/min)	92.34 ± 8.73[b]	99.30 ± 10.85[a]	91.13 ± 64.43[b]
Respiratory rate (breaths/min)	99.83 ± 11.57[b]	104.09 ± 12.81[a]	95.80 ± 13.96[c]
Skin temperature (℃)	37.19 ± 0.45[a]	37.15 ± 0.40[a]	38.81 ± 21.92[a]
Rectal temperature (℃)	38.70 ± 0.40[a]	37.56 ± 0.67[c]	38.59 ± 0.35[b]
Body weight (Kg)	8.35 ± 1.48[c]	19.76 ± 1.87[a]	13.83 ± 5.38[c]

[a,b,c] Means with the same superscript within the same row do not differ significantly (P>0.05) WAD= West African Dwarf goats RS=Red Sokoto goats SH= Sahel goats

HSP 70 concentration was significantly different (P<0.05) across the breed of Nigerian Indigenous goat, RS goat had the highest concentration, followed by SH and WAD goat.

Table 2: Mean Values of HSP 70 concentration in extensively managed goats in Nigeria under heat stressed conditions

Breed	Mean+SD (ng/mL)	P-value
WAD	82.79+61.33[b]	0.0176
RS	124.44+59.10[a]	0.0176
SH	94.84+47.05[b]	0.0176

[a,b] Means with the same superscript within the same column do not differ significantly (P>0.05) WAD= West African Dwarf goats RS=Red Sokoto goats SH= Sahel goats

Discussion

Rectal temperature, pulse and respiration rates are important indicators of thermal balance and are used to evaluate the impact of heat stress (Spiers *et al*., 2004). Avendano-Rayes *et al*. (2006) reported that increased body temperature, pulse and respiration rates are a normal mechanism by which animals diffuse heat from their bodies to maintain thermoregulation in hot ambient conditions. In the present study, heart rate ranged from 107.00 - 92.20 beats/min which was significantly different across the breed with RH goats (107.00 beats/min) been the highest, followed by WAD goats (99.36 beats/min) and SH goats (92.20 beats/min) the lowest. This was lower than the values obtained by Antonio *et al*. (2014) for Santa Ines and Dopa sheep between October and November in Brazil. Quesada *et al*. (2001) also found higher valued for HR in Novada and Santa Ines sheep after 6 hour period of exposure to the sun. The lower heart rate found in the present study is probably because under heat stress, animals tend to reduce their movement and other metabolic activity so as to reduce metabolic heat production. This agrees with the report of Barkai *et al*., (2002) that there is a correlation between HR and metabolic heat production.

Pulse rate was not significantly different between WAD goats (92.34 beats/ min) and SH goats (91.13 beats/ min) but both were significantly different from RS goats (99.30 beats/min). This was higher than the 65 - 80 beats/ min basal pulse rate reported by Davendra (1987) for goats. This was however similar to the observation of Sanni *et al*., (2013) who observed pulse rate of 131.37 beats/min for RS goats, 124.97 beats/min for WAD goats and 112.33 beats/min for SH goats. The higher significant pulse rate could be due to the redistribution of blood to peripheral tissues during heat exposure in goats. These findings is in accordance with the report of Okoruwa (2014) of a pulse rate of 91.04 beats/ min for WAD goat exposed to the sun between 1-6pm,

RR can be used to estimate the adverse effects of environmental temperature and as an indicator of heat stress (Haidary *et al.,* 2012). Okoruwa *et al*. (2014) also reported that RR is a practical and reliable measure of heat load and stated that respiratory rate above 12 - 20 breaths/min in sheep and goats is an indicator of heat stress. Thus the observed increase in the RR of all breeds goats in this study indicated that they were exposed to severe heat stress. This respiratory response arises from direct heat stimulation of the peripheral receptors which transmit nervous impulses to the thermal centre in the hypothalamus.

Rectal temperature (RT) is a good indicator of general status of an animal's health. It remains fairly consistent under non- stressed condition. It is recognized as an important measure of physiological status as well as an ideal indicator for assessment of stress in animals. (Lefcourt *et al*., 1986 Otoikian *et al*., 2009). In all farm animals, only goats can maintain their rectal temperatures below 38.5 ℃ , which is considered normal (Devendra, 1987 Avendano-Reyes *et al*., 2006). Change in rectal temperature has been considered an indicator of heat storage in animal's body and may be used to assess the adversity of thermal environment, which can affect growth, lactation and reproduction of dairy animals (West *et al*., 1999 Hansen and Arechiga, 1999). Even a rise of 1 ℃ in rectal

temperature was enough to reduce performance in most livestock species (Ganaie *et al.,* 2013). Rectal temperature was significantly different between the breeds with WAD goats having the highest value (38.70℃) and RS goats the lowest (37.56℃). The observed elevation of RT in WAD goat above the basal level of 38.50℃ (Avendano-Reyes *et al*., 2010) indicated that they were the most heat stressed. This may also be due to the coat colour of WAD goat which is predominantly black or brown with patches compared with the RS goat which is predominantly brown and SH goat which is predominantly white.

In the present study, it was observed that HSP 70 concentration in tropical Nigerian goats was found to be significantly different, with HSP 70 concentration in RS goat (124.44ng/mL) significantly higher than SH goat (94.84ng/mL) and WAD goat (82.79ng/mL). Lacetera *et al*. (2006), Liu *et al*. (2010), Patir and Upadhyay (2007, 2010), Mishra *et al*. (2010) reported increased heat stress induced HSP 70 expression in the bovine lymphocytes.

RS had the highest HSP 70 concentration, it also has the highest HR, PR and RR, while SH had the least with WAD been intermediate, This may indicate that RS goat made more effort to physiologically ameliorate the impact of heat stress, while SH goat was still stable physiologically under the heat stressed condition. The significantly higher concentration of HSP 70 in RS goats may therefore be an indication of a better cellular response to heat stress compared to the other two breeds (SH goats and WAD goats) although result of the study suggest SH goat was superior in maintaining thermal balance physiologically and cellular level.

Conclusion

This study showed that under heat stress conditions, Sahel goat maintained a fairly stable physiological condition while West African Dwarf goat has the highest physiological imbalance. HSP 70 was found to be highest is Red Sokoto goat thus suggesting breed differences in HSP concentrations. This must however be verified in future experiments.

KEYWORD : Goat, Heat stress, Temperature-Humidity Index, HSP70

REFERENCES

Antonio G.J, Erika S.S., Rafael S.F., Fabio D.C.H., Vicente P.S.O., Gesualdi A.C.L.S. (2014) Effects of heat stress on the physiological parameters and productivity of hair sheep in tropical and coastal environment. *Research. Brasilia.Zootechnology*, 43(10):556-560.

Avendano-Reyes, L., F. D. Alvarez-Valenzuela, A. Correa- Clederon, J. S. Saucedo-Quintero, P. H. Robinson and J. G. Fadel. (2006). Effect of cooling Holstein cows during the dry period on postpartum performance under heat stress conditions. *Journal Livestock Science*, 105:198-206.

Barkai, S., A. Landau, Brosh et al. (2002). Estimation of energy intake from heart rate and energy expenditure in sheep under confinement or grazing condition. *Livestock Production Science*, 73: 237–46

Devendra C. (1990). Comparative aspects of digestive physiology and nutrition in goats and sheep. In: Devendra C, I mazumi. E.(Ed.), *Ruminant Nutrition and Physiology in Asia.* pp. 45-60.

Ganaie A.H., Shanker G., Bumla N.A., Ghasura R.S., Mir N.A. (2013) Biochemical and Physiological Changes during Thermal Stress in Bovines. *Journal of Veterinary Science and Technology*, 4: 126.

Haidary S.M., Corcoles E.P., Ali N.K. (2012). Nanoporous Silicon as Drug Delivery Systems for Cancer Therapies. *Journal of Nanometerials*, vol 2012, Article ID:830503.

Hansen J.R.S. and Arechiga C.F. (1999) Strategies for Managing Reproduction in the Heat Stressed Dairy Cow. *Journal of Dairy Science*, 77: 36-50.

Lacetera N., Bernabucci U., Scalia D., Basirico L., Morera P., Nardone A. (2006) Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. *Journal of Dairy Science* 89:4606–4612.

Lefcourt A.M., Bitman J., Wood D.L., Stroud B. (1986) Radiotelemetry system for continuously monitoring temperature in cows. *Journal of Dairy Science* 69: 237-242.

Liu, Y.X., Li, D.Q., Cui, Q.W., Shi, H.X. and Wang, G.L. (2010). Analysis of HSP70 mRNA level and association between linked microsatellite loci and heat tolerance traits in dairy cows. Yi Chuan, 3, 935–941.

Mishra, A., Hooda, O.K., Singh, G. and Meur, S.K. (2010). Influence of induced heat stress on HSP70 in buffalo lymphocytes. Journal of Animal Physiology and Animal Nutrition, 95, 540–544.

Okoruwa M.I. (2014). Effect Of Heat Stress On Thermoregulatory, Live Bodyweight And Physiological Responses

Of Dwarf Goats In Southern *Nigeria. European Scientific Journal* Vol.10, No.27

Otoikhian C.S.O., Orheruata J.A., Imasuen, J.A. and Akporhuarho O.P. (2009). Physiological response of local (West African Dwarf) and adapted Switzerland (White Bornu) goat breed to varied climatic conditions in South-South Nigeria. *African Journal of General Agriculture*, 5 (1): 1-6.

Patir, H. and Upadhyay, R.C. (2007). Interrelationship between heat shock protein 70 (HSP70) and lymphocyte proliferation in thermal exposed buffalo heifers. *Italian Journal of Animal Science*, 6, 1344–1346.

Patir, H. and Upadhyay, R.C. (2010). Purification, characterization and expression kinetics of heat shock protein 70 from Bubalus bubalis. *Journal of Veterinary Research Science*, 88, 258–262.

Quesada,M., McManus, C. and Cuoto, F.A.D. (2001). Tolerancia ao calor de duas eacas de ovinos deslanados no Distrito Federal. *Revista Brasiliera de Zootecnia*, 30:1021-1026.

Sanni TM, Onasanya GO, Adefenwa MA, Yakubu A, Ikeobi CON, Adebambo OA, Talabi AO, Ozoje MO, Wheto M, Takeet MI, Peters SO, Donato MD, Thomas BN, Imumorin IG. (2013). Molecular Diagnosis of Sunclinical African Trypanosoma vivax Infection and Association with Physiological Indices and Serum Metabolites in Extensively Managed Goats in the Tropics. *Open Journal of Veterinary Medicine*, 3:39-45.

Spiers, D. E., J. N. Spain, R. P. Sampson and R. P. Rhoads. (2004). Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. *Journal of Thermal Biology*, 29:759-764.

West J.W, Hill GM, Fernandez JM, Mandebvu P, Mullinix BG (1999) Effects Of Dietary Fiber On Intake, Milk Yield, And Digestion By Lactating Dairy Cows During Cool Or Hot, Humid Weather. *Journal Dairy Science*, 82: 2455-2465.

O-29-6

Whole Transcriptome Analysis of Thoroughbred and Jeju Native Horse by Exercise using RNA-sequencing

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INTRODUCTION

The horse (Equus caballus) is a subspecies of the family Equidae and has evolved over the last 45-55 million yrs, from a small multi-toed mammal into the large single-toed animal it is today. Wild horses were domesticated in Central Eurasia 5500-6000 years ago and were primarily bred for their endurance, strength, and speed. This makes the horse not only a useful biological model to study the physiology of exercise, but also to identify the molecular mechanisms of adaptive responses to exercises. There are currently .300 breeds of horse, and humans use them in a wide range of activities. Since domestication, at around 3500 B.C.E, horses have mainly been used for riding and racing(Collins et al., 1997). One domesticated breed of horses, the Thoroughbred, has been specifically bred for speed, endurance, and strength since the 18th century. The extreme selection for these traits has resulted in a highly adapted athlete (OUTRAM et al, 2009) with very high aerobic capacity (WADE et al., 2009), and high skeletal muscle mass (LING et al, 2011), which comprises over 55% of total body mass (Marini and Veicsteinas, 2010). The skeletal muscle of the thoroughbred horse comprises over 55% of its total body mass (Marini and Veicsteinas, 2010, LING et al, 2011) and has remarkable functional and structural plasticity (WADE et al., 2009).

horses are very valuable animals to preserve historically and economically and it is very important to investigate unique genetic characteristics of Jeju horses (WADE et al., 2009, LING et al, 2011). Jeju horses have been isolated for more than 700 years and it is estimated that their homozygocity of genotype increased by inbreeding and genetic drift. The increase of recessive homozygosity caused inbreeding and decreased growth and reproductive performance(BARREY et al, 2006, Marini and Veicsteinas, 2010).

In previous study, we conducted whole in vitro transcriptome analysis of six Thoroughbred horses before and after exercise using RNA-sequencing in skeletal muscle and blood. We identified 20,428 novel unigene clusters, 878 up-regulated and 285 down-regulated genes in the muscle and 62 up-regulated and 80 down-regulated genes in the blood. (Park et al., 2012). Comparative analysis to detected Evolutionary selected gene in Throughbred and Jeju-horse using skeletal muscle.

In this study, we conducted RNA-sequencing analysis of Jeju-horse first time, using Jeju-hoses before and after exercise muscle sample. We show the evolutionary selected gene in the Jeju-horse skeletal muscle and validation the gene expression, we identified differentially expressed genes (DEGs), and detect selection signals of three evolutionary layers between Jeju-horse and Throughbred horse. We investigated exercise stress related pathways in Jeju-horse DEG using DAVID tools and selected genes (CCL4, CXCL2, FOS, IL6, OSM). We identified the DEG in jeju horse muscle in vitro.

MATERIALS AND METHODS

Analysis of horse DNA re-sequencing data

After 14 Thoroughbreds and 6 Jeju native horses (Equus caballus) whole genome sequencing using HiSeq2000 (Illumina, Inc), we remained sequencing approximately 15.87 x coverages on average, with a total of approximately 39 billion bp in 40 million reads per sample after removing the potential adapter sequence using Trimmomatic-0.32. Using the Burrows-Wheeler Aligner with the default setting, pair-end sequence reads were mapped to the reference horse genome (EquCab 2.66) originated from the Ensemble database with an overall alignment rate of 94.58%. After that, we used the following open-source software packages Picard Tools, SAMtools (Li et al, 2009) and the Genome analysis toolkit(McKenna et al, 2010) for downstream processing and variant calling. All calls with a Phred-scaled quality of, were filtered out. For each chromosome, we inferred the phased

haplotype and imputed the missing alleles for the entire set of Thoroughbred populations simultaneously using BEAGLE (Browning, 2011). We finally remained a total of ~12.9 million autosomal SNPs.

Total RNA isolation

Total RNA samples for investigation of DEGs were extracted from three Thoroughbred. Tissues (from skeletal muscle, kidney, heart, liver, lung, colon, and spinal cord) were extracted for polymerase chain reaction (PCR) analysis. The various tissues sampled from the horses were crushed in a mortar and pestle by using 50-100 mg, or 3 mL in the case of blood, and mixed with 9 mL of red blood cell (RBC) lysis buffer (Solgent Co., Ltd., Daejeon, Korea) to remove red blood cells. The cells were then dissolved using 1 mL of TRIzol (Invitrogen, Karlsruhe, Germany) and 200μL of chloroform was added to remove cells from the organic solvent. The mixture was then shaken for 10 s and left at 4℃ for 5 min. Centrifugal separation was carried out at 4℃ for 15 min, and the supernatant removed to a new test tube and mixed with the same amount of isopropanol. The test tube was left at 4℃ for 15 min to produce RNA pellets. Isopropanol was removed by carrying out centrifugal separation at 4℃ for 15 min and the sample was then sterilized with 85% ethanol and dissolved with RNase-free water. The purity of extracted RNA was confirmed by measuring the absorbance at 230 nm and 260 nm using a spectrophotometer (ND-100, Nano Drop Technologies Inc., Wilmington, DE, USA) and only the extracted RNA with purity (OD value of 230 nm/260 nm) over 1.8 (found via quantitative analysis) was used. The selected RNA was stored at -70℃ until the experiment occurred.

cDNA synthesis

In order to synthesize cDNA, 2μg of RNA, 1μL of oligo-dT (Invitrogen), and 1μL of RNase-free water were added, denatured at 80℃ for 3 min, and cDNA was synthesized using 4μL of 5 x RT buffer, 5μL of 2 mM deoxynucleotide (dNTP), 0.5 μL of RNase inhibitor (Promega Corporation, Madison, USA), and 1 μL of moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, USA).

Polymerase Chain Reaction

The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and Ensembl Genome Browser (www.ensembl.org) were utilized for the desired gene sequence information, and the primer used for checking the single nucleotide polymorphism was synthesized using PRIMER3 software (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR was carried out using cDNA under the following conditions: to amplify target genes on cDNA, 1.8μL dNTP, 2μL 10 X buffer, 0.2μL HS-Taq, and 12μL distilled water were added to 2μL 50 ng/μL diluted DNA, 5 pmol/μL diluted forward primer and reverse primer, and PCR was carried out with a total of 20μL. The PCR conditions were denaturation carried out at 94℃ for 10 min, and the second denaturation at 94℃ for 30 s, annealing at 58℃ for 30 s and extension at 72℃ for 30 s. This was repeated for 40 cycles, and then the extension occurred at 72℃ for 10 min. The band was confirmed on UV using a 1.5% SeaKem LE agarose gel (Lonza, Rockland, USA).

Real time qPCR amplification

The RT-qPCR was carried out using the C1000 Thermal Cycler (Bio Rad, Hercules, CA) in order to measure the relevant expression of target genes. 25μL of reaction solution was used and the solution was produced as follows: 2μL and 5μL of distilled water, and 2μL of diluted cDNA (50 ng/μL) were added to 14μL of SYBR green master mix (Bio Rad, Hercules, CA), and 5 pmol/μL each of diluted forward primer and reverse primer. The RT-qPCR conditions were as follows: the first denaturation was carried out at 94℃ for 10 min, and then the second denaturation was at 94℃ for 10 s, the annealing occurred at 60℃ for 10 s and the extension at 72℃ for 30 s. This was carried out repeatedly 40 times. All measurements were performed in triplicate for all specimens, and the comparative method used was the 2- δ δ Ct method (Livak et al., 2001). The relevant expression of target genes was calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalizer.

RESULTS AND DISCUSSION

Differentiation of DEGs(differntly Expressed Genes) by exercise in Thoroughbred and JeJu horses.

To investigate differentiation of DEGs following exercise by species, we conducted RNA-sequencing with thoroughbred and Korean Native horse(JeJu horse). Result shows that 99 of Up-regulated genes, 25 of down regulated genes in Jeju horse muscle, and 878 up-regulated genes, 285 of down regulated genes in thoroughbred muslce (Figure 1). We conducted comparison analysis between Jeju horse DEGs and thoroughbred horse DEGs. As

a result, we found 15 of jeju horse specific genes and 34 of thoroughbred specific genes used an arbitrary cut-off of a >6-fold (log2FC>6)(Figure 2).

Positive selection during the domestication period: XP-CLR and XP-EHH

To detect a selection signal during the domestication of the horse, we performed a com-parative genome-wide statistical analysis based on the protein-coding genes of 14 Thorough-breds and 6 Jeju ponies using 10-fold re-sequencing data. In this study, we aimed to detect the selection signatures of the two evolutionary phases of Jeju native horse using XP-CLR and XP-EHH. These two evolutionary statistics are directional evolutionary statistics. So we could estimate only an extreme positive selective region in Jeju native horse using Thorough-bred as control group. The first phase, calculated by XP-CLR, shows the relatively later selection that occurred during the domestication of the Jeju native horse. The second phase, calculated by XP-EHH, indicates the most recent selective sweep. We used Thoroughbred as a comparative group because this breed displays pronounced phenotypic differences with the Thoroughbreds, especially with regard to body shape and racing performance. XP-CLR statistics are generally more sensitive to older selection events of an intermediate to high frequency, while the XP-EHH test is used to detect evidence of a recent, stronger, positive selection. Using top 1 percent of XP-CLR score, we identified significantly 448 selective region, which were associated with 275 differentiated protein-coding genes. Using an empirical p-values of 0.01 of XP-EHH, we identified significantly 447 selective region, which were associated with 266 differentiated protein-coding genes. Thorough Gene Ontology analysis, the XP-CLR phase shows enhanced gene directional differentiation mainly in the protein metabolic process, organ development and immune response, while the most recent selection events indicated by the XP-EHH value involve the genes of the protein metabolic process, nervous system development and ion transport (Figure 3).

We found that the enriched Gene Ontology terms in DEGs which are directly related to immune and inflammation system-wide response to exercise (Table 1). Additionally we found that some genes belong to skeletal muscle cell differentiation system in Gene Ontology network.

Differentially expressed genes Validation

We analysis RT-PCR to validation of gene expression detected the RNA-seq data (Figure 4). Genes were selected by using David tool and the pathway was associated exercise stress. Candidate genes were all up regulated in muscle RNA-seq data. We conducted RT-PCR analysis using Jeju horses and thoroughbred muscle to identified the gene expression before and after exercise. qPCR Ct values were calculated by the $2^{-\delta\delta}$ Ct method.

After exercise, skeletal muscle produces and releases cytokines called myokines and the tissue cell secretes myokines as part of the extracellular signaling pathway in response to factors such as exercise, and the secreted factors can participate in nutrient generation, mediating angiogenesis, and regulating myogenesis in which exercise-induced myokines. Concordantly, we found many up-regulated myokines (containing cytokines and chemokines) and inducer in the muscle of “After exercise”, including CCL2, CCL4, CXCL2, IL-6, OSM and SOCS3 (Pedersen et al, 2007, Pedersen et al, 2008, Pedersen et al, 2009).

KEYWORD : RNA-sequencing, Transcriptome, Thoroughbred, Jeju Native horse, Gene Expression

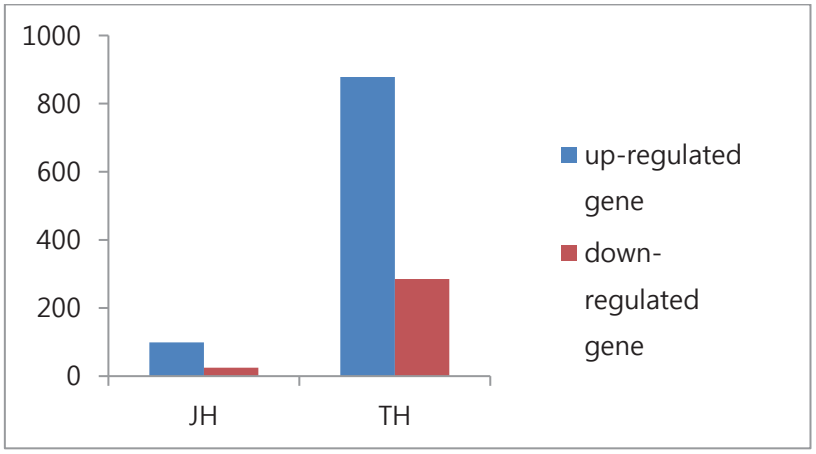

Figure1. DEG comparison Jeju native horse with Thoroughbred

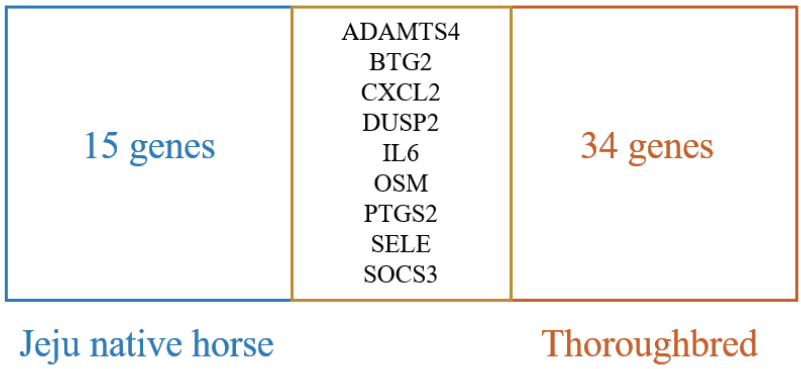

Figure 2. DEG comparison Jeju native horse with Thoroughbred

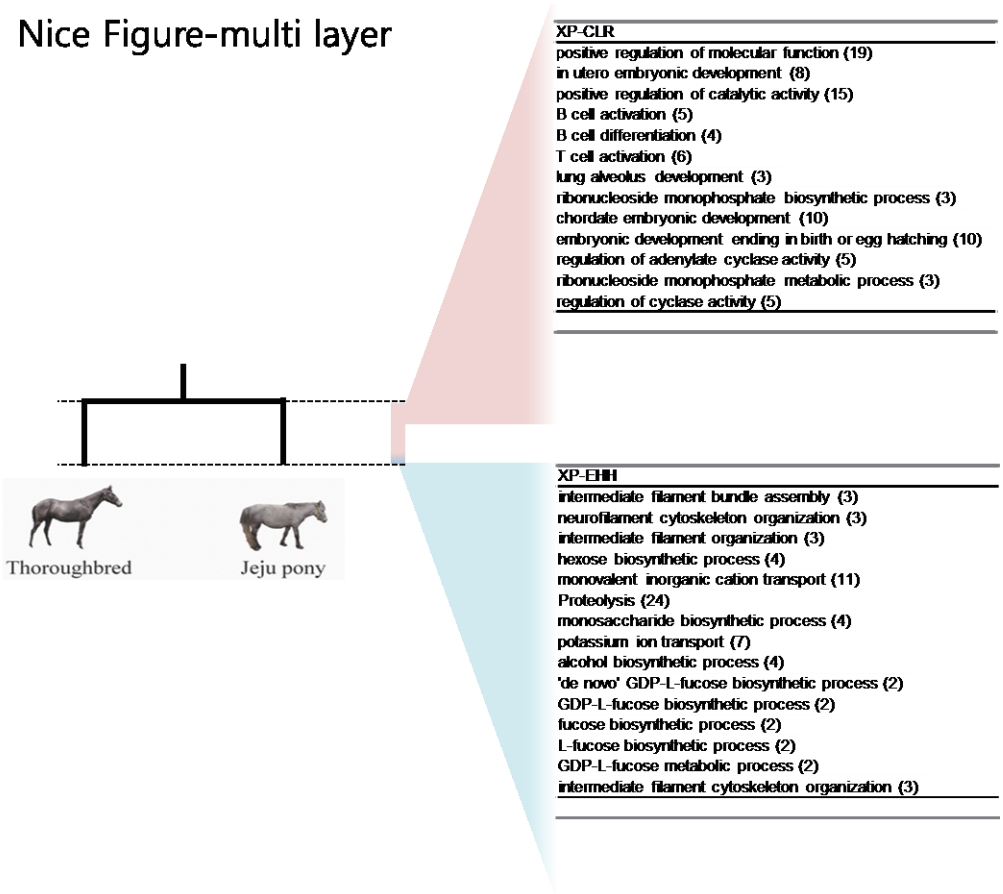

Figure 3. XP-CLR & XP-EHH Gene Ontology between Jeju horse and Thoroughbred

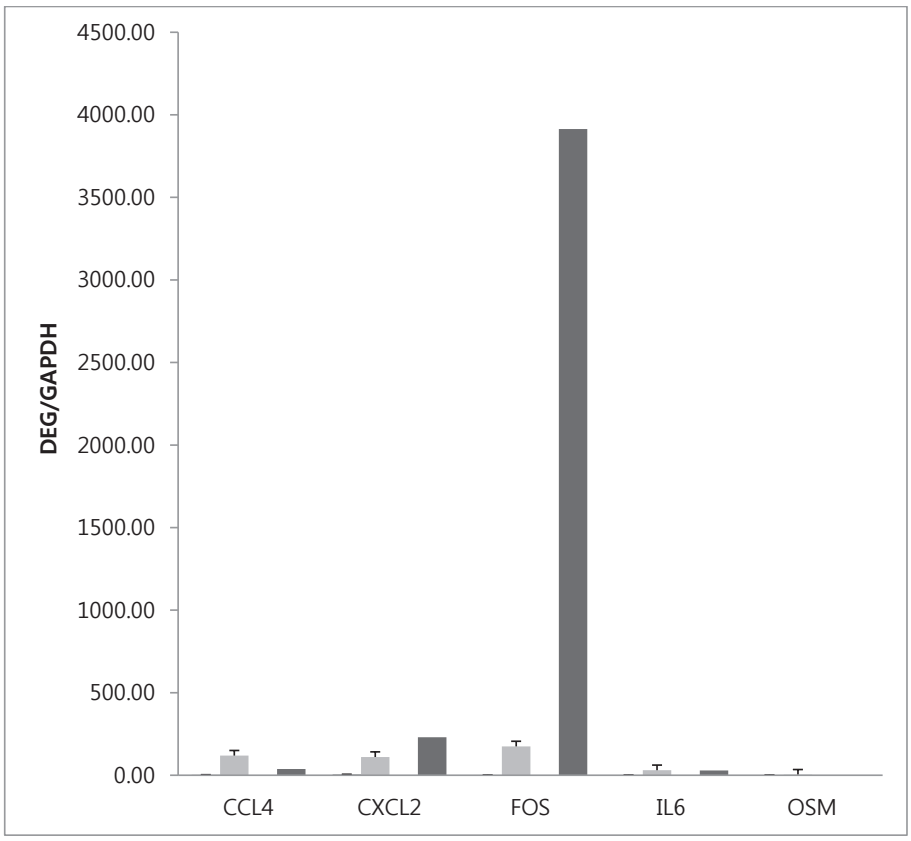

Figure 4. qRT-PCR validation of differentially expressed genes (DEGs) identified from Jeju horse and Thoroughbred before and after exercise.

Table 1. DEG Gene Ontology

Category	Gene Ontology Term	P-value	#Genes	Genes
skeletal muscle cell differentiation	skeletal muscle cell differentiation	2.36E-06	4	BTG2, EGR1, EGR2, FOS
positive regulation of leukocyte migration	regulation of granulocyte chemotaxis	3.19E-05	3	CCL2, CXCL2, CXCL8
	cellular response to interleukin-1	2.03E-09	6	CCL2, CCL4, CXCL8, EGR1, ICAM1, IL6
	response to interleukin-1	1.87E-10	7	CCL2, CCL4, CXCL8, EGR1, ICAM1, IL6, SELE
	chemokine-mediated signaling pathway	4.20E-06	4	CCL2, CCL4, CXCL2, CXCL8
	cellular extravasation	3.86E-05	3	CCL2, ICAM1, SELE
	neutrophil chemotaxis	7.56E-06	4	CCL2, CCL4, CXCL2, CXCL8
	positive regulation of leukocyte chemotaxis	9.36E-08	5	CCL2, CCL4, CXCL2, CXCL8, IL6
	regulation of leukocyte chemotaxis	2.80E-07	5	CCL2, CCL4, CXCL2, CXCL8, IL6
	regulation of leukocyte migration	1.71E-09	7	CCL2, CCL4, CXCL2, CXCL8, ICAM1, IL6, SELE
placenta blood vessel development	labyrinthine layer development	1.51E-05	3	CYR61, JUNB, SOCS3
response to progesterone	cellular response to calcium ion	4.62E-05	3	FOS, FOSB, JUNB
	response to corticosterone	6.99E-06	3	FOS, FOSB, JUNB
	response to mineralocorticoid	1.39E-05	3	FOS, FOSB, JUNB
response to gamma radiation	response to gamma radiation	7.10E-05	3	CCL2, EGR1, SOCS3
positive regulation of inflammatory response	positive regulation of nitric oxide metabolic process	3.63E-05	3	ICAM1, IL6, PTGS2
	positive regulation of reactive oxygen species biosynthetic process	5.47E-05	3	ICAM1, IL6, PTGS2
	maternal process involved in female pregnancy	1.38E-04	3	CCL2, JUNB, PTGS2
	positive regulation of nitric oxide biosynthetic process	3.63E-05	3	ICAM1, IL6, PTGS2
	regulation of nitric oxide biosynthetic process	7.46E-05	3	ICAM1, IL6, PTGS2
	response to cold	5.47E-05	3	FOS, IL6, PTGS2
	regulation of vascular endothelial growth factor production	1.06E-05	3	CCL2, IL6, PTGS2
	vascular endothelial growth factor production	1.27E-05	3	CCL2, IL6, PTGS2
	positive regulation of acute inflammatory response	1.06E-05	3	IL6, OSM, PTGS2

REFERENCES

BARREY, E., et al. Gene expression profiling in blood cells of endurance horses completing competition or disqualified due to metabolic disorder. EQUINE VETERINARY JOURNAL-SUPPLEMENT-, 2006, 36: 43.

Browning, B.L. and S.R. Browning, A fast, powerful method for detecting identity by descent. The American Journal of Human Genetics, 2011. 88(2): p. 173-182

Li, H., et al., The sequence alignment/map format and SAMtools. Bioinformatics, 2009. 25(16): p. 2078-2079.

LING, Y. H., et al. Evaluation of the genetic diversity and population structure of Chinese indigenous horse breeds using 27 microsatellite markers. Animal genetics, 2011, 42.1: 56-65.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(Delta Delta C(T)) method. Methods 25:402-408.

McKenna, A., et al., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research, 2010. 20(9): p. 1297-1303.

MARINI, Marina; VEICSTEINAS, Arsenio. The exercised skeletal muscle: a review. European Journal of Translational Myology, 2010, 20.3: 105-120.

OUTRAM, Alan K., et al. The earliest horse harnessing and milking. Science, 2009, 323.5919: 1332-1335.

PARK, Kyung-Do, et al. Whole transcriptome analyses of six thoroughbred horses before and after exercise using RNA-Seq. BMC genomics, 2012, 13.1: 473.

Pedersen, B.K. and M.A. Febbraio, Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiological reviews, 2008. 88(4): p. 1379-1406.

Pedersen, B.K., Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines. Journal of applied physiology, 2009. 107(4): p. 1006-1014.

Pedersen, B.K., et al., Role of myokines in exercise and metabolism. Journal of applied physiology, 2007. 103(3): p. 1093-1098.

VAN DE GOOR, L. H. P.; VAN HAERINGEN, W. A.; LENSTRA, J. A. Population studies of 17 equine STR for forensic and phylogenetic analysis. Animal genetics, 2011, 42.6: 627-633.

WADE, C. M., et al. Genome sequence, comparative analysis, and population genetics of the domestic horse. Science, 2009, 326.5954: 865-867.

O-29-10 EFFECT OF PRESYNCH-OVSYNCH/SUPEROVULATION USING A SPLIT-SINGLE INTRAMUSCULARLY OF FOLLICLE-STIMULATING HORMONE IN RUSA DEER

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Introduction

Several states were consciously concerned about the reduction in wildlife deer populations over the past several decades because there are no appropriate conservation measures. Thailand used to be the habitat of Schomburgk's deer (*Rucervus schomburgki*) before it was extinct around 1932. In order to maintain the remaining deer species such as Eld's deer which have been counted as reserved species under the Thai Wildlife Preservation and Protection Act (1992) and are also classified as endangered species by IUCN Red List, the ex situ conservation programs have been strongly proposed to prevent genetic loss and to increase the prolificacy of remaining individuals. However, the application of reproductive biotechnologies such as superovulation and embryo transfer in deer has been limited, and almost protocols were adapted from proven cattle and sheep protocols (Argo et al., 1994 Fenessy et al., 1994). Even those protocols were successfully useful in some commercial deer (Wang et al., 2012), however these technical developments are rarely directly transferable from farmed to some wildlife and endangered species because of interspecies variations and management distinction which should be designed to eliminate as much handling stress possible.

The traditional superovulation programs normally need CIDR® insertion and twice daily injections of FSH (Looney et al., 1981 Monniaux et al., 1983 Walsh et al., 1993). Therefore more attention by personnel and capture strategies for immobilization are required, increasing in undue stress and harmful to wildlife species especially deer. In order to minimize animal handling, this study was designed to evaluate the ovarian responses after treatment by the novel program includes Presynch-Ovsynch and split-single doses of FSH injection protocols that allowed for estrus synchronization and superstimulation, respectively.

Materials and Methods

Animals

Six hinds which were located in Khon Kaen zoo were used in this study. They were over 2 years of age when conducted the experiment, and were housed together in a paddock.

Estrus Synchronization and Superovulation

Initially, the estrus cycle of the hinds at unknown stages were synchronized with two injection of PGF2 α intramuscularly at an interval of 12 days by darting, then began the Ovsynch protocol (GnRH-7d-PGF2 α -48h-GnRH) 10 days later. The first injection of GnRH was determined as Day 0.

Follicular developments of hinds were then randomly superstimulated by injecting of FSH (total dose = 180 mg) with one of two different treatments at Day 2. Group 1 received 8 injections of FSH every 12 h (multiple injection treatment) Group 2 received twice injections of FSH dissolved in MAP-5 50 mg (for slow released effect) with a 48 h interval (split-single injection treatment). The estrus signs were observed by zoo keeper.

Evaluation

Nine to Ten days after the hinds shown estrus signs, laparoscopy was conducted to determine the appearance of corpus luteum and unruptured follicles. The hinds were starved for 18 h before anesthetized using xylazine and Zoletil®. The midline ventral laparotomy was performed. The number of corpus luteum and unruptured follicles were recorded and compared between two superovulation treatments.

Statistical Analysis

The data were analyzed using the SAS system version 9.0. Before statistical analysis data were tested for normality and homogeneity of variance, and then were performed by ANOVA using PROC GLM.

Results

All hinds exhibited estrus behaviors. The mean number of ovulation (4.3 and 3.0), unruptured follicle (4.0 and 4.3), and total follicular stimulation (8.3 and 7.3) subjected to multiple and split-single injections of FSH for superstimulation did not differ between groups ($P > 0.05$ Figure 1).

Discussion

This study evaluated the efficacy of a split-dose single injection of FSH to superstimulate the ovarian activities in terms of the number of corpus luteum and unovulated follicles after treatment by the novel program of estrus synchronization includes Presynch-Ovsynch in order to minimize animal handling in Rusa deer. Also we hypothesized that the ovarian responses would be similar for hinds treated with split-single injection of FSH compared with those treated with a multiple injection of FSH. Overall, the results were demonstrated that the ovarian responses were successfully by Presynch-Ovsynch together with both FSH treatments. The total follicular stimulation in this study was higher than a previous report in brown brocket deer (approximately 1.4-4.8) which firstly mentioned about single injection of FSH in deer but unsuccessfully (Zanetti and Duarte, 2012). However, those gonadotropins and materials for single injection of FSH preparation were different with our study. It was speculated that a single injection of FSH dissolved in 30% PVP perhaps was no longer able to maintain sufficient FSH activity to sustain the growth of multiple follicles (D'Alessandro et al., 2001). For our treatment, the additional injection of FSH (the second dose) was adopted to continue growth to an ovulatory size as similarly as single injection (Tríbulo et al., 2012).

In conclusion, it implied that Presynch-Ovsynch with slow released FSH stimulation would be more beneficial in terms of reduce animal stress and human handling, which is feasible to be applied in other endangered Cervidae.

Acknowledgements

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KEYWORD : Gonadotropins, Ovarian response, Superstimulation, Cervidae

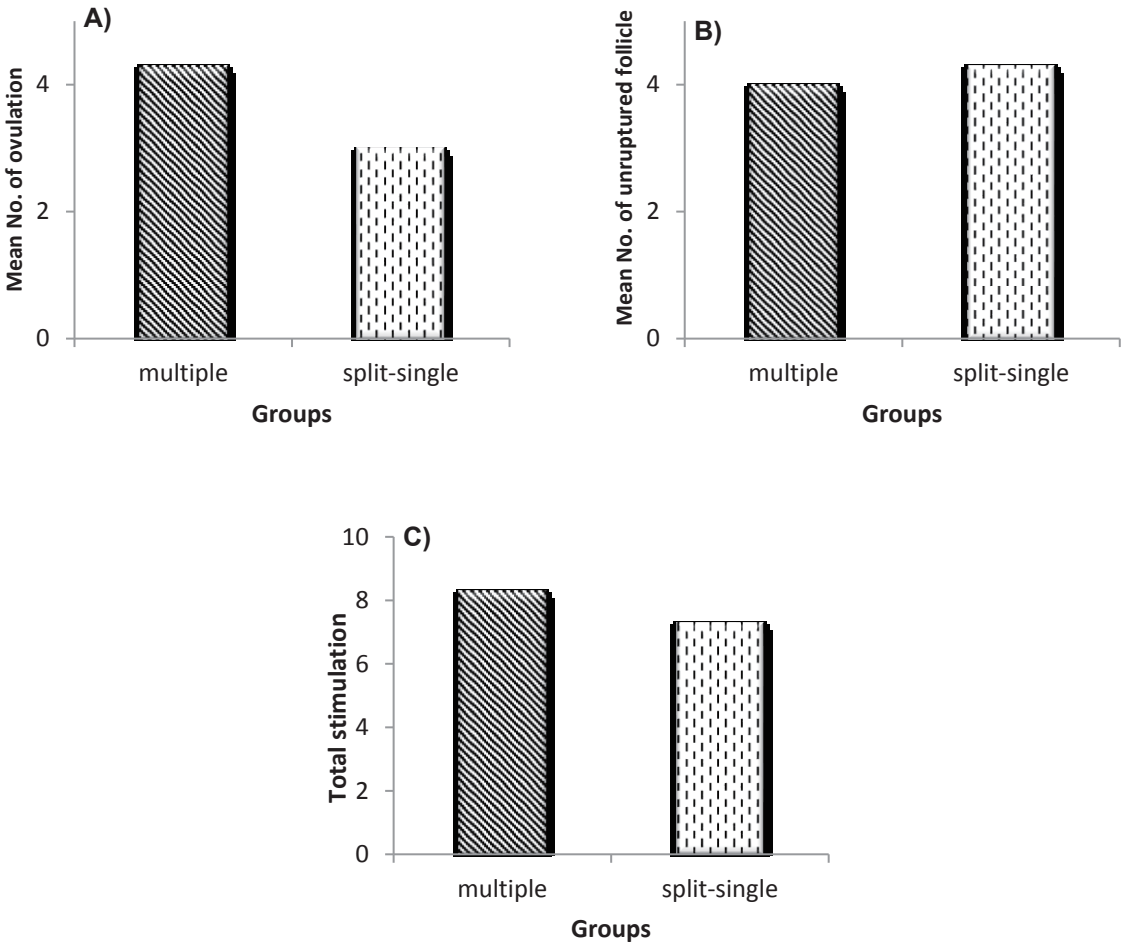

Figure 1 The ovarian follicular response in terms of the mean number of ovulation (A), the mean number of unruptured follicle (B), and the total stimulation (C) of Rusa deer subjected to multiple and split-single injections of FSH for superstimulation; ($P > 0.05$).

REFERENCES

Argo CM, Jabbour HN, Goddard PJ, Webb R, Loudon AS. 1994. Superovulation in red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*), and fertilization rates following artificial insemination with Père David's deer semen. J Reprod Fertil. 100: 629 –36.

D'Alessandro AG, Martemucci G, Colonna MA, Borghese A, Terzano MG, Bellitti A. 2001. Superovulation in ewes by a single injection of pFSH dissolved in polyvinylpyrrolidone (PVP): effects of PVP molecular weight, concentration and schedule of treatment. Anim Reprod Sci. 65(3-4):255-64.

Fennessy PF, Asher GW, Beatson NS, Dixon TE, Hunter JW, Bringans MJ. 1994. Embryo transfer in deer. Theriogenology. 41:133– 8.

Looney CR, Boutle BW, Archibald LF, Godke RA, 1981. Comparison of once daily FSH and twice daily FSH injections for superovulation of beef cattle. Theriogenology 15: 13–22.

Monniaux D, Chupin D, Saumande J. 1983. Superovulatory responses of cattle. Theriogenology 19: 55–81.

Tríbulo A, Rogan D, Tríbulo H, Tríbulo R, Mapletoft RJ, Bo GA. 2012. Superstimulation of beef cattle with a split-single intramuscular administration of Folltropin-V in two concentration of hyaluronan. Theriogenology. 77: 1679-85.

Wang L, Zhou GB, Shi WQ, Shi JM, Tian XZ, Gao C, Zhang L, Zhu SE, Zhang TT, Zeng SM, Liu GS. 2012. First live offspring born in superovulated sika deer (*Cervus nippon*) after embryo vitrification. Theriogenology. 78(7):1627-32

Walsh JH, Mantovani R, Duby RT, Overstrom EW, Dobrinsky JR, Enright WJ, Roche JF, Boland MP. 1993. The effects of once or twice daily injections of p-FSH on superovulatory response in heifers. Theriogenology 40: 313–21.

Zanetti Edos S, Duarte JM. 2012. Comparison of three protocols for superovulation of Brown brocket deer *(Mazama gouazoubira*). Zoo Biol. 31(6):642-55.

O-30-1

The Microbial Analyses of Fermented Sausage from Tetelan Beef using Probiotic Culture Starter

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INTRODUCTION

*Tetelan*beef is the carcass in the first forequarters path to the tenth ribs, with the meat and fat proportions are 65% and 35%. Therefore this part in slaughter house usually called forequarters 65 CL beef. *Tetelan*beef in market well-known has the low price, besides that Indonesian people have long time using this to process into other kinds of meat processing in the household or sell them to the public consumers. The lower price makes*tetelan*beef fancied by many people. However, the quality of food products is not as good as the price. Furthermore,*tetelan*beef has the potency to process into a functional food, so then the products have a good price while a good quality.

*Tetelan*beef by using the probiotic starter culture, such as*Lactobacillus plantarum*2C12 or *Lactobacillus acidophilus*2B4 followed by adding herbs inside called the fermented sausage. Probiotic bacteria are the good bacteria which can grow in human intestinal. In related to improve the*tetelan*beef quality, produce fermented sausage intestine health products, utilize*tetelan*beef as the solutions to longer the shelf-life, safety, and has sell high value. Fermented sausage from*tetelan*beef needed to analyze its microbial quality. The aim of this research was to examine the microbial quality including pathogenic bacteria inside of fermented sausage that using*L. plantarum*2C12 or *L. acidophilus*2B4 as the probiotic culture starter.

MATERIALS AND METHODS

Materials

*Tetelan*beef of forequarter 65 CL from Brahman Cross with the postmortem 24 hours, bought from PT. Elders, Dramaga, Bogor slaughter house. Other ingredients in producing fermented sausage were sugar, salt, ginger, subtle nutmeg, white pepper, black pepper, lime leaves and probiotic starter culture (*L. plantarum*2C12 and *L. acidophilus*2B4). The other materials were non-edible sausage casing diameter 4.5 cm, skim milk, yolk egg, alcohol 70%, and potassium tellurite. In addition, the media for microbial analyses were: Buffer Peptone Water (BPW) de Man Rogosa Sharp Broth (MRSB) de Man Rogosa Sharp Agar (MRSA) Eosin Methylene Blue Agar (EMBA) Xylose Lysine Deoxycholate Agar (XLDA) Bacteriological Agar (BA) and Baird-Parker Agar (BPA).

Methods

Fermented sausage production

Arief (2000) revealed that the process started by minced the beef then chilled within 24 h, then minced in bowl cutter machine, added the ingredients and probiotic culture starter based on treatments, final temperature was not more than 2°C. The final dough was around the grain of rice, and next was mixed into stuffer then put compactly inside a casing, kept the temperature around 2°C, continued by conditioning 24 h at room temperature (± 27°C). In the end, cooling smoked during three days with temperature 28 - 30°C in 4 hours per day.

Quantitative analyses of bacteria

Prepared 25 g samples, diluted by 225 mL BPW until fifth dilution (10^{-6}). Total LAB counted with aseptically pipetting between $10^{-1}$and 10^{-3}dilution, poured 1 mL into a sterile petri dish (did it twice) then poured MRSA, in the end, incubated all the media for 24 hours at 37°C. Growth colony was white and yellowish (Fardiaz, 1989). Likewise*E.coli*, aseptically pipetting between $10^{-1}$and 10^{-3}dilution and poured it 1 mL into a sterile petri dish (duplo), then poured EMBA. The media for*Salmonella*counts was by using XLDA,*Staphylococcus aureus*by using BPA. Finally, all the media were incubated 24 h at 37°C. *E.coli*colonies which can grow were blue to purple. *Salmonella*was muddy yellow with a black spot inside,*S. aureus*color was black with yellow ring (APHA, 1992).

Statistical Analysis

Statistical analysis by using ANOVA completely randomized design, continued by non-parametric Kruskal-Wallis test and Tukey test (Steel and Torrie, 1991). The software was Minitab 14.

RESULTS AND DISCUSSION

Microbial quality of tetelan beef

Tetelan beef in the first forequarters part before producing the fermented sausage, firstly its microbial quality were analyzed (Table 1). The microbial analyses were including lactic acid bacteria (LAB) calculation and pathogenic bacteria population count (*Salmonella*, *E.coli*, and *S. aureus*). LAB numbers are 6.05 x 10^2 CFU g-1, due to LAB has many advantages for human health, also the amounts are good for body and origin microflora growth in meat, expected the high number is increasing during the production process. Total of LAB number was normal even above the standard.

The calculation of pathogenic bacteria population showed that there was no *Salmonella*, *E.coli* and *S. aureus* in *tetelan* beef as a primary raw material for fermented sausage production, based on the data, *Salmonella* was negative or not detected, *E.coli* and *S. aureus*, both of them, were 0 CFU g-1. The numbers were below the safety standard, which means *tetelan* beef was safe to further process.

Probiotic culture starter population count

Lactobacillus spp.. is the biggest group of LAB genus (Axelsson, 1993). Genus *Lactobacillus* is gram positive and could not generate spore, anaerobic facultative, optimally growth at around 30 - 40℃ and pH 5.5 - 5.8, this genus growth at around 5 - 35℃ and pH less than 5. *Lactobacillus* spp. Mostly in fermented food, contribute to preservation, nutrition, and flavor for products (Salminen and Wright, 2004). Starter culture put in fermented sausage was the probiotic starter which has the suitable viability and moisture content, can kill pathogenic bacteria and spoilage bacteria (Varnam and Sutherland, 1995).

LAB gets enormous attention because many genera have advantages for body health, that was called probiotic. Probiotic defined as the live microorganism which can be eaten by human or animals in an appropriate amount, then giving health advantages for the host (FAO/WHO, 2002). Probiotic has many health functions such as preservative and therapeutic for diarrhea, reducing lactose intolerance, protecting from inflammation and arthritis, avoiding hypertension and cancer, and improving immunity system (Parvez et al., 2006). Probiotic also has a function for completing digestive system by protecting it from pathogenic bacteria (Agostoni, 2004).

The research started by refreshed probiotic culture starter *L. plantarum* 2C12 and *L. acidophilus* 2B4, then continued by calculation of LAB population. According to Table 2, *L. plantarum* 2C12 culture had outset population at 1.08 x 10^9 CFU mL-1 and *L. acidophilus* 2B4 1.83 x 10^9 CFU mL-1. Arief et al. (2000) said that minimum limit LAB population to be a probiotic culture starter must be around 10^8 - 10^9 CFU mL-1, seen from this calculation, so both of these probiotic culture starters had fulfilled the criteria.

LAB mechanism on eliminating pathogenic bacteria during fermented sausage production

Fermented sausage is a functional food (Aberle et al., 2001), produced by combining the minced beef in bowl cutter with ingredients and either using culture starter (*L. plantarum* 2C12 or *L. acidophilus* 2B4) or without culture starter. Culture starter added gradually in this process, started from a little dough into all part of the dough to mix evenly throughout the dough.

Ingredients combined have plenty of advantages, salt was for flavoring and keeping the particular condition of LAB to grow well, otherwise pathogenic bacteria and spoilage bacteria were going to resist the growth. Sugar as a fermentation substrate was for LAB growing, generate flavor and sausage texture. Ginger, nutmeg, pepper and lime leaves are herbs and spices had advantages for natural preservatives in food, especially in this fermented sausage production.

All living creatures, including microorganisms, need water. The moisture content of the *tetelan* beef was 73.54%, after being fermented sausage product water content was 41.71-44.29%, the reduction was 29.25%. It also affects the availability of water for microorganisms. Microorganisms need water called a_w (activity water), some bacteria can not grow well on a_w less than 0.91, but minimum a_w for growing was various. For instance, minimum a_w for *Salmonella* was 0.94, *S. aureus* was 0.86, and *E.coli* was 0.96 (Lechowich, 1971 Forest et al., 1975). Based on calculations performed, a_w of beef was 0.88, whereas when the processed into sausage fermentation, a_w become 0.80-0.82, a_w value range of meat and products can be said to be safe from the growth of pathogenic bacteria, including *Salmonella*, *S. aureus*, and *E.coli*.

Fermented sausage after smoking 4 hours each day, then it was stored at room temperature (±27 ºC) to optimize the growth of the LAB product. LAB can grow with or without air, but it will very quickly produce acids when cultured in the absence of air (anaerobic facultative) (Food Safety and Inspection Service, 2005). LAB can change some sugar into lactic acid and other metabolic results, this event is called homofermentative. LAB produced an enzyme that plays a role in chemical changes that can lead to high acidity so that the pH value and redox

potential were small. It can inhibit the growth of other microorganisms, especially pathogenic or spoilage bacteria. Bacteriostatic or bactericidal in phenolic compounds during the smoking process, it can also inhibit the growth of pathogenic and spoilage bacteria. If the growth of pathogenic and spoilage bacteria were inhibited, then the resulting product would become safer for consumption, and fermented sausage products will have a longer shelf life.

Microbial quality of fermented sausage product within or without probiotic culture starter

Table 3 shows microbiological quality sausage fermented product fermented sausages without culture (P1), the use of *L. plantarum* culture 2C12 (P2) and *L. acidophilus* culture 2B4 (P3). Values contained in Table 3 were transformed into $\log_{10}$ CFU g^{-1}. Values obtained from the calculation using the plate count method BAM (2002) for three dilutions elected, the population calculated using this approach was only the bacterial population with the colony between 25 and 250 CFU mL^{-1}. The number under 25 CFU mL^{-1} assume has a number below 25 CFU mL^{-1}, and number above 250 CFU mL^{-1} considered has a number above 250 CFU mL-1. Values were averaged and calculated the standard deviation of each treatment with four replications.

Proven contained *Salmonella* in fermented sausage products without culture, whereas in the sausage product of fermentation with culture (*L. plantarum* 2C12 and *L. acidophilus* 2B4) not contained *Salmonella*. The danger of *Salmonella* according to Jay (2000) evidenced by the many species of *Salmonella* that can cause various health problems, among which are *Salmonella* typhi and *Salmonella* paratyphoid serotypes that cause fever or even death. According to the MLA (2003), the product is safe if there are not *Salmonella* bacteria, related to this statement the fermented sausage products without culture (P1) contained *Salmonella* then the product was not safe for consumption. Unlike the products P1, fermented sausage products with the use of *L. plantarum* culture 2C12 (P2) and *L. acidophilus* 2B4 (P3) were safe, because there was no *Salmonella* bacteria contamination.

Fermented sausages without culture (P1) contained bacteria *S. aureus* as much as 0.67 $\log_{10}$ CFU g^{-1} or 116.25 CFU g^{-1}, even though tolerance limit of *S. aureus* based on MLA standard (2003) for meat products of fermentation are 100 CFU g^{-1}. In other words, a product without culture (P1) was not safe for consumption. Fardiaz (1992) stated that *S. aureus* as indicators of the sanitary quality because its presence indicates contamination of workers, slaughterhouse or animal origin. *S. aureus* called pathogen bacterial because it often causes intoxication in food through enterotoxin that resistant to heat from its production, there are lots of meat, meat products, or even high protein foods. Sausage fermentation with the use of culture *L. plantarum* 2C12 (P2) and *L. acidophilus* 2B4 (P3) proved not contain *S. aureus* because a number of bacteria in the product was 0 CFU g^{-1}. This result showed the optimum LAB metabolism. Furthermore, it can inhibit pathogenic bacteria in the product.

The population of *E.coli* in three treatments fermented sausage products were in the safe range (the number of sub-standard), which was less than 3 $\log_{10}$ CFU g^{-1}. According to Fardiaz (1992) *E.coli* is the indicator of food or water contaminated by bacteria, because *E.coli* is normal flora contamination channels. These bacteria also are part of the healthy microflora facultative anaerobic digestive tract of humans and warm-blooded animals. Fermented sausages without culture (P1) contaminated with bacteria *E.coli* was 1.05 $\log_{10}$ CFU g^{-1}. At the same time, sausage products fermented with *L. plantarum* 2C12 (P2) infected with *E.coli* was 0.35 $\log_{10}$ CFU g^{-1} and sausages fermented with *L. acidophilus* 2B4 (P3) contaminated with *E.coli* was 0.62 $\log_{10}$ CFU g^{-1}. The population of *E.coli* that acceptable into the body is 3.6 $\log_{10}$ CFU g^{-1}(MLA 2003). Judging from the results of tests performed, we can conclude fermented sausage products were safe from *E.coli*.

Based on the microbiological analysis have been done, fermented sausage products safe for consumption were products with the probiotic starter culture, i.e., P2 and P3. Due to antimicrobial probiotic starter culture of *L. plantarum* called plantasin 2C12 and at *L. acidophilus* 2B4 called lactasin.

LAB growth during Fermented Sausage production

The use of probiotic culture starter has proven to inhibit the growth of pathogenic bacteria in the product. The population of LAB in the product without culture (P1) was 2.91 x 10^9 CFU g^{-1}, while the total of LAB in fermented sausage product with *L. plantarum* 2C12 (P2) amounted 8.26 x 10^9 CFU g^{-1} and the culture of *L. acidophilus* 2B4 (P3) was 3.83 x 10^9 CFU g^{-1}. *L. plantarum* 2C12 was a starter culture that can inhibit pathogenic bacteria more effectively than *L. acidophilus* 2B4. It was shown from LAB growing population during the study (Figure 1) and also the ability of probiotic culture starter of *L. plantarum* 2C12 in inhibiting *E.coli*.

Figure 1 shows the growth curve of *tetelan* beef using LAB probiotic to be fermented sausage products. Overall it can be seen that the population of LAB in *tetelan* beef may increase during the production process of fermented sausages. Fermented sausages without culture (P1) have a not significantly different increasing, even a population of almost stable during the smoking process. Due to the absence of a culture so that the fermentation process was

only done by the bacteria present in meat. In the sausage product of fermentation with culture (P2 and P3), the fermentation process was faster and more significantly increasing in LAB, due to the additional assistance of the culture used.

Each bacterial growth as Figure 2 was starting from the adaptation phase, then the logarithmic phase, until the death phase. LAB growth had seen increasing in population from *tetelan* beef into the dough, during this process LAB was on the adaptation period. When in admixture product, LAB adjusted the native atmosphere into the new environment, and then the dough was treated by the smoking, the bacteria began to enter the logarithmic phase, and growth began to rise sharply, but the next stage was smoking, which indirectly reduce the number of LAB, as LAB were the situated a new environment again. LAB population declined in smoking day 1. Smoking in the 2nd day LAB resumed its growth phase by re-adapting so that in the next step of LAB population continues to increase, to obtain fermented sausage products.

CONCLUSION

Fermented sausage from *tetelan* beef has proven as a safety functional food based on microbial analyses to inhibit pathogenic bacteria such as *E.coli*, *Salmonella* and *S. aureus*. *Lactobacillus* plantarum 2C12 has proven more efficiently as probiotic culture starter in fermented sausage.

KEYWORD : Fermented sausage, Lactobacillus plantarum 2C12, Lactobacillus acidophilus 2B4, Microbial quality, Tetelan beef

Table 1. Microbial quality of *tetelan* beef (CFU g^{-1})

Microbes	Total	Standard
Salmonella	Negative	Negative**
*E. coli***	0.00	5 x 10^1**
S. aureus	0.00	1 x 10^1**
LAB	6.05 x 10^2	Minimal < 10^1*

Notes: *Chenoll (2006) ** BSN (2008) *** MPN g^{-1}

Table 2. Outset population of probiotic culture starter (CFU mL^{-1})

LAB	Population (CFU mL^{-1})
L. plantarum 2C12	1.08 x 10^9
L. acidophilus 2B4	1.83 x 10^9

Table 3. Microbial quality of fermented sausage (log_{10} CFU g^{-1})

Microbes	P1	P2	P3
Salmonella	Positive*	Negative	Negative
S.aureus	0.67*	0.00	0.00
E.coli	1.05***	0.35*	0.62**
LAB	9.36 ± 0.33	9.83 ± 0.33	9.45 ± 0.36

Notes: P1 (without culture), P2 (using 2% *L. plantarum* 2C12) and P3 (using 2% *L. acidophilus* 2B4).*) 1 sample contaminated from 4 sample analysed, **) 2 out of 4 contaminated sample ***) 3 out of 4 contaminated sample.

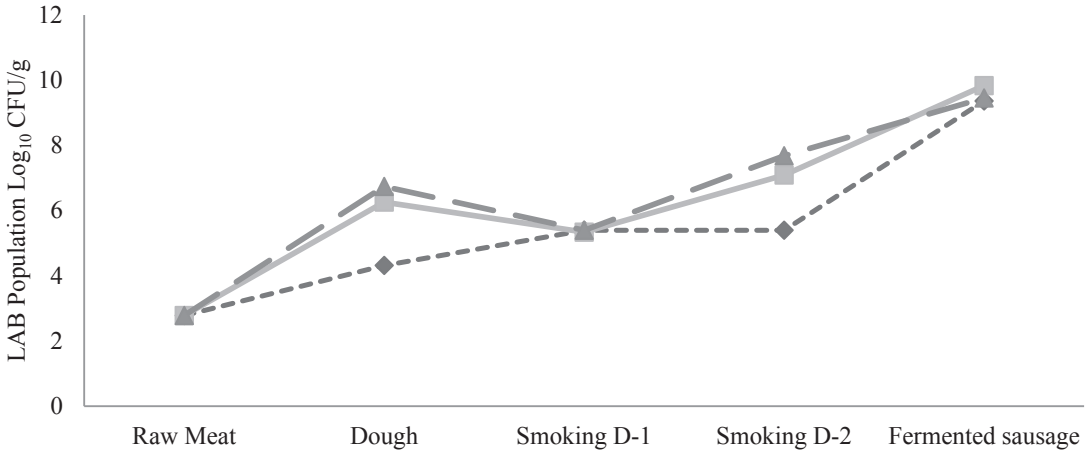

Figure 1. Chart of LAB population from raw material to fermented sausage based on treatments. (♦) P1: without probiotic culture starter, (■) P2 using 2% of *L. plantarum* 2C12 and (▲) P3 using 2% of *L.acidophius* 2B4.

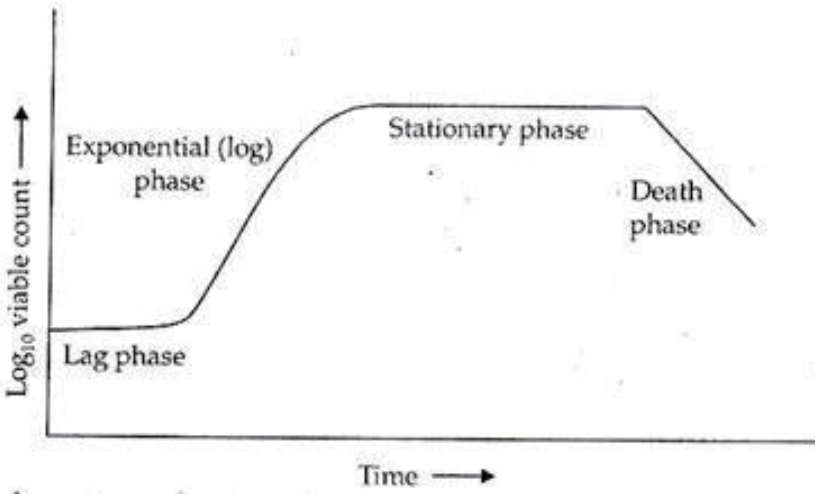

Figure 2. Bacterial lag phase

REFERENCES

Aberle, E.D., Forrest, J.C., Gerard, D.E., Mills, D.E., Hendrick, H.B., Judge, M.D., Merkel, R.A. 2001. *Principle of Meat Science*. Ed ke-4. Iowa (US): Kendall Hunt Publishing Company.

Agostoni, C. 2004. Probiotic bacteria in dietetic products for infants acommentary by the ESPHGHAN Committee on Nutrition. *J.Pediatr Gastroenterol Nutr*. 28: 365-374.

[APHA] American Public Health Association. 1992. *Standard Methods for the Examination of Water and Wastewater*. Ed ke-18. Washington DC (US): American Public Health Assoc.

Arief, I.I. 2000. Pengaruh aplikasi kultur kering dengan beberapa kombinasi mikroba terhadap kualitas fisiko - kimia dan mikrobiologi sosis fermentasi [thesis]. Bogor (ID): Institut Pertanian Bogor.

Axelsson, L. 1993. *Lactid Acid Bacteria: classification and physiology*. In: Salminen S, Wright AV, editor. *Lactid Acid Bacteria: Microbiology and Functional Aspects*. Ed ke-2, Revised and Expanded. New York (US): Marcell Dekker Inc.

[BAM] Bacteriological Analytical Manual. 2002. GRAS Exemption Claim and Exemption Notification for the Use in Infant Formula of Bifidobacterium, Lactobacillus and Streptococcus thermophilus. BAM [Internet]. [Downloaded 2013 Nov 12]; Volume 1. Available at: http://www.accessdata.fda.gov/ scripts/fcn/gras_ notices/grn000049A.pdf

[BSN] Badan Standarisasi Nasional. 2008. *Mutu Karkas Daging Sapi* (SNI 3932-2008). Jakarta (ID): Badan Standarisasi Nasional.

Chenoll, E., Macian, M.C., Elizaquivel, P., Aznar, R. 2006. Lactic acid bacteria associated with vacuum-packed cooked meat product spoilage: population analysis by rDNA-based methods. *J App Microbiol* ISSN 1364-5072. 102 (2). doi:10.1111/j.1365-2672.2006.03081.x.

[FAO/WHO] Food and Agriculture Organisation and World Health Organisation. 2002. Guidelines for the evaluation of probiotics in food. Report of Joint FAO/WHO Working Group on drafting Guidelines for the evaluation of probiotics in food. Canada (US): London Ontario.

Fardiaz, S. 1992. *Mikrobiologi Pangan I*. Jakarta (ID): Gramedia Pustaka Utama.

Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D., Merkel, R.A. 1975. Principles of Meat Science. San Fransisco (US): W. H. Freeman and Co.

[FSIS] Food Safety and Inspection Service. 2005. *Microbiology*. Ed ke-4. New York (US) : Mc-Graw-Hill Book Co.

Jay, M.J. 2000. *Modern Food Microbiology*. Ed ke-6. Maryland (US): Aspen Publisher Inc.

Lechowich, R.V. 1971. The Science of Meat and Meat Products. Ed ke-2. Price JF, Schweigert BS, editor. Hal 230-286. San Fransisco (US): W. H. Freeman and Co.

[MLA] Meat and Livestock Australia. 2003. *Through chain risk profile for the Australian red meat industry. PRMS.038c, part 1: risk profile*. North Sydney (AU): Meat and Livestock Australia.

Parvez, S., Malik, K.A., Kong, S.A., Kim, H.Y. 2006. Probiotics and their fermented food products are beneficial for health. Review article. *J App Microbiol* 100: 1171-1185.

Salminen, S., Wright, A.V. 2004. *Lactic Acid Bacteria. Microbiology and Functional Aspects*. Ed ke-2, Revised and Expanded. New York (US): Marcell Dekker Inc.

Steel, R.G.D., Torrie, J.H. 1991. *Prinsip dan Prosedur Statistik. Suatu Pendekatan Biometrik*. Ed ke-2. Sumantri, B., translator. Jakarta (ID): PT. Gramedia. Translation from: Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed.

Varnam, A.N., Sutherland, J.P. 1995. *Meat and Meat Products. London* (GB): Chapman and Hall.

O-30-2 MICROBIOLOGY QUALITY OF BEEF DENDENG TREATED BY CURING WITH OR WITHOUT SODIUM NITRITE SOLUTION AT DIFFERENT STORAGE AND AFTER FRYING

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INTRODUCTION

Dendeng is an Indonesian dried meat product processed using some spices and drying process to reduce its moisture. Using of high sugar, antioxidant and antimicrobe rich spices, as well as drying process could increase shelf life of dendeng compared to moisture meat products. Some dendeng producers use sodium nitrite as curing agent to improve the color and increase the shelf life of dendeng (Suryati *et al*., 2012) since the function of nitrite salt as color improver, antioxidant, and antimicrobes agent (Honikel, 2008 Suryati *et al*., 2014). Nevertheless dendeng as a meat product has high risk if it is produced without safety and hygiene procedures. Microbiology contamination on dendeng product is very susceptible from livestock slaughtering, meat processing to preparation before consumption. Some pathogen microorganism like *E. coli*, *Salmonella sp*. and *S. aureus* have to be controlled to meet regulation microbiology quality (Food Standard Australia New Zealand, 2001 BSN, 2013 Center for Food Safety, 2014).

The presence of *E. coli* in ready to eat product like dendeng is undesirable because it indicates poor hygienic condition which have lead to contamination or inadequate heat treatment. Levels of *E. coli* exceeding 100 per gram are unacceptable and indicate a level of contamination which may have introduced pathogens or that pathogens. *S. aureus* levels of $\square 10^4$ cfu/g are considered as potentially hazardous as foods with this level of contamination may result in food borne illness if consumed (Australia New Zealand Food Standard, 2001). Dendeng should be free of *Salmonella* (BSN, 2013) as consumption of food containing this pathogen may result in food borne illness. The presence of this organism indicates poor food preparation and handling practices such as inadequate cooking or cross contamination (Australia New Zealand Food Standard, 2001).

Drying process and short frying process in preparation before consumption (Suryati *et al*., 2012 and Suryati *et al*., 2014) may increase the safety of dendeng as ready to eat food, but this need deep investigation to assure the safety of dendeng as food. Drying process at 60-70°C in dendeng making (Suryati *et al*., 2014) may be inadequate to eliminate the pathogens, but shortly frying in oil at 150°C (Suryati *et al*., 2012 Suryati *et al*., 2014) could reduce microbiology contamination on dendeng. Besides that dendeng has long shelf life at room temperature because of low its moisture and water activity (Suryati *et al*. 2012 Suryati *et al*., 2014), but for safety purpose the frozen storage is often chosen. This study was conducted to evaluate microbiology quality of cured or noncured beef dendeng stored at 0 mo, 10 mo and after short frying to assure its safety.

MATERIALS AND METHODS

Materials

Beef used in this study was obtained from round of Brahman Cross 1.5 yr. The spices i.e. galangal, coriander, garlic, tamarind, pepper were bought from local market, Indonesia. Agar media used in microbiological analysis involved buffered peptone water (BPW), plate count agar (PCA) (Difco™, USA), xylose-lysine deoxycholate agar (XLDA) (Oxoid LTD, England), eosin methylene blue agar (EMBA) (Oxoid LTD, England), potato dextrose agar (PDA) (Oxoid LTD, England), Baired-Parker agar (BPA) (Difco™, USA).

Dendeng Making and Sample Preparations

Dendeng ingredient formula (Table 1) and its making procedure used in this research followed Suryati *et al*. (2014) with little modification in drying duration. The procedure covered preparation of ingredient and spices grilling, slicing beef with 0.5 cm of thickness, incubation of meat in spices mixture for 12 h, and drying at 60°C for 3 h, continued at 70°C for 7 h. Wet cured dendeng was incubated in sodium nitrite solution (125 mg/L) for 12 h before it was mixed with spices mixture. Dried dendeng was exposured at room temperature to decrease its temperature, and then it was packed in sealed plastic.

Raw packed dendeng was stored at freezer (-10 to -18°C) for 0 (24 hr) and 10 mo as raw dendeng samples. Fried

dendeng samples were prepared according to Suryati *et al*. (2014). Dendeng was fried in oil at 150°C for 1.5 min after soaked in potable water for 5 min and drained for 15 min.

Determination of Dendeng Microbiology Quality

Microbiological analyses were conducted to evaluate the microbiology quality of dendeng during storage and after short frying as preparation before consumption. Microbiological analyses involved total plate count (Maturin dan Peeler, 2001), mold and khamir (Tournas *et al*., 2001), *Escherichia coli* (Feng *et al.*, 2002), *Salmonella sp*. (Andrews dan Hammack, 2007), *Staphylococcus aureus* (Bennett dan Lancette, 2001) with some modifications.

Experimental Design and Data Analysis

Experiment was arranged in factorial design 2 x 3 with 2 factors, i.e. curing factor (curing or non-curing with sodium nitrite solution) and status condition of dendeng factor (dendeng storage for 0 mo (24 hr)), raw dendeng with 10 mo, and fried dendeng). Data were evaluated by anaysis of variant (ANOVA), and continued with Tukey test, if the variable was significantly affected by the treatment.

RESULTS AND DISCUSSION

Total mold and yeasts, total plate count (TPC), *E. coli* dan *Salmonella sp.* were affected by interaction of storage/ preparation condition treatment and curing treatment of dendeng (Table 2). The interaction between the treatment did not affect population of *S. aureus*. The number of *S. aureus* was affected by condition of dendeng (storage and preparation/frying) only. The curing treatments did not influence the number of *S. aureus*. *E. coli*, *Salmonella* sp. dan *S. aureus*. *S. aureus*. *E. coli*, *Salmonella* sp. dan *S. aureus* were pathogen indicators often found in meat product, like dendeng. *E. coli* indicated hygiene of product during processing and storage. *E. coli*, *Salmonella* sp. and *S. aureus* were foodborne pathogens could be found in meat product (Food Standard Australia New Zealand, 2001 Center for Food Safety, 2014).

Total mould and yeasts were not different between cured and noncured dendeng at 0 mo, 10 mo storage and after frying process (Table 2). Total mould and yeasts of cured dendeng decreased after frying process that were not different with noncured dendeng after frying. One of critical quality of dendeng is a presence of mould that could be seen by naked eye. Dendeng stored up to 10 mo did not show the mold that can be seen by naked eye. This indicated that the dendeng still qualified to consume.

TPC either of cured or noncured dendeng was not different at 0 mo, 10 mo storage but decreased after frying process ($p < 0.01$) (Table 2). TPC of cured dendeng was not different with noncured dendeng during storage and after frying. Based on Indonesian standard, TPC of dendeng was 1×10^5 cfu/g (BSN, 2013). Statistically dendeng stored for 0 mo and 10 mo were not different, but the dendeng stored for 10 mo has met Indonesian Standard. This indicated that lower moisture, aw, high spice of dendeng and frozen condition during storage could decrease TPC of dendeng. Frying process could reduced TPC due to heating that could eliminate most of microbes.

The number of *E. coli* on noncured dendeng at 10 mo storage significantly increase ($p < 0.05$) compared to its begining. Nevertheless frying process of noncured dendeng for 1.5 min could eliminate *E. coli* until not detected. The other way *E. coli* on cured dendeng has different fenomena with noncured dendeng (Table 2). The number of *E. coli* on cured dendeng at early storage (0 mo) satistically was not different either with dendeng at 10 mo storage or fried dendeng, but fried dendeng actually decrease up to under limit of *E. coli* for ready to eat food (Food Standard Australia New Zealand, 2001 Center for Food Safety, 2014).

Salmonella sp. either on cured or noncured dendeng at early storage (0 mo) until 10 mo storage and after frying process was not detected (Table 2). The absence of *Salmonella sp*. on all of dendeng samples were appropriate with SNI regulation (BSN, 2013), Hongkong guideline microbiology of ready to eat food (Center for Food safety, 2014), and in Australia (Food standard Australia New Zealand, 2001). This indicated that dendeng production in this experiment has fulfilled the hygiene procedure.

S. aureus number both of dendeng until 10 mo storage were not different that reached 1×10^3 cfu/g. Eventhough the reduction of *S. aureus* population after frying was not significant compared to raw dendeng, but it could decrease until $< 10^2$ cfu/g (Table 2). The number of *S. aureus* that did not reach satisfactory level (1×10^2 cfu/g) (Food Standard Australia New Zealand, 2001 BSN, 2013 Center for Food Safety, 2014) indicated that the hygiene of dendeng making process need to improve. Nevertheless frying process as preparation before consumption could reduce *S. aureus* population until satisfactory level (Food Standard Australia New Zealand, 2001 BSN, 2013 Center for Food Safety, 2014) thereforefried dendeng was safe to be consumed.

CONCLUSION

There were no differences of total molds/yeasts, total plate count, *Salmonella sp.* and *S.aureus* between noncured or cured dendeng with sodium nitrite solution during storage. Frying process before consumption at 150°C for 1.5 min resulted all of microbiology variables of dendeng below maximum limit to consume. It is concluded that dendeng stored at freezer temperature for 10 mo and fried shortly was safe for consumption.

ACKNOWLEDGEMENT

This research was funded by Ministry of Research, Technology and Higher Education, Indonesia through Penelitian Unggulan Perguruan Tinggi (PUPT) Program 2016.

KEYWORD : Beef Dendeng, Microbiology Quality, Dendeng Safety, Dendeng Storage, Fried Beef Dendeng

Table 1. Composition of dendeng ingredients

Ingredients	Percentage based on beef (%)	Ingredients for 1 kg beef (g)
Salt	2.5	25
Galangal	8.5	85
Coriander	2.0	20
Garlic	10.0	100
Brown sugar	16.5	165
White sugar	16.5	165
Tamarind	0.3	3
Pepper	0.3	3
Sodium nitrite (150 ppm)	0.015	0.15

Source: Suryati *et al*. (2014)

Table 2. Quality of dendeng microbiology treated by curing with sodium nitrite for 0 and 10 months storage as well as after frying

Variables/Curing Treatments	Dendeng Status		
	0 mo storage	10 mo Frozen Storage	Fried Dendeng
Total Molds/Yeasts			
Non-cured Dendeng	4.37 ± 0.96^{ab}	5.16 ± 0.94^{a}	4.13 ± 0.87^{ab}
Wet Cured Dendeng	5.18 ± 0.33^{a}	4.76 ± 0.43^{a}	2.44 ± 0.63^{b}
Total Plate Count			
Non-cured Dendeng	5.58 ± 0.22^{a}	4.94 ± 1.03^{ab}	3.44 ± 0.71^{bc}
Wet Cured Dendeng	6.08 ± 0.23^{a}	4.68 ± 1.09^{ab}	2.58 ± 0.64^{c}
E. coli			
Non-cured Dendeng	0.53 ± 0.93^{b}	2.68 ± 0.34^{a}	0 ± 0.00^{b}
Wet Cured Dendeng	2.58 ± 0.13^{a}	2.64 ± 0.11^{a}	1.17 ± 1.04^{ab}
Salmonella sp			
Non-cured Dendeng	0 ± 0	0 ± 0	0 ± 0
Wet Cured Dendeng	0 ± 0	0 ± 0	0 ± 0
S. aureus			
Non-cured Dendeng	3.39 ± 0.78	3.33 ± 1.14	2.28 ± 1.29
Wet Cured Dendeng	3.89 ± 0.53	3.24 ± 0.06	1.01 ± 1.76
Average	3.64 ± 0.66^{a}	3.28 ± 0.73^{ab}	1.65 ± 1.54^{b}

Note: Different superscripts at the same row and column for all variables, except *S. aureus* means significant different ($p < 0.05$). Different superscript at the same row at *S. aureus* variables means significant different ($p < 0.05$).

REFERENCES

Andrews, W.H. and Hammack, T.S. 2007. Salmonella. In: [BAM] Bacteriological Analytical Manual. 8th Ed. USA: Food and Drug Administration.

Bennett, R.W. and Lancette, G.A. 2001. *Staphylococcus aureus*. In: [BAM] Bacteriological Analytical Manual. 8th Ed. USA: Food and Drug Administration.

[BSN] Badan Standardisasi Nasional. 2013. Dendeng Sapi. Standar Nasional Indonesia 2908: 2013. Jakarta: Badan Standardisasi Nasional.

Center for Food Safety. 2014. Microbiological Guidelines for Food: For Ready-to-Eat Food in General and Spesific Food Items. Center for Food Safety, Food and Environmental Hygiene Department, Hongkong.

Feng, P., Weagant, S.D., and Grant, M.A. 2002. Enumeration of Escherichia coli and the Coliform Bacteria. In: [BAM] Bacteriological Analytical Manual. 8th Ed. USA: Food and Drug Administration.

Food Standard Australia New Zealand. 2001. Guidelines for The Microbiological Examination of Ready-to-Eat Food. Food Standard Australia New Zealand, New Zealand.

Honikel, K. O. 2008. The use and control of nitrate & nitrite for the processing of meat products. Meat Sci. 78: 68–76.

Maturin, L.J. and Peeler, J.T. 2001. Aerobic Plate Count. In: [BAM] Bacteriological Analytical Manual. 8th Ed. USA: Food and Drug Administration.

Suryati, T., Astawan, M., Lioe, H. N. and Wresdiyati, T. 2012. Curing ingredients, characteristics, total phenolic, and antioxidant activity of commercial Indonesian dried meat product (dendeng). Med. Pet. 35(2):111-116.

Suryati, T., Astawan, M., Lioe, H. N., Wresdiyati, T., and Usmiati, S. 2014. Nitrite residue and malonaldehyde reduction in *dendeng* – Indonesian dried meat – influenced by spices, curing methods and precooking preparation. Meat Sci., 96(3):1403-1408.

Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., and Bandler, R. 2001. Yeasts, Molds and Mycotoxins. In: [BAM] Bacteriological Analytical Manual. USA: Food and Drug Administration.

O-30-3

Improvement of intestinal health by probiotic fermented beef product

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INTRODUCTION

Functional foods market has grown rapidly in the past decade. A probiotic is a viable microbe which supplements or maintains a beneficial balance of the natural microorganisms present in the human gastrointestinal tract. *Lactobacillus plantarum* is one of probiotic lactic acid bacteria (FAO/WHO, 2001). *L. plantarum* IIA-2C12 is one of probiotic which was isolated from Indonesian local beef (Arief, 2011). It has been found that many bioactive peptides are also produced during the processing of meat like fermentation and hydrolysis so the generation of these compounds and subsequent enrichment in meat products can prove beneficial for human health (Arihara, 2006). In meat sector use of meat starter cultures has been done for improving the safety of meat rather than introducing functional or physiological qualities. The aim of the study was conducted to evaluate the functional properties of probiotic *L.plantarum* IIA-2C12 as starter culture for fermented meats.

METHODOLOGY

A total of 20 male rats Sprague Dawley were used in this study. They were devided into 2 groups control was administrated by casein as protein source and treatment was administrated by fermented meats using *L.plantarum* IIA-2C12. Data was collected and analyzed by ANOVA and tukey for further analysis.

Sampling and Making Preparations for Histological Intestine

Sampling intestine (jejunum and colon) in rats on day-20. Paraffin method and hematoxylin-eosin staining were used in the manufacture of histological preparations intestine jejunum and colon. Making the histological preparations included several stages of fixation, dehydration, clearing, embedding, sectioning, staining, and mounting. Bowel preparations were then made measurements and taking pictures by using a light microscope objective lens 10x and 20x. The parameters observed in this study were high villi in the small intestine (jejunum), thick mucous layer, muscular layer thickness, and diameter of the intestine (jejunum and colon) in the rat (Rattus norvegicus).

RESULT

The surface morphology of the small intestine villi very important role in absorbing the material feed, so that the morphology of the small intestine has the optimal structure to absorb nutrients. Fermented meat rations given in rats does not affect histological jejunum villi height (P> 0.05) (Table 1, Figure 1). This means that the process of absorption of nutrients by the villi of rats given meat fermentation run optimally, not unlike the rats given the control diet. Rats fed control diet tend to have higher villi than rats fed a ration of meat fermentation. It can be caused by different sources of protein used in the ration. Control diet using casein as a protein source which is the standard of pure protein that can be digested and absorbed optimally by the digestive system of the body (almost 100%). Nutrition of rations given flesh fermentation can be digested and absorbed by the villi can be caused by the role of *Lactobacillus plantarum* that help break down the meat protein into amino acids that facilitate the absorption of nutrients by the villi. *Lactobacillus plantarum* can act as probiotics by providing an enzyme capable of digesting crude fiber, protein, and fat (Gibson and Roberford, 1995). *Lactobacillus plantarum* can produce aminopeptidase. *Lactobacillus plantarum* used as starter cultures in the fermented sausage has proteolytic function. Rats fed control diet and diet with the use of fermented meat relative had villi which taper in the apical portion.

Rats fed by fermented meat in the rations had mucosal thickness that were not different from rats fed a control diet (Table 1). This indicated that the protein casein and protein from meat fermentation could be digested and absorbed easily by the villi to the growth of mucosal cells. Bolton (1969) reported that feeding was easy to digest, could cause mucosal cells become longer and the apical portion of villi shaped taper, whereas on feed containing crude fiber was higher (difficult to digest), the cells became shorter and parts apical villi became blunt.

Rats fed by fermented meat had colonic mucosa thicker than rats fed by control diet (Table 1, Figure 2). This is thought to occur reabsorption of water and mineral salts were high compared with the control diet to keep the

amount of fluid that comes out through the feces.
The histological features thick muscular layer of the jejunum and colon. Tunica muscularis in the intestine jejunum of rats by administration of fermented meat in the rations tend to be thicker than the control diet. It could be caused by the use of corn starch in different amounts in the rations. Control diet using corn starch in an amount more than the ration of meat fermentation. Corn starch is a material that is fermentable. Fermentable material can cause muscular atrophy. Therefore, the tunica muscularis jejunum and colon in rats fed by control diet tend to be thinner than rats fed by diet containing fermented meat.

Administration of fermented meats using *L. plantarum* IIA-2C12 could maintenance health of intestinal with thick villi, high mucosal and large diamatere of jejunum and colon and high population of lactice acid bacteria were colonized on jejunum and colon. The health benefits of probiotics are anchored in the presence of beneficial viable strains of microorganisms, which, when ingested in ample quantity, boost the health of the host. Probiotic food products which provide health benefits in addition to nutrition have captured a considerable percentage of functional food market. Based on this experiments, administration of fermented meats with *L. plantarum* IIA-2C12 could display probiotic effect on rats.

CONCLUSION

Administration to rats proved that probiotic fermented meat with *L. plantarum* IIA-2C12 as starter culture could maintain the health of rat's intestinal.

ACKNOWLEDGEMENTS

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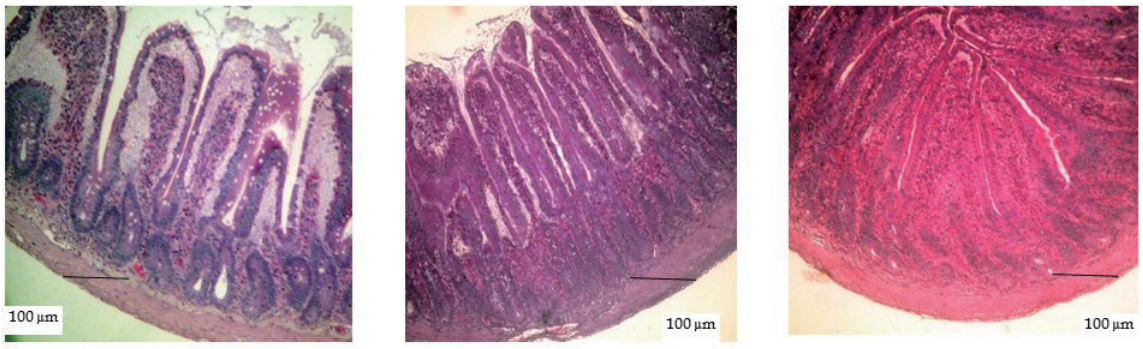
KEYWORD : fermented beef, probiotic, intestinal health

Table 1. Paramaters of jejunum and colon of rat fed by probiotic fermented sausage

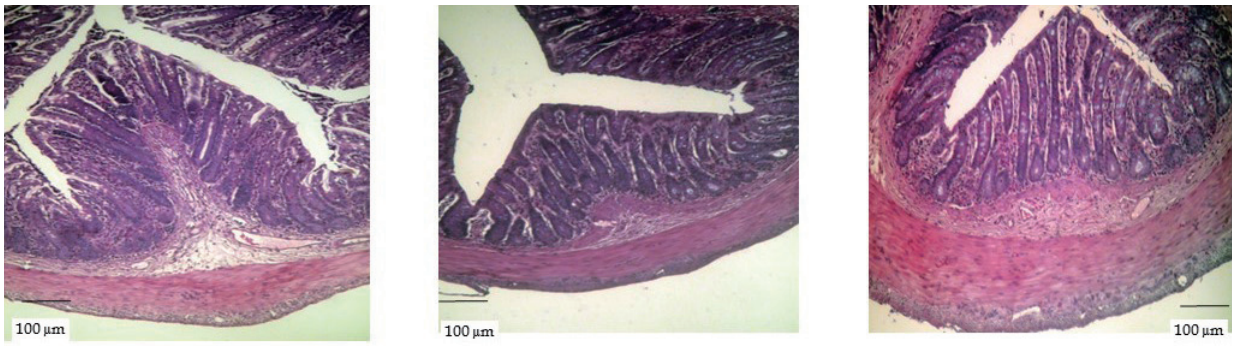
Unit (μm)	Before treatment	After treatment	
		Control (casein)	Fermented sausage
Villous height of jejunum	382.7± 63.6 [a]	377.24± 22.3 [a]	425.9± 43.3 [b]
Mucosal height of jejunum	579.2± 79.3 [a]	670.7± 50.8 [b]	673.5± 53.5 [b]
Mucosal tickness of jejunum	59.59± 6.80 [a]	53.77± 7.72 [a]	72.32± 5.12 [b]
Diamatere of jejunum	2569.5± 141.2 [a]	2879.0± 86.1 [b]	2907.0± 47.7 [b]
Mucosal height of colon	266.4± 34.7 [a]	277.7± 21.8 [a]	381.8± 55.2 [b]
Mucosal tickness of colon	181.7± 14.0 [a]	182.7± 38.4 [a]	257.1± 47.7 [b]
Diamatere of colon	2957.0± 270 [a]	2725.0± 258 [a]	3122.0± 204 [b]

The values followed by the different letters at same rows indicated those were significantly different ($p < 0.05$).



1.a 1.b 1.c

Figure 1. Histological structure of jejunum of rat : (a). Before treatment, (b) after treatment casein (control) and (c) after treaftment fermented meat with LAB population on mucus was 5.0 x 10^7 cfu/cm^2



2.a 2.b 2.c

Figure 2. Histological structure of colon of rat : (a). Before treatment, (b) after treatment casein (control) and (c) after treaftment fermented meat with LAB population on mucus was 8.0 x 10^8 cfu/cm^2

REFERENCES

Arief, I.I. 2011. Characterization of Indigenous Lactic Acid Bacteria from Beef as Probiotic and Identification by 16S rRNA gene sequencing. Ph.D. Dissertation. Bogor Agricultural University. Indonesia.

Arief, I.I., Z. Wulandari, E.L. Aditia, M. Baihaqi, Noraimah, and Hendrawan. 2014. Physicochemical and Microbiological Properties of Fermented Lamb Sausages using Probiotic *Lactobacillus plantarum* IIA-2C12 as Starter Culture. Procedia Environmental Sciences 20 : 352 – 356.

Arihara, K. (2006). Strategies for designing novel functional meat products. Meat Science, 74, 219–229.

Fadda, S., Y. Sanz, G. Vignolo, M. C. Aristoy, G. Oliver and F. Toldrá. 1999. Characterization of Muscle Sarcoplasmic and Myofibrillar Protein Hydrolysis Caused by *Lactobacillus plantarum*. Applied and Environmental Microbiology. 65: 3540-3546.

FAO/WHO. 2001. Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.

Gänzle, M. G., Hertel, C., Vossen, J. M., and Hammes, W. P. (1999). Effect of bacteriocin producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic model of the stomach and the small intestine. International Journal of Food Microbiology, 48(1), 21–35.

Gibson, G. R. dan M. B. Roberford. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of probiotics. J. Nutr. 125: 1401-1412.

Holzapfel, W. H., and Schillinger, U. (2000). Introduction to pre- and probiotics. Food Research International, 35, 109–116.

O-30-4

Difference of Gelatin Quality Goat Leather and Fur

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OBJECTIVE

The objectives of the present study were to prepare goat skin gelatin and fur gelatin from goat leatherPeranakanEttawah (PE) males aged 2 years and fur from New Zealand White rabbits male aged 4 months and to compare them at the protein content, ash content, water activity,carbohydrates, yield, viscosity, water content, pH and fat content. The hypothesis is that goat skin gelatin may have comparable properties to fur gelatin based on proximate analysis.

METHODOLOGY

Materials. The research material was goat leatherPeranakanEttawah (PE) males aged 2 years, fur from New Zealand White rabbits male aged 4 months and liming $Ca(OH)_2$10%.

Preparation of gelatin. Gelatin was prepared by the base extraction method (Ockerman and Hansen, 2000). The raw material (goat skin and fur) were washed and soaked in the liming (10%) for 24 hour. After soaked, samples were unhairing and degreasing with teepol 2% for 1 hour, then soaked in curing solution ($Ca(OH)_2$) 10% for 4 hour. After curing, samples were neutralized with HCl 5% for 6 hour to pH 7-8. Then washed, the extraction process with aquadest (1:2) were performed on three steps (temperature 55-60^{o}C 3 hour, 60-65^{o}C 3 hour, 65-75^{o}C 3 hour). Solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extraction gelatin were concentrated at 70^{o}C for 7 hour, then dried at 50^{o}C for 20hour until the gelatin sheet solid. Gelatin sheets were milledand packaged in vacuum plastic and store in a desicator for subsequent process.

Experimental design and data analysis. The research were determined by one way analysis of variance (ANOVA) using 7 replications. Differences between pairs of means were assessed on the basis of confidence intervals by using Least Significant Difference (LSD) test, followed by the t-test. The level of significance was considered at ($p<0.05$).

Parameter. The characteristics parameters of this research were protein content, ash content, water activity (A_w), carbohydrates,yield,viscosity, water content, pH and fat content value gelatin. The protein content, ash content, carbohydrates, water content, pH and fat content based on AOAC(1995), water activity (A_w) (Sudarmadji et al, 2007), yield (Gimenes et al, 2005), viscosity (Arnesen and Gildberg, 2002).

RESULTS

Gelatin has broad functions, for example in food products, pharmaceuticals, cosmetics and photography. Gelatin source can be derived from mammals (cow, ox bone, pork skin) or fish (Haug et al., 2004 Haug and Draget, 2009). Haug and Draget (2009) explains that there are two types of gelatin in terms of the manufacturing process, types A through the process of acid and type B through the base (alkali), which depends on the source of gelatin used. Type A to Type B gelatin from pigs and cattle for gelatin. This study uses a goatskin and rabbit skins as raw material for the manufacture of gelatin, and a solution of lime (Ca (OH) 2 as much as 10% as a curing material, then gelatin produced than quality.

The manufacturing process and different raw materials in the manufacture of gelatin (skin, bones, types of livestock, cattle age) will affect the quality and composition of the resulting gelatin, such as gel strength, viscosity, composition of amino acids and others. This is related to the protein content of collagen as the main source of making gelatin. According to Swatland (1984), increasing age of the animal protein collagen-growing and getting stronger collagen fibers. Gelatin is distinguished by the immersion process (curing) is carried out prior to the extraction process and depending on the source of gelatin used, are gelatin type A (acidic), especially of pork and gelatin type B (base), especially of cattle (Haug and Draget, 2009). The acid used egg hydrochloric acid, sulfuric acid, sulphurous acid and phosphoric acid, while the base used is a solution of lime (Ca (OH) 2) and NaOH (Imeson (1999) and Marchaban (1992). The process of making gelatin after soaking in a solution acid or base is neutralization, extraction and concentration (evaporation), drying, milling or crushed into smaller particles. According to Ward and Court (1977) de Wolf (2003), there are three main stages to the main process of making gelatin is under preparation raw material (removal of non-collagen components), phase conversion of collagen

into gelatin, which is the third stage of purification and recovery of dry gelatin.
The quality gelatin from leather goat and rabbit skin using a lime solution curing of 10% can be seen in Table 1.below:

The table show that the quality gelatin goat leatherhad some different variables but other variables did not differ and gelatin quality of the goat leather orfur were highly significant differences (t<1%) in the protein content, ash content, carbohydrates, different significant (t<5%) to yield, viscosity and water content but not significant (P >5%) on pH and fat content. The average gelatin of leather goats compared fur gelatin were protein levels (75.182% : 57.328%), ash content (1.166% : 3.171%), A_w (0.615 : 0.482), carbohydrates (17.486% : 31.960%), yield (13.436% : 12.686%), viscosity (4.429 cP : 5.429%), moisture content (8.381% : 7.281%), pH (2.417 : 2,633) and fat content (1.853% : 1.894%).
Gelatin contains high levels of protein (about 84 to 86%) because it is made from the basic ingredients of collagen which is a protein, low fat content (hardly any), the water content of about 8 to 12% and minerals about 2 to 4%. Gelatin contains ten essential amino acids the body needs, but does not contain the amino acid tryptophan (King et al., 2011). The protein content of the research ranged between (75 182%: 57 328%).
Factors that affect the characteristics, properties of gelatin and production processes gelatin, among others: the quality of raw materials (eg, species, race, age and type of feed from animals), storage conditions of raw materials, pH, the presence of organic substances, methods of extraction, temperature and the concentration of the curing (Parker, 1982 Ward and Court, 1977). Johnson-Bank (1990) Gilsenan and Ross-Murphy (2000) adds that the gel strength, viscosity and melting gelatin numbers depending on the source of raw materials and the molecular weight distribution of the amino acid composition of gelatin. Karim and Bhat (2008) adds that there are two kinds of functional properties of gelatin, the first relating to the process of forming a gel, such as gel strength, gelling time, the melting temperature, viscosity, viscosity, texture and water content. The second is related to the surface properties of gelatin, including the shape and stabilization of emulsions, protective colloid, shape and stability of the foam, as well as the shape of the film adhesion and cohesion.
Sompie et al (2015) said that concentration acetic acid solution (CH_3COOH 0.5M for 3%, 5% and 7% v/v) had significant effect on the yield, gel strength, viscosity on protein content of native chicken legs skin gelatin but had no significant effect on water content an pH value.

CONCLUSSION

Raw materials for gelatin from leather goat and rabbit skin using a lime solution 10% curing produce different qualities in terms of protein content, ash content, A_w and carbohydrates, yield, viscosity and water content but no significant in pH value and fat content.

KEYWORD : Gelatin, Goat Leather, Fur

Table 1. Differences in the quality of goat leather and fur using lime solution 10%.

Gelatin	Protein content (%)	Ash content (%)	A_w	Carbohydrates (%)	Yield (%)	Viscosity (cP)	Water content (%)	pH	Fat content (%)
Goat leather	75.182[a]	1.166 [a]	0.615 [a]	17.486 [a]	13.436 [a]	4.429 [a]	8.831 [a]	2.417	1.853
Fur	57.328[b]	3.171 [b]	0.482 [b]	31.960 [b]	12.686 [a b]	5.429 [a b]	7.281 [a b]	2.633	1.894

Description: highly significant (t> 0.01) in the protein content, ash content, A_w, carbohydrates, different significant (t> 0.05) on the yield, viscosity and water content but no significant (P <0.05) on pH and fat levels using a test t (t 0.01 = 3.06 and t 0.05 =2.18).

REFERENCES

AOAC. 1995. Official Methods of Analysis The Association of Officials Analysis Chemist, 14th ed. Assoc. Agric. Chemist, Washington, D.C.

Arnesen, J.A. and A. Gildberg. 2002. Preparation and Characterisation of gelatin from the skin of harp seal (*Phocagroendlandica*). J. Bioresource Tech. 82: 191-194

Gilsenan, P.M. and Ross-Murphy. 2000. Rheological of characterisation of gelatin from mammalian and piscine collagens. J. Rheol. 44(4):871-883.

Gimenez, B., M.C. Gomez-Guillen, and P. Montero. 2005. Storage of Dried fish skins on quality characteristics of extracted gelatin. J. Food. Hydro. 12: 958-963.

Haug, I.J, K.I. Draget, and O. Smidsrot. 2004. Physical and rheological properties of fish gelatincompared to mammalian gelatin. J. Food Hydro. 18:203-213.

Haug, I.J. and K.I. Draget. 2009. Gelatin, In: G.O. Philips and P.A. Williams, Ed., Handbook of Hydrocolloids Second Edition, Woodhead Publishing Limited. Cambridge. 142-163.

Immeson, A. 1999.Thickening and Gelling Agents for Food. Aspen Publisher Inc. New York.

Johnson-Banks, F.A. 1990. Gelatin: In P. Harris (Ed), Food Gels. Elsevier Applied Science. London. 233-289.

Karim, A.A and R. Bhat. 2008. Gelatin alternative for the food industry : recent development, chalanges and prospects. Trend in Food. Sci & Tech. Article in Press. Doi: 10. 1016/J.Food Hydrocolloid.

Marchaban. 1992. Gelatin. Inter-University Center for Food and Nutrition. GadjahMada University. Yogyakarta. Indonesia.

Ockerman, H.W., and C.L. Hansen. 2000. Animal By Products Processing and Utilization. CRC Press. USA.

Parker, A.L. 1982.Principles of Biochemistry.Worth Publishers Inc., Sparks, Maryland.

Raja. M.H.R.N., C.M. Yaakob, I. Amin, and A. Noorfaizan. 2011.Chemical and functional properties of bovine and porcine skin gelatin. J. Inter. Food Res. 18: 813-817.

Sompie, M., S.E. Surtijono and J.H.W. pontoh. 2015. The Effect of Acetic Acid Concentration and Curing Time on the Characteristic of Native Chicken Legs Skin gelatin. Proceeding The 6th International Seminar on Tropical Animal Production Integrated Approach in Developing Sustainable Tropical Animal Production. October 20-22, 2015, Yogyakarta, Indonesia.

Sudarmadji, S.B. Haryonoand Suhardi.2007. Analysis of Food and Agriculture. Liberty Second Edition. PAU Food and Nutrition. GadjahMada University. Yogyakarta. Indonesia.

Swatland, H.J. 1984.Structure and Development of Meat Animals. Prentice-Hall Inc, Englewood Cliffs, New Jersey.

Ward, A.G., and A. Court. 1977. The science of technology of gelatin. London: Academic Press inc. Ltd. London.

de Wolf, F.A. 2003. Collagen and gelatin. Progress in biotechnology, Elsevier Science B.V, Amsterdam, Netherlands, 133-218.

O-30-5

Preliminary study of ethanol extract of Phyllanthus acidus L. (Skeels) leaves as natural antioxidant in raw and cooked ground pork

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ABSTRACT

The objectives of this study were to evaluate the use of ethanol extract of *P. acidus* leaves as natural antioxidant in ground pork. The experiment was divided into two points. The first, total phenolic content and DPPH radical scavenging activity of ethanol extract of *P. acidus* leaves were determined. The second, application of *P. acidus* extract at the concentrations of 0.15%, 0.2% and 0.25 % (w/w) in pork were compared to non-treated control and butylated hydroxytoluene(BHT) on free radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and sensory evaluation. The result was expressed that total phenolic content of *P. acidus* extract was 4870.59 ± 2.07 mg GAE/100 g DW. In DPPH assay, IC_{50} value of *P. acidus* extract compared to ascorbic acid and BHT were 95.32 ± 1.22, 3.16 ± 0.03 and 21.82 ± 0.43 mg/l, respectively. In particular, the results of application of ethanol extract of *P. acidus* leaves in both raw and cooked pork showed that inhibiting properties of DPPH were significantly higher ($P < 0.05$) than the non-treated control and BHT. The sensory evaluation of pork burger revealed that at the 0.15%, 0.2 % and 0.25% concentrations of *P. acidus* extract had no different significance in overall appearance, color, odor and texture when compared to the control. The findings demonstrated strong potential for ethanol extract of *P. acidus* leaves as natural antioxidant in meat.

INTRODUCTION

Nowadays, the topic of functionally healthy food, especially ground pork, is increasingly interested because the consumers are increasingly aware of diet related to health problems and the safety of synthetic compounds is questioned over the past few years. This awareness has moved consumers to become more health-conscious, driving a trend towards healthy and nutritious foods with additional health promoting functions. Therefore, solutions of industrial meat must concentrate on improvement in the healthy meat to satisfy the consumer concerns. As for the use of antioxidants, a growing interest has been directed towards plant-based extracts as sources of natural phenolic antioxidants (Kahkonen et al., 1999). Shahidi and Zhong (2010) reported that most natural antioxidants are obtained from plant resources including culinary herbs, fruits, vegetables, and oilseed products. *Phyllanthus acidus* (L.) Skeels (Euphorbiaceae) belong to the Phyllanthaceae family. The *P. acidus* leaves are 2 to 7.5 cm long and thin, they are green and smooth on the upper side and blue-green on the underside. It is analgesic, antipyretic, antirheumatic, cures jaundice, small pox, itching, gum infection and as liver tonic and blood purifier. Many previous studies demonstrated that *P. acidus* leaves contain some important chemical constituents including kaempferol, hypogallic acid, gallic acid, quercetin, alkaloids, tannins, flavonoids, phenolics and terpenes (Sousa et al., 2007). Therefore, the objectives of this study were performed with a view to evaluate the traditional use of ethanol extract of *P. acidus* leaves as a natural antioxidant agent to improve the functional value in ground pork.

MATERIALS AND METHODS

Antioxidant activity of ethanol extract of P. acidus leaves

Dried *P. acidus* leaves were ground into small pieces and soaked in ethanol for 3 days. After filtering, the stock solution was concentrated by using a rotary evaporator and stored at 4°C. The total phenolic content was determined according to the method of Chumyam et al. (2013) with the results were calculated on the basis of the calibration curve of gallic acid standard and expressed as mg gallic acid equivalent per 100 gram dry weight (mg GAE/100g DW) of the plant material. DPPH free radical scavenging of ethanol extract of *P. acidus* leaves was performed by the method of Ebrahimzadah et al. (2008). The absorbance was measured at OD 517 nm and compared to ascorbic acid and BHT as reference standards. The results were expressed as IC_{50} values which required scavenging 50% inhibition of DPPH radical.

DPPH radical scavenging activity of raw and cooked pork treated with P. acidus extract

Ground pork contains 30% fat were assigned and homogenized with one of the 4 treatments including BHT

0.02% and 0.15%, 0.2%, 0.25% (w/w) of ethanol extract of *P. acidus* leaves. Cooked samples were prepared by boiling at 95°C for 20 min. No antioxidant agent used as control sample. DPPH radical scavenging activity of the supplements was estimated by the method of Qwele et al. (2013). The reaction mixture was measured at OD 517 nm. The scavenging activity of meat sample against DPPH radical was expressed as percentage activity for DPPH technique and calculated as follow:

Inhibition of DPPH (%) = [1 - (OD_{sample} - OD_{blank}) / $OD_{control}$] x 100.

Sensory evaluation

Twenty panelists were asked to evaluate the samples for overall appearance, color, odor, texture, flavor and overall quality of pork burger products that added the treatments as above described. The sensory evaluation was organized as soon as the samples were completed. A seven point hedonic scales (7 = extremely like 6 = very like 5 = like 4 = neither like nor dislike 3 = dislike 2 = very dislike 1 = extremely dislike) was used to score the samples (Meilgaard et al., 1987).

Statistic analysis

The statistical comparison among the groups were performed with one way analysis of variance test using SPSS program. The significance of the difference between means was determined at ($p<0.05$) significant level. All the assays were conducted in triplicate.

RESULTS AND DISCUSSIONS

Antioxidant activity of ethanol extract of P. acidus leaves

The total phenolic content of ethanol extract of *P. acidus* leaves was 4870.59 ± 2.07 mg GAE/100 g of DW and the IC_{50} values of DPPH radical scavenging activity of *P. acidus* extract, BHT and ascorbic acid were 95.32 ± 1.22, 21.82 ± 0.43 and 3.16 ± 0.03 mg/l, respectively (Table 1). The result was similar to the results of Jain and Singhai (2011) who reported that IC_{50} value of *P. acidus* extract was 69.60 ± 0.23 mg/l, while ascorbic acid was 21.80 ± 0.34 mg/l. Phenolic compounds are the major constituents of plants that contribute to their antioxidant capacity. Phenolic compounds included phenolic acids, flavonoids, and tocopherols (Wong et al., 1995). Several studies have demonstrated that phenolic compounds scavenge free radicals. Therefore, one of the most important factors affecting antioxidant activity is the total amount of phenolic compounds.

Table 1 Total phenolic content and DPPH free radical scavenging activity of *P. acidus* extract compare to ascorbic acid and BHT

Table 1. Total phenolic content and DPPH free radical scavenging activity of *P. acidus* extract compare to ascorbic acid and BHT

Extract	DPPH radical scavenging activity (IC_{50} mg/l)	Total phenolic content (mg GAE/100 g of DW)
Ethanol extract	95.32 ± 1.22[a]	4870.59 ± 2.07
BHT	21.82 ± 0.43[b]	
Ascorbic acid	3.16 ± 0.03[c]	

[a-c] Means sharing different letters in the same column are significantly different ($P < 0.05$)
All the values are mean ± SD; SD: standard deviation.

DPPH radical scavenging of ground pork treated with ethanol extract of P. acidus leaves

The inhibited percent of DPPH in ground pork was showed in Table 2. Overall, both raw and cooked samples containing *P. acidus* extract treatments had significantly higher radical scavenging activity than the control and BHT treatments. The inhibited abilities of DPPH increased following the increase of *P.acidus* extract concentrations. DPPH radical scavenging activity was quantified in terms of percentage inhibition of a pre-formed free radical by antioxidants in each sample. Interaction of antioxidants with DPPH, either the transfer of an electron or a hydrogen atom to DPPH, neutralizes its free radical character (Naik et al., 2003). In addition,

efficacies of antioxidants are often associated with their ability to scavenge stable free radicals. Therefore, the high DPPH scavenging ability of pork supplemented with *P. acidus* extract may be attributed to its high hydrogen donating ability causing the difference about phenolic content (Sreelatha and Padma, 2009).

Table 2 DPPH free radical scavenging activity of *P. acidus* extract in raw and cooked pork

Table 2. DPPH free radical scavenging activity of P. acidus extract in raw and cooked pork

Treatments	Inhibition of DPPH (%)	
	Raw meat	Cooked meat
Control	15.36 ± 0.19^{e}	15.99 ± 2.50^{d}
BHT 0.02%	48.71 ± 1.85^{c}	50.04 ± 4.65^{c}
P. acidus extract 0.15%	42.94 ± 2.57^{d}	50.30 ± 2.03^{c}
P. acidus extract 0.2%	56.09 ± 2.09^{b}	62.90 ± 4.73^{b}
P. acidus extract 0.25%	67.50 ± 1.64^{a}	69.02 ± 2.27^{a}

$^{a-e}$ Means sharing different letters in the same column are significantly different ($P < 0.05$)
All the values are mean ± SD; SD: standard deviation.

Sensory evaluation

Overall, although the addition of *P. acidus* extract showed change in flavor but these results suggested that they were considered acceptable by the panelist. Moreover, the *P. acidus* extract treated samples, the sensory evaluation of burger products showed that at the 0.15%, 0.2% and 0.25% concentrations of ethanol extract of *P. acidus* leaves had no different significance in overall appearance, color, odor and texture when compared to control (Table 3).

Table 3 Mean scores of sensory evaluation of burger products treated with *P. acidus* extract

Table 3. Mean scores of sensory evaluation of burger products treated with *P. acidus* extract

Treatments	Burger					
	Overall appearance	Color	Ordor	Texture	Flavor	Overall quality
Control	5.30 ± 1.30^{a}	5.05 ± 1.05^{a}	5.40 ± 1.19^{a}	5.20 ± 1.36^{a}	5.45 ± 0.23^{a}	5.35 ± 1.23^{a}
0.15%	4.90 ± 1.02^{a}	4.95 ± 1.28^{a}	4.90 ± 1.15^{a}	4.55 ± 1.70^{a}	3.95 ± 1.54^{b}	4.55 ± 1.36^{ab}
0.2%	4.65 ± 1.35^{a}	4.85 ±1.23^{a}	4.80 ± 1.28^{a}	4.45 ± 1.47^{a}	3.30 ± 1.53^{b}	4.00 ± 1.17^{b}
0.25%	4.75 ± 1.41^{a}	4.45 ± 1.61^{a}	4.50 ± 1.02^{a}	4.10 ± 1.71^{a}	2.95 ± 1.36^{b}	3.80 ± 1.36^{b}

$^{a-b}$ Means sharing different letters in the same column are significantly different ($P < 0.05$)
All the values are mean ± SD; SD: standard deviation.

CONCLUSIONS

Extracts from *Phyllanthus acidus* leaves are effective antioxidants and especially in inhibiting meat oxidation. Therefore, these findings of present study suggested that *P. acidus* extract have a potential source as natural antioxidant in meat. Additional studies should be conducted to determine the composition and structure of the active polyphenolic compounds of the extracts.

KEYWORD : DPPH, ethanol extract, Phyllanthus acidus leaves, ground pork

REFERENCES

Chumyama, A., K. Whangchai, J. Jungklang, B. Faiyue and K. Saengnil. 2013. Effects of heat treatment on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. Sci. Asia. 39: 246-251.

Ebrahimzadeh, M. A., N. Pourmorad and A. R. Bekhradnia. 2008. Iron chelating activity, phenol and flavonoid content of some medicinal plant from Iran. Afr. J. Biotechnol. 18: 3188-3192.

Jain, N. K and A. K. Singhai. 2011. Protective effects of *Phyllanthus acidus* (L.) Skeels leaf extracts onacetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pac. J. Trop. Med. 4: 470-474.

Kahkonen, M. P., A. I. Hopia, H. J. Vourela, J. P. Rauha, K. Pihlaja, T. S. Kujala and M. Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.

Meilgaard, M., G.V. Civille and B.T. Carr. 1987. Sensory evaluation techniques. Florida. CRC Press.

Naik, G. H., K. I. Priyadarsinia, J. G. Satavb, M. M. Banavalikarc, D. P. Sohonic, M. K. Biyanic and H. Mohana. 2003. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry. 63: 97-104.

Qwele, K.,A. Hugo, S. O. Oyedemi, B.Moyo,P. J. Masika and V. Muchenje. 2013. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. Meat Sci. 93: 455-462.

Shahidi, F and Y. Zhong. 2010. Novel antioxidants in food quality preservation and health promotion. Eur. J. Lipid Sci. Technol. 112: 930-940.

Sousa, M., J. Ousingsawat, R. Seitz, S. Puntheeranurak, A. Regalado and A. Schmdit. 2007. An extract from the medicinal plant *Phyllanthus acidus* and its isolated compounds induced airway chloride secretion: A potential treatment for cystic fibrosis. Mol. Pharm. 71: 366-376.

Sreelatha, S and P. R. Padma. 2009. Antioxidant activity and total phenolic of*Moringa oleifera*leaves in two stages of maturity. Plant Foods Hum. Nutr. 64: 303-311.

Wong, J. W., K. Hashimoto and T. Shibamoto. 1995. Antioxidant activities of rosemary and sage extracts and vitamin E in a model meat system. J. Agric. Food Chem. 43: 2707-2712.

O-30-7

Effect of Ractopamine Supplementation in Diets on Longissimus Muscle Characteristics of Finishing Pigs

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Introduction

Recently, consumers have a high demand for leaner pork with less fat. Thus, nutritional technology such as ß-adrenergic agonist has become important for improving carcass quality to meet consumer demand (Almeida et al., 2012). Ractopamine hydrochloride (RAC) is a ß-adrenergic agonist in the phenethanolamine class of chemicals (Patience et al., 2009), with structure similar to the catecholamines, epinephrine and norepinephrine. RAC is an exogenous substance that alters the animal metabolism and distribution of nutrients favoring muscle deposition in relation to fat deposition (Ferreira et al., 2013).

RAC has been used to improve feed efficiency and increase carcass lean meat yield (Xiong et al., 2006). According to the study of Carr et al. (2005a), RAC has been shown to increase carcass lean content and acts directly to nutrients from fat deposition toward protein deposition. However, the finding of Athayde et al. (2012) reported that supplementing swine diets with 10 mg/kg of RAC had negatively affected on tenderness of loin muscle. As well as, Patience et al. (2009) reported the addition of RAC at the level of 5 mg/kg diet slightly reduced tenderness of pork. Moreover, the study of Carr et al. (2005b) and Xiong et al. (2006) reported that dietary RAC supplemented increased toughness of pork.

RAC has been obtained regulatory approval as a growth promoting feed additive for swine in the United States, Canada and Brazil. However, RAC is not authorized for using as growth promotion in the European Union or China (Almeida et al., 2012) and also in Thailand due to the public concern about its residual effects. Regarding on meat quality, there are several studies have been shown the effect of RAC on pork quality in many countries. However, the information in Thailand is still limit. Therefore, the objective of this study was to evaluate the effect of RAC onLD muscle characteristics of finishing pigs.

Materials and methods

Animals and experimental diets

A total of 15 borrows and 15 gilts (Large White × Landrace × Duroc), average initial bodyweight 60 kg, were assigned to randomized completed block design (sex as blocks). The animals were divided into 3 groups control diet (without RAC), pigs fed with 20 ppm RAC, and pigs fed with 40 ppm RAC diets, with 5 borrows and 5 gilts in each group. All pigs were offered with commercial diet and drinking water *ad libitum*. Management and routine vaccination were administered.

Meat characteristic measurements

At approximately 100 kg bodyweight, all pigs were slaughtered and *Longissimus dorsi* (LD) muscle pH at 45 min and 24 hours post-mortem were measured directly at 10-11th thoracic vertebra of the carcass by pH meter (Metler-Toledo: SevenGo^{TM} pH meter SG2, China). After 24 hours chilling, LD muscle from each carcass was collected and cut into 1.0 cm thickness LD steak to determine drip loss percentage. Furthermore, 1¼ inches thickness LD steak were cut and kept in vacuum bag at -20˚C to determine thawing loss, cooking loss and Warner-Bratzler shear force (WBSF).

Statistical Analysis

All data were analyzed as a randomized complete block design using the General Linear Model (GLM) procedure. Diet groups were considered as main effect and sex was used as the block. Means of each diet group were compared by Duncan's multiple range test.

Results and Discussion

The result of this study was shown in Table 1. There were no significantly different (P>0.05) in pH 45 min, pH 24 hours post-mortem, drip loss, thawing loss and cooking loss percentage between diets groups. This was similar to the finding of Fernández-Dueñas et al. (2008), who reported that, the RAC did not affect ultimate pH and cooking loss of pork loin. Carr et al. (2005a) and Athayde et al. (2012) reported a lower drip loss percentage in the RAC fed pigs while Carr et al. (2005b) found no significant difference in drip loss between 10 ppm of RAC added and control. The results of this study showed that pigs fed with 20 ppm and 40 ppm supplementation in diets RAC had a higher (P<0.05) WBSF values than there fed control group. This result was in agreement with the study of Carr et al. (2005b), who reported a higher WBSF in RAC fed pigs than in control. Moreover, Xiong et al. (2006) reported that dietary RAC at 20 ppm supplemented in diet increased toughness of pork. In addition, they also found RAC-fed pigs showed a slower protein degradation rate than control pigs. The tougher meat in RAC fed pigs might be due to beta agonists promote muscle protein accretion by promoting the expression of calpastatin (the inhibitor of major post mortem protelytic enzyme, calpains) that limits the calpain-dependent protein turnover (Wheeler and Koohmaraie, 1992). After death, the suppression of calpains continued as the study of Koohmaraie et al. (1996) that reported lower calpain but higher calpastatin activity in beta agonists-fed lamb. The reduced proteolytic activity corresponded with a slower protein degradation.

Conclusion

Under the conditions of this current study, the supplementation of RAC in diets did not affect pH and water holding capacity but it affected meat tenderness as the increasing WBSF of pork LD muscle.

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KEYWORD : Ractopamine, Drip loss, Thawing loss, Cooking loss, Warner-Bratzler shear force

Table 1 Effect of RAC supplementation in diets on meat characteristics of LD muscle.

Traits	RAC supplementation in diets			SEM	*P-value*
	0 ppm	20 ppm	40 ppm		
pH 45 min	5.93	5.89	5.98	0.18	0.549
pH 24 hours	5.74	5.69	5.84	0.49	0.815
Drip loss (%)	5.99	5.42	4.51	1.58	0.128
Thawing loss (%)	9.39	9.29	7.91	1.87	0.160
Cooking loss (%)	21.89	23.64	21.69	2.39	0.156
WBSF (kg)	5.51^{b}	7.34^{a}	7.26^{a}	1.38	0.009

^{a,b} Different superscripts in the same row represent significant differences (P<0.05).

REFERENCES

Almeida, V.V., A.J.C. Nuñez and V.S. Miyada. 2012. Ractopamine as a metabolic modifier feed additive for finishing pigs: a review. Braz. Arch. Biol. Technol. 55(3): 445-456.

Athayde, N.B., O.A. Dalla Costa, R.O. Roca, A.L. Guidoni, C.B. Ludtke and G.J.M.M. Lima. 2012. Meat quality of swine supplemented with ractopamine under commercial conditions in Brazil. J. Anim. Sci. 90: 4604-4610.

Carr, S.N., D.J. Ivers, D.B. Anderson, D.J. Jones, D.H. Mowrey, M.B. England, J. Killefer, P.J. Rincker and F.K. McKeith.

2005a. The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. J. Anim. Sci. 83: 2886-2893.

Carr, S.N., P.J. Rincker, J. Killefer, D.H. Baker, M. Ellisand and F.K. McKeith. 2005b. Effects of different cereal grains and ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. J. Anim. Sci. 83: 223-230.

Koohmaraie, M., S.D. Shackelford and T.L. Wheeler. 1996. Effect of ß-adrenergic agonist (L644, 969) and male sex condition on muscle growth and meat quality of callipyge lamb. J. Anim. Sci. 74: 70-79.

Fernández-Dueñas, D.M., A.J. Myers, S.M. Scramlin, C.W. Parks, S.N. Carr, J. Killefer, and F.K. McKeith. 2008. Carcass, meat quality, and sensory characteristics of heavy body weight pigs fed ractopamine hydrochloride (Paylean). J. Anim. Sci. 83: 3544-3550.

Ferreira, A.S., G.M.O. Júnior, F.C.O. Silva, R.F.M. Oliveira and E.P. Silva. 2013. Ractopamine for pigs: a review about nutritional requirements. J. Basic Appl. Sci. 9: 276-285.

Patience, J.F., P. Shand, Z. Pietrasik, J. Merrill, G. Vessie, K.A. Ross and A.D. Beaulieu. 2009. The effect of ractopamine supplementation at 5 ppm of swine finishing diets on growth performance, carcass composition and ultimate pork quality. Can. J. Anim. Sci. 89: 53-66.

Wheeler, T.L. and M. Koohmaraie. 1992. Effect of ß-adrenergic agonist L644, 969 on muscle protein turnover, endogenous proteinase activities and meat tenderness in steers. J. Anim. Sci. 70: 3035-3043.

Xiong, Y.L., M.J. Gower, C. Li, C.A. Elmore, G.L. Cromwell and M.D. Lindemann. 2006. Effect of dietary ractopamine on tenderness and postmortem protein degradation of pork muscle. Meat Sci. 73: 600-604.

O-30-8

Effect of dipping in Longan seed extract phenolic solution on the shelf life and quality of pork under refrigerated storage

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1. Introduction

The shelf life of pork is very short due to spoilage by faster microbial growth and oxidative stress on meat. The nutritive value contains with high protein and moisture level supports microbial spoilage of meat, and also the presence of air induces oxidation of lipid as well as protein. Preventing microbial growth and retarding lipid and protein oxidation during storage and retail display is essential to maintain the quality and safety of meat. Synthetic preservatives are being currently used to curtail the microbial growth and thereby extending the shelf life of meat. Synthetic preservatives used in extending shelf life of meat are less preferred over bio-preservatives by the consumers. Bio-preservatives are mainly derived from plant extracts.

Longan seed extracts contain phenolic compounds as secondary metabolites. Biological significance of these compounds is immense due to enormous reducing power of free hydroxyl groups (antioxidant property) and protein binding capacity (causes inhibition of microbial growth-antimicrobial property). The antioxidant activity exhibited by phenolic compounds has been reported in fresh as well as in processed meat in terms of reduced lipid oxidation (Naveena, Sen, Vaithiyanathan, et al., 2008a Naveena, Sen, Kingsly, Singh, & Kondaiah, 2008b Naveena, Muthukumar, Sen, Babji, & Murthy, 2006 Vaithiyanathan et al., 2009). Similarly, phenolic compounds can also exhibit antioxidant activity by reducing protein oxidation. A significant reactive site can easily be oxidized by oxidizing agents generated during meat storage. Then the oxidation state is of great importance regarding biological activity (Batifoulier, Mercier, Gatellier, & Renerre, 2002). During refrigerated storage, oxidative stress has long been known to cause lipid oxidation.

Hence there is a possibility of using phenolic compound from edible plant parts as biopreservative in extending the shelf life of meat. Therefore the objective of this work is to study the effect of dipping pork in Longan seed extraction solution on the shelf life under refrigerated storage (4℃).

2. Materials and Methods

2.1. Meat sample

Fifteen commercial fattening pigs were obtained separately for each batch. Fifteen pigs were used for each replication. Fresh LD muscles were obtained from slaughtered by the commercial pig slaughter house with the standard of good manufacturing practice for abattoir of Thai agricultural and food standard. Swine processing unit and brought to the lab within half an hour for analyzed.

2.2. Preparation of Longan seed extraction

Dry longan seed obtained from the dry longan industry were washed and air dry. The longan seed were extract for the phenolic solution according the procedure of Vaithiyanathan et al. (2011). Briefly, the longan seed was mixed with 70% acetone (acetone: distill water 70:30 v/v) in the ratio of 1:5 and extracted by homogenizing for three times with 30 s bursts at 10,000 rpm and filtered through 4 layers of muslin cloth. The filtrate was placed in separating funnel and was extracted with equal amount of diethyl ether to remove lipids and other pigmets. The aqueous extract containing phenolics was collected in lower portion and the procedure was repeated 4-times. The acetone in aqueous phase was evaporated in vacco

2.3. Preparation of samples

Hot muscles were dipped (1:2 w/v muscle: liquid) for 60 s either in sterile distilled water or in sterile distilled water with 0.2% or 0.4% (v/v) longan seed extraction. The temperature of dipping solutions was maintained at room temperature (25 ℃). Fifteen samples in each treatment with 3 replicates were used in this experiment. After dipping, the samples were drained, wiped with sterile tissue paper and packed in low-density polyethylene pouches under aerobic conditions, before being kept in a household type refrigerator at 4 ℃ with digital display

of internal temperature. Lots were removed on 0, 4 and 8 days of storage and analyzed for pH, TBA reactive substances (TBARS), CIELAB color, drip loss, cooking loss, shear force at each storage interval.

2.4. Analysis of samples

Meat pH (pH meter model HI981400, Hanna Instruments) and meat color using colorimeter (Konica - Minolta, CR-400, Osaka, Japan) were determined in LD on day 0 day 4 and day 8 post mortem. The water holding capacity (WHC) was assessed via substance losses occurring during different procedures.

Cooking losses were determined using meat sample from LD (2.5 cm thickness, weight approximate 50 g). Meat samples were dried with soft tissue before weighing and put in polyethylene bags. Samples were sealed in heat-resistant plastic bags and boiled in water bath (80℃) until an internal temperature of 70℃. Cooking loss was evaluated by the different before and after cooking weight.

Drip loss determined using 1.5×1.5 inch^2 of LD samples dried with soft tissue before weighing the samples and put in polyethylene bags. The samples were place in cooling room at 4℃. Drip loss was evaluated by the different of weight loss before and after chilling. The boiled meat sample from cooking loss analyze, shear force were measured after cooling at room temperature. A steel hollow-core device with a diameter of 1.27 cm was punched parallel to the muscle fiber to obtain five pieces from each muscle sample. Measurement were using a Warner-Bratzler shear.

The TBARS value(mg of malonaldehyde/kg) of meat was determined by using the extraction method described by Witte, Krauze, and Bailey (1970) with slight modification, because slurry was centrifuged at $3,000 \times g$ for 10 min instead of filtration through Whatman filter paper No. 42 (Whatman International Ltd., Maidstone, UK).

2.5 Statistical analysis

All data were statistically analyzed as completely randomize design (CRD) using the General Linear Model (GLM) procedures of SAS (SAS Inst. Inc., Cary, NC). Significant differences between treatments were determined using Duncan's New Mutiple Range Test (DMRT) according Steel et al. (1997).

3. Results and Discussion

Results on the chemical nature of aqueous extract obtained from longan seed are presented in Table 1. The dipping with longan seed extraction were not had an effect on pH, Drip loss, Lightness (L), cooking loss and TBARS of pork ($P>0.05$). However, the dipping with longan seed extraction improved the redness color on day0 and day 4 of meat storage ($P<0.05$) and also yellowness on day 0 of meat storage ($P<0.05$). The pork dipping with longan seed extraction were showed the lower TBA reactive substances value in day 4 and day 8 of the experiment ($P<0.05$).

Gordon (1990) reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. Phenolics present in longan seed extraction may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and to terminate free radical chain reaction. The TBARS values of pork dipping with longan seed extract and without dipping in longan seed extraction were below the accepted sensory threshold limit (TBARS less than 1 mg/kg) according Ockerman (1976).

4. Conclusion

Pork dipped with longan seed extract phenolic solution could improving color appearance of meat by increasing the redness (a*) of meat on day 0 and day 4, whereas, yellowness (b*) were decrease on day 0. Moreover, pork dipped with longan seed extract could decrease thiobarbituric acid reactive substance (TBARS) under refrigerated storage at 4℃ on day 4 and day 8 after storage. Pork samples dipped with longan seed extract phenolic solution reduced lipid oxidation up to 8 days of refrigerated storage.

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KEYWORD : Longan seed, phenolics, shelf life, pork

Table 1 the pork quality characteristic treated with longan seed extraction after storage time.

Item	Storage time	T1	T2	T3	SEM	P-value
pH	Day 0	5.65	5.72	5.67	0.07	0.75
	Day 4	5.92	5.59	5.93	0.06	0.27
	Day 8	5.89	5.9	5.98	0.04	0.29
Drip loss (%)	Day 0	2.36	2.45	2.15	0.74	0.45
	Day 4	2.43	2.24	2.36	0.83	0.41
	Day 8	2.22	2.41	2.25	0.22	0.35
L	Day 0	56.25	56.31	55.11	0.63	0.38
	Day 4	54.98	56.31	56.35	1.47	0.76
	Day 8	57.07	56.43	53.99	1.15	0.22
a*	Day 0	19.30^{b}	19.88^{a}	19.73^{ab}	0.18	0.01
	Day 4	16.15^{b}	18.08^{a}	17.12^{ab}	0.38	0.03
	Day 8	16.44	16.88	16.09	0.42	0.46
b*	Day 0	0.50^{a}	0.03^{b}	0.32^{ab}	0.08	0.02
	Day 4	7.62	9.63	8.80	0.90	0.35
	Day 8	9.75	9.86	9.43	0.70	0.91
Cooking loss (%)	Day 0	29.23	25.8	24.73	2.15	0.52
	Day 4	27.05	27.84	24.33	1.25	0.37
	Day 8	25.94	24.91	24.96	1.04	0.82
WBSF (kg)	Day 0	3.40^{b}	4.79^{a}	3.39^{b}	0.17	0.00
	Day 4	3.72	3.45	3.36	0.43	0.80
	Day 8	3.16	3.23	3.36	0.5	0.95
TBARS (mg/kg)	Day 0	0.11	0.04	0.06	0.38	0.91
	Day 4	0.29^{a}	0.19^{b}	0.21^{b}	0.12	0.01
	Day 8	0.64^{a}	0.48^{b}	0.49^{b}	0.08	0.03

Mean values bearing different superscripts in a row differs significantly ($P<0.05$)

REFERENCES

Batifoulier, F., Mercier, Y., Gatellier, P., and Renerre, M. 2002. Influence of vitamin E on lipid and protein oxidation induced by H2O2-activated MetMb in microsomal membranes from turkey muscle. Meat Science, 61, 389 — 395.

Gordon, M. H. 1990. The mechanism of antioxidant action in vitro. In B. J. F. Hudson (Ed.), Food antioxidants (pp. 1 — 18). London: Elsevier.

Kumar, R., and Vaithiyanathan, S. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Animal Feed Science and Technology, 30, 21 — 38.

Ockerman, H. W. 1976. Quality control of post-mortem muscle and tissue. Colombus OH: The Department of Animal Science, The Ohio State University.

Naveena, B. M., Muthukumar, M., Sen, A. R., Babji, Y.and Murthy, T. R. K. 2006. Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. Meat Science, 74, 409 — 415.

Naveena, B. M., Sen, A. R., Vaithiyanathan, S., Babji, Y. and Kondaiah, N. 2008. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Science, 80, 1304 — 1308.

Naveena, B. M., Sen, A. R., Kingsly, R. P., Singh, D. B. and Kondaiah, N. 2008. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. International Journal of Food Science & Technology, 43, 1807 — 1812.

Pin-Der Duh. 1998. Antioxidant activity of Budrock (Arctium lappa L.): Its scavenging effect on free radical and active oxygen. Journal of American Oil Chemist Society, 75, 455 — 461

Steel, R. G. D. and J. h. Torrie. 1980. Principle and Procedure of statistics : MC Graw-Hill Book company, New poek. 631p.

Vaithiyanathan, S., Naveena, B. M., Muthukumar, M., Girish, P. S., Sen, A. R., Babji, and Y.,et al. (2009). Effect of methyl gallate on physico-chemical qualities of spent hen meat patties. Journal of Food Science and Technology, 46, 577 — 580.

Vaithiyanathan S., B.M. Naveena, M. Muthukumar, P.S. Girish, and N. Kondaiah.2011. Effect of dipping in pomegranate (Punica granatum) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 ℃). Meat Sci. 88: 409 - 414.

O-30-10

Myosin Heavy Chain Expression of Purebred and Crossbred Pig

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Abstract

The aims of this study were to examine and compare the myosin heavy chain (MyHC) isoform expression in purebred and crossbred pigs. The animals used were from 4 groups of 1) Duroc purebred (D), 2) three-way crossbreds [Duroc x (Large white x Land race form England)] (DLL_e), 3) three-way crossbreds [Duroc x (Large white x Land race form England and France)] (DLL_{ef}), and 4) two-way crossbred [Large white x Land race from England] (LL). There were 10 pigs in each group and slaughtered at the weight of 110 kg. The Longissimus dorsi (LD) muscle was taken to process for RNA and cDNA synthesis for measuring the myosin heavy chain (MyHC) isoform expression. The results revealed that there were not significantly different in the relative percentage of MyHC I and MyHC IIa ($p>0.05$). The expression of MyHC IIx from Duroc purebred pig was significantly higher than from two-way and three-way crossbred pigs. In contrast, the amount of MyHC IIb expression from two-way and three-way crossbred pigs were higher than from Duroc purebred.

Introduction

Genetics is one of the major factors that influences on animal development, growth and meat quality (Wimmer et al., 2008). Meat quality is commercially important and it can be manipulated to produce higher quality to meet particular market demands. Aspects of meat quality such as color, water-holding capacity, marbling, and texture depend basically on muscle fiber characteristics (Totland et al., 1988). Muscle fibers are classified into type I (slow oxidative), type IIa (fast-twitch oxidative glycolytic), and type IIb (fast-twitch glycolytic) by using conventionally histochemical techniques that are based on the ATPase activity of the fibers (Brooke and Kaiser, 1970). This*method howeveris time consuming* and requires large amounts of muscle sample. Therefore *alternative methods such*as electrophoresis (Picards et al., 2011) and q PCR (Wimmers et al., 2008) have also been used. These two alternative methods classify muscle fiber type based on the different myosin heavy chain isoforms. There are 4 adult isoforms (MyHC I, MyHC IIa, MyHC IIx, and MyHC IIb) that express in the skeletal muscle of many species, with each isoform originating from a different gene (Weiss and Leinwand, 1996). Wimmers et al (2008) reported the highly significant correlations between the corresponding MyHC isoforms evidenced the consistency of results between histological and quantitative real-time assays. In addition, quantitative real-time PCR is more accurate muscle typing due to it can classify muscle fibers into type I, IIa, IIx and IIb while histochemistry method can not distinguish type IIx and IIb fibers.

Regarding genetic factor has highly impacted on muscle fiber types thereby affects meat quality. Marbling is one of the most important traits in terms of meat quality. Duroc has high meat quality because it has high marbling. Therefore, the investigation of muscle fiber type in Duroc and their offsprings is required for more clear understanding about factors that might impact on meat quality. The aims of the current study were to examine and compere the expression of MyHC isoform in Duroc purebred and Duroc crossbred with real-time PCR technique.

Material and methods

Animals and Muscle Sampling

In this study, there were 4 group of purebred and crossbred pigs 1) Duroc purebred (D), 2) three-way crossbreds [Duroc x (Large white x Land race form England)] (DLL_e), 3) three-way crossbreds [Duroc x (Large white x Land race form England and France)] (DLL_{ef}), and 4) two-way crossbred [Large white x Land race from England] (LL). There were 10 pigs in each group and slaughtered at the weight of 110 kg. Fresh Longissimus dorsi (LD) muscle sample taken at the 13^{th} to 14^{th} ribs were snapped in liquid N_2 and then stored at -40 for RNA and cDNA synthesis at laboratory of the Department of Agricultural Education, Faculty of Industrial Education, King Mongkut's Institute of Technology Ladkrabang.

RNA Extraction and cDNA Synthesis

Total RNA was extracted using Trizol reagent according to the protocol of the manufacturer (Invitrogen, Paisley,

UK). Total RNA was treated with deoxyribonuclease, and then first-strand cDNA was generated from 0.5 ug. of total RNA by using random primers and Moloney murine leukemia virus reverse transcriptase in a 20 ul final volume as described by the manufacturer (Thermo Scientific, USA).

Primer used

The list of primers used to quantify myosin heavy chain (MyHC) isoform were shown in the Table 1.

Quantitative Real Time PCR

First-strand cDNA generated from each LD muscle was diluted 1:5, and from this, a pool of cDNA was generated for each sample and a dilution series made and used as a standard curve individual samples were further diluted 1:4 for analyzing gene expression. The reaction mixture consisted of 3.5 µl cDNA, 0.4 µl of forward and reverse primers, and 5µl SYBR Green Universal PCR Master mix (SensiFastTM SYBR, BIOLINE). Reactions were carried out in duplicate on a 96-well plate run on a Bio-Rad CFX96 system (Bio-Rad, USA): 95℃ for 2 min, and then 40 cycles of 95℃ for 5 s and 55℃ for 15 s fluorescence was detected in real time. Quantification cycle (Cq) values were calculated using Biorad-Rad CFX Manager 3.1 (Bio-Rad Laboratories).
Relative standard curves were generated for each MyHC isoform primer set to calculate PCR efficiency. The Cq values (y-axis) were plotted against $\log_{10}$ ng equivalent RNA (x-axis), and PCR reaction efficiencies (E) were calculated from the standard curve as $10^{(-1/\text{slope})}$-1. (Čikoš et al., 2007). An average Cq value was obtained for each primer set of each sample, and this was used to calculate the relative expression ratio (rER).

Because of the nature of the analysis, any one of the primer sets could be set MyHC control gene, with the expression of each other MyHC target gene being calculate relative to this. The rER of the MyHC control gene was set as a constant value of 1. The sum of rER values for the different primer sets was calculated and the relative contribution of each primer set rER was determined as a percentage. Data were expressed as the percentage of total adult MyHC expression (Hemmings et al., 2009).
For four group comparison of each MyHC isoform expression data was analyzed statistically using the Proc GLM offered in SAS software (SAS Institute Inc., Cary, NC, USA). The model used in this procedure includes animal group as a treatment factor. Least-square mean was separated by using PDIFF option ($P<0.05$).

Results and Discussion

The relative percentage of 4 MyHC isoform of different pig groups were shown in Table 2. The results showed that there were not significantly different in MyHC I, and MyHC IIa ($p>0.05$). The relative percentage of MyHC IIx from D was significantly higher while the relative percentage of MyHC IIb was significantly lower than from DLL_e, $DLL_{ef,}$ and LL_e ($P<0.01$).
In this study Duroc pig had lower MyHC IIb while had higher MyHC IIx than DLL_e, $DLL_{ef,}$ and LL_e, which may affect meat quality especially in terms of marbling. Normally, slow type I muscles contain more intramuscular fat than fast type IIb muscle. In beef, Hwang et al. (2010) reported a negative correlation between fat content and fiber number percentages of type IIa and IIb (r = -0.40 and -0.33, respectively). While in pig there was no relationship between intramuscular fat (IMF) and fiber type composition (Lefaucheur, 2010). However, Kim et al (2013) reported a positive relationship between the proportion and size of type IIB fibers and IMF content in porcine longissimus muscle. They also pointed out that pigs which contained high small and normal size of muscle fiber type IIb had higher IMF than those with larger muscle fiber type IIb.

Conclusion

Duroc purebred has different muscle fiber type from Duroc crossbred that might be affected meat quality. Therefore, the further meat quality study of Duroc purebred and crossbred are required to carry on to be able to improve meat quality by using this specific pig breed.

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KEYWORD : Purebred Pig, Crossbred Pig, Myosin Heavy Chain Isoform Expression

Table 1 List of primers used to quantify myosin heavy chain (MyHC) isoform

Gene	Primer sequence (5'to3')	Annealing temperature, ºC	GenBank accession no.
MyHC I	Forward: AAGGGCTTGAACGAGGAGTAGA Reverse: TTATTCTGCTTCCTCCAAAGGG	60	AB053226
MyHC IIa	Forward: GCTGAGCGAGCTGAAATCC Reverse: ACTGAGACACCAGAGCTTCT	60	AB025260
MyHC IIx	Forward: AGAAGATCAACTTGATGAACT Reverse: AGAGCAGAGAAACTAACGTG	60	AB025262
MyHC IIb	Forward: ATGAAGAGGAACCACATTA Reverse: TTATTGCCTCAGTAGCTTG	57	AB025261

Wimmers et al., 2008

Table 2 Least square mean (LSM) of relative percentage of myosin heavy chain isoform

Trait[1]	LSM[2]				SE	P-value
	D	DLL_e	DLL_{ef}	LL_e		
MyHC I	0.14	0.13	0.39	0.21	0.10	0.227
MyHC IIa	2.42	2.23	2.30	2.96	0.43	0.633
MyHC IIx	73.0^a	43.1^b	34.11^b	27.38^b	6.12	<.0001
MyHC IIb	24.45^b	54.54^a	63.2^a	69.46^a	6.21	<.0001

[1]MyHC = myosin heavy chain isoform

[2]D : Duroc purebred, DLL_e : Duroc x (Large white x Land race form England), DLL_f : Duroc x (Large white x Land race form England and France), and LL_e : (Large white x Land race from England)

REFERENCES

Brooke M.H., and Kaiser K.K. 1970. Three "myosin adenosine triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. Journal of Histochemistry and Cytochemistry 18, 670–672.

Čikoš, Š., Bukovská, A., and Koppel, J. 2007. "Relative quantificationof mRNA: Comparison of methods currently used for real-time PCR data analysis". BMC Molecular Biology. 8:113.

Hemmings, K. M., Parr, T., Daniel, Z. C., Picard, B., Buttery, P. J., and Brameld, J. M. 2009. "Examination of myosin heavy chain isoform expression in ovine skeletal muscles". Journal of Animal Science, 87(12), 3915-3922.

Hwang, Y.H., Kim, G.D., Jeong, J.Y., Hur, S.J., and Joo, S.T. 2010. "The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers". Meat Science, 86:456-461.

Kim, G.D., Jeong J.Y., Jung E.Y., Yang H.S., Lim H.T., and Joo S.T. 2013. "The influence of fiber size distribution of type IIB on carcass traits and meat quality in pigs". Meat Science, 94(2) : 267-273.

Lefaucheur L. 2010. A second look into fibre typing — Relation to meat quality. Meat Science, 84, 257–270.

Picard B., Barboiron B., Chadeyron D. and Jurie C. 2011. Protocol for high-resolution electrophoresis separation of myosin heavy chain isoforms in bovine skeletal muscle. Electrophoresis 32, 1804-1806.

Totland G.K., Kryvi H., and Slinde E. 1988. Composition of muscle fibre types and connective tissue in bovine M. semitendinosus and its relation to tenderness. Meat Science 23, 303–315.

Weiss, A., and Leinwand L. A. 1996. "The mammalian myosin heavy chain gene family". Annual Review of Cell and Developmental Biology. 12:417-439.

Wimmers K., N.T. Ngu, D.G. Jennen, D. Tesfaye, E. Murani, K. Schellander and S. Ponsuksili. 2008. "Relationship between myosin heavy chain isoform expression and muscling in several diverse pig breeds". Journal of Animal Science. 86(4) : 795-803.

O-31-1

Quality of Liquid Organic Fertilizer from Rabbit fs Urine with The Addition of Nitrifying Bacteria, Urea, and Leucaena leucocephala

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INTRODUCTION

Rabbit *(Oryctolagus cuniculus)* is herbivore which is very good in feed conversion (Lebas *et al.*, 1997). Since they barely drink water and only consume forage, rabbit's urine contains high levels of nitrogen. For a day, a rabbit can produce 100 ml of urine a bunny can produce 25 ml. Urine contains ammonia (NH_3)--a colorless gas which is lighter than air and possesses strong odor. Ammonia and other nitrogenous gases results from the digestion of protein, part of which is lost in manure and urine (Atia *et al.*, 2005). Solid and liquid animal waste is a type of waste that can be used as liquid organic fertilizer through a fermentation process (Salisbury and Ross, 1995). Additional treatment of microbial decomposers can intensively improve the form of organic fertilizer. *Pseudomonas* is a group of bacteria that is most important in denitrification (Tortora *et al.*, 2001). *Candida* is yeast that is commonly found in soil with high organic contents (Prasad, 2005). Urea is inorganic fertilizers, shaped as white crystalline solid, which is highly soluble in water and contents 46% of nitrogen (Boswell *et al.*, 1997). The aim of *Leucaena leucocephala* addition is to increase the nitrogen content in the organic fertilizer. Therefore, it can be used as nutrient growth for decomposers.

MATERIALS AND METHODS

Growth in liquid condition

The organisms, *Pseudomonas* sp. LS3K and *Candida* sp. LS3T, were grown in basal salt medium (g/L): meat extract, 0.1 biological peptone, 0.1 and NaCl, 0.05. The cultivation was performed at 28℃ at 120 rpm on a shaking incubator for 48 h. Every 3 h, 1 ml sample was read by spectrophotometer (600nm).

Organic fertilizer production

The fertilizer was made by fermented aerobic process. Each substance (microbial, urea, *L. Leucocephala*, and control) was used at 1% (v/v) concentration which is put into 500 ml of rabbit's urine. Fermented process was conducted for a week. Boric acid was also provided to determine ammonia concentration in liquid organic fertilizer.

Ammonia concentration

Ammonia was caught by boric acid which analyzed using Nessler method. Every day, 1 ml sample was read by spectrophotometer (425nm).

Physical, chemical and microbiology parameters

Physical parameters were tested based on physical condition of liquid organic fertilizer, such as pH, color, odor, volume, and temperature. Nitrogen, phosphor, organic carbon, and potassium were measured as chemical parameters of liquid organic fertilizer. Nitrogen phosphor organic carbon was measured by spectrophotometer method (636 889 591 nm). For microbiology parameters, the organisms were grown in basal salt medium (g/L): meat extract, 1.0 biological peptone, 1.0 NaCl, 0.5 and agar powder, 1.5. Isolated microbial (*Pseudomonas* sp. LS3K and *Candida* sp. LS3T) was grown by spread plate method. After 3 d incubation, isolated microbial was able to shape colonies.

RESULTS AND DISCUSSION

The ability of all strains in growing in liquid medium was shown in Fig. 1A it showed different profiles from *Pseudomonas* sp. LS3K and *Candida* sp. LS3T. Based on the Fig. 1A, *Pseudomonas* sp. LS3K directly grew into log phase, while lag phase of the growth of *Candida* sp. LS3T was occurred at 0 h until 3 h. Log phase of *Pseudomonas* sp. LS3K confirmed faster compared with the log phase of *Candida* sp. LS3T log phase of *Psedomonas sp.* LS3K

started from 0 h until 18 h and *Candida* sp. LS3T started from 3 h until 36 h. The differences in the growth profiles affected by genetic potential of microorganisms, medium, and the growth conditions (Prescott *et al*., 1999). In the stationary phase, microorganism only actives in metabolic activity, and the cell division is stopped (Prescott *et al.*, 1999 Tortora *et al*., 2001). After 48 h incubation, the color of liquid medium was changed from clear medium into a murky yellow medium.

The principle of Nessler method is reaction between Nessler reagent with ammonia in alkali condition that will form a murky brown colloidal dispersion. Based on Fig. 1B, the addition of microbial showed a lower pattern of liquid organic fertilizer it means that the microbial is the best treatment to reduce ammonium concentration in rabbit's urine compared with the others treatment and control. The data were then analyzed using completely randomized design of statistical analysis. Based on the results, different treatment given on liquid organic fertilizer affected the ammonia concentration (ppm). However, there was no an effect on ammonia concentration when *L. Leucocephala* was added. Ammonia concentration in the microbial medium was significantly different ($P<0.05$) with urea medium, meanwhile it was not significantly different with *L. leucocephala* medium and control. The average of ammonia released per day (ppm) by each treatment was 5936.5 (microbial) 7789.63 (control) 7972.5 (*L. leucocephala*) and 30529.02 (urea). It is because of 46% nitrogen contained within the urea that caused ammonia production was higher than other treatments. Ammonia used by microbial as an-organic nitrogen source to cell growth through enzymatic reaction (McCrory and Hobbs, 2001 Satoh et al., 2004).

Liquid organic fertilizer from rabbit's urine was fermented by aerobic process for 8 days. It must be examined for physical analysis every day. The result of physical analysis can be seen in Table 1. The explanation as follow: 1) The volume of liquid organic fertilizer was decreased because the oxygen supply of aerator caused evaporation liquid organic fertilizer with additional treatments has a different temperature and pH. 2) The temperature of liquid organic fertilizer with the addition of microbial and *L. leucocephala* was showed higher than control and urea. 3) The highest pH found in in the medium in which the microbial was added it reached 10.75. However, pH contained within the medium was the lowest when *Leucaena leucocephala* is added. Organic compounds degraded by microbial that will produce organic acids. Hence, pH will be decreased. In further step, the microbial will degrade the organic acids. Therefore, pH will be increased and make alkali conditions.

From the Table 1, we can see that the medium with the addition of microbial has no odor because microbes play an important role in both production and reduction of malodors (Zhu, 2000). Microbial treatments have been extensively used in municipal livestock waste to degrade organic matter (Low and Chase, 1999) and microbial treatments are emerging to treat livestock waste, since degradation of organic matter in livestock waste relies on microorganisms (Sund *et al.*, 2001). Some of bacteria have the ability to reduce nitrate in aerobic condition, such as genus *Paracoccus*, *Pseudomonas*, *Bacillus*, and *Alcaligenes* (Wu *et al.*, 2013). On the contrary, the medium with the addition of urea had odor because the organic compounds that contained in rabbit's urine were not degraded.

Besides the physical parameters, the liquid organic fertilizer also examined for chemical parameters. Based on the Fig. 2A, the nitrogen concentration of urea that contained within the medium had highest percentage (0.88%) it caused by the nitrogen within the urea was not degraded well and the nitrogen itself was solubility in rabbit's urine. On the other hand, the lowest nitrogen concentration is in control and microbial medium (0.05%). The result of statistical analysis showed that the nitrogen concentration in liquid organic compounds with the additional treatments was significantly different ($P<0.05$) with control medium. Organic carbon in liquid organic fertilizer of rabbit's urine with the additional treatment was shown in Fig. 2B. From the percentage, we can see that the addition of *Leucaena leucocephala* was the highest organic carbon (0.014%) contained within the medium. Conversely, urea contained within the medium was the lowest organic carbon (0.01%). Based on statistical analysis, organic carbon concentration in liquid organic compounds with the additional treatments was not significantly different with the control.

Based on the Fig. 2C, the medium with the addition of *L. leucocephala* showed the highest phosphor (0.025%) and the lowest phosphor concentration is the medium which contained urea (0.02%) it caused by urea that only contained nitrogen. The result of statistical analysis showed that phosphor concentration in liquid organic compounds with the additional treatments was not significantly different with control medium. Microorganisms used organic carbon as carbon source to produce energy, and when the microorganisms were died, the organic carbon will be released as carbon dioxide/CO_2 (Sholikah *et al.*, 2013). Potassium in liquid organic fertilizer from rabbit's urine with the additional treatment was shown in Fig. 2D. The highest potassium (0.82%) found in the medium that contained of microbial while the lowest potassium found in control medium (0.63%). Based on statistical analysis, potassium concentration in liquid organic compounds with additional treatments was

significantly different (P<0.05) with control. Hidayati *et al.* (2011) stated that potassium in substrate substances used by microbial as catalyst. Potassium was bonded and stored within cell by bacteria or fungi and when it was re-degraded, the potassium will be reappeared.

Microbiology parameter was analyzed by colony growth in solid medium. Isolated bacteria were grown and maintained by spread plate method. After 3 days incubation, the bacteria were able to shape, and then the colonies were count. The result of colonies computation can be seen in Table 2. The medium with the addition of microbial absolutely showed the best result which was 17.3 x 10^4 CFU/ml and medium that contained urea showed the worst result, 0.2 x 10^4 CFU/ml. It definitely caused by *Pseudomonas* sp. LS3K and *Candida* sp. LS3T contained within the liquid organic fertilizer. *Pseudomonas* sp. LS3K and *Candida* sp. LS3T can grow in the medium with ammonia concentrated. Jenie and Rahayu (1993) stated that microbial growth was influenced by multiple factors, such as the source of energy, protein, mineral, pH, and temperature.

CONCLUSIONS

Pseudomonas sp. LS3K can grow faster than *Candida* sp. LS3T. However, the growth of those bacteria was not significantly different. The best treatment to reduce ammonia was using microbial addition. Furthermore, the addition of microbial was significantly different in potassium concentration and microbiology parameter. Then, the addition of urea was significantly different in nitrogen concentration, but it was not significantly different in other parameters. It was unfortunate that the addition of *Leucaena leucocephala* was not significant in any parameters.

KEYWORD : Animal waste, Liquid organic fertilizer, Nitrifying bacterium, Urea, Leucaena leucocephala

Figure 1. Comparison of microbial growth in liquid medium

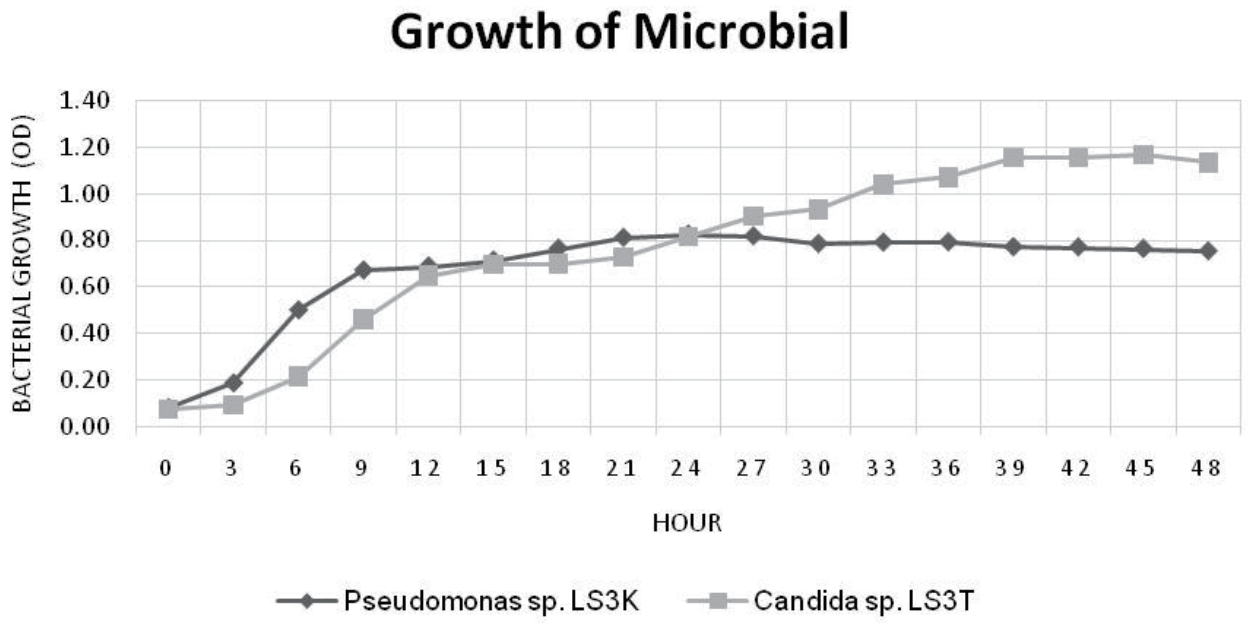

Figure 2. Ammonia concentration in liquid organic fertilizer

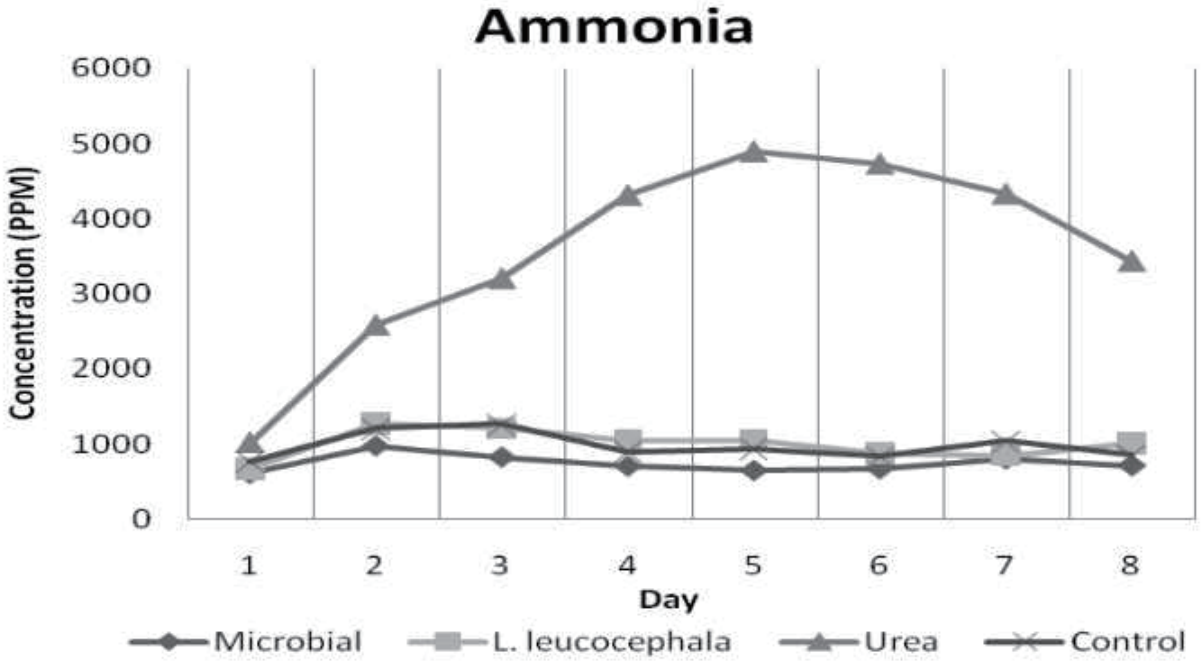

Figure 3. Profile of liquid organic fertilizer chemical analysis =
(A) Nitrogen, (B) Organic Carbon, (C) Phosphor, (D) Potassium

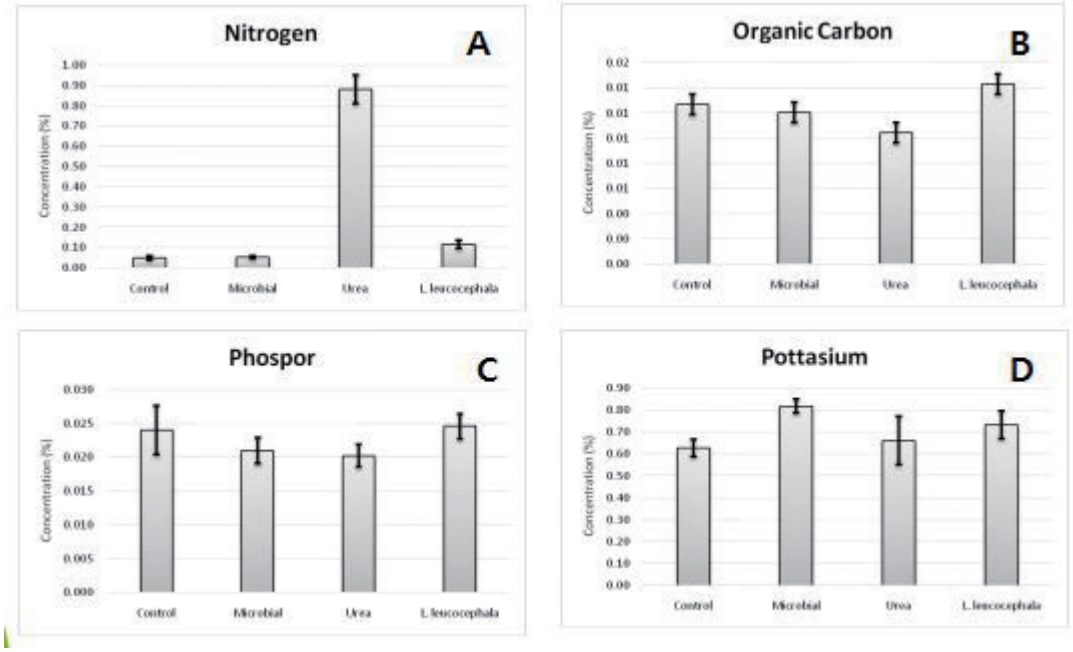

Table 1- Profile of liquid organic fertilizer physical analysis

Additional treatment	Rep.	Volume (ml)	Temp. (°C)	pH	Color	Odor
Microbial	1	300	29	10.75	Brownish black	No odor
	2	270	29	10.75	Brownish black	No odor
	3	250	29	10.75	Brownish black	No odor
Leucaena leucocephala	1	300	29	9	Black	Bit odor
	2	320	29	9	Black	Bit Odor
	3	290	29	9	Black	Bit Odor
Urea	1	400	29	9.25	Brownish black	Odor
	2	240	28	9.25	Brownish black	Odor
	3	310	28	9.25	Brownish black	Odor
Control	1	300	28	10	Black	Bit Odor
	2	340	28	10	Black	Bit Odor
	3	400	28	10	Black	Bit Odor

Table 2- Profile of liquid organic fertilizer microbiology analysis

Additional treatment	Colonies (CFU/ml)
Microbial	17.3×10^4
Leucaena leucocephala	2.6×10^4
Urea	0.2×10^4
Control	6.6×10^4

REFERENCES

Atia, A., K. Haugen-Kozyra, et al. (2005). "Ammonia and hydrogen sulfide emissions from livestock production." Manure research findings and technologies: From science to social issues [Online]. Available at wwwl. agric. gov. ab. ca/$ department/deptdocs. nsf/all/epw8313/$ FILE/chapter7. pdf (accessed 25 Apr: 226-272.

Boswell, F., J. Meisinger, et al. (1997). Produksi, Pemasaran dan Penggunaan Pupuk Nitrogen. Dalam Engelstad, OP 1985. Fertilizer Technology and Use (terjemahan DH Goenadi, 1997. Teknologi dan Penggunaan Pupuk). Edisi Ketiga, UGM Press, Yogyakarta.

Hidayati, Y. A., T. B. A. Kurnani, et al. (2011). "Kualitas Pupuk Cair Hasil Pengolahan Feses Sapi Potong Menggunakan Saccharomyces cereviceae (Liquid Fertilizer Quality Produced by Beef Cattle Feces Fermentation Using Saccharomyces cereviceae)." Jurnal Ilmu Ternak 11(2).

Jenie, B. S. L. and W. P. Rahayu (1993). Penanganan limbah industri pangan, Kanisius.

Lebas, F. and P. Coudert "de Rochambeau h., Thébault RG 1997. The rabbit husbandry, health and production." FAO Animal Production and Health Series(21).

Low, E. W. and H. A. Chase (1999). "Reducing production of excess biomass during wastewater treatment." Water research 33(5): 1119-1132.

McCrory, D. and P. Hobbs (2001). "Additives to reduce ammonia and odor emissions from livestock wastes." Journal of environmental quality 30(2): 345-355.

Prasad, G., S. Mayilraj, et al. (2005). "Candida digboiensis sp. nov., a novel anamorphic yeast species from an acidic tar sludge-contaminated oilfield." International journal of systematic and evolutionary microbiology 55(2): 967-972.

Prescott, J. F., J. Harley, et al. (1999). "Antimicrobial chemotherapy." Microbiology. 4th ed. McGraw-Hill, New York: 678-696.

Salisbury, F. and C. Ross (1995). Fisiologi tumbuhan. Jilid 3. Terjemahan DR Lukman dan Sumaryono, Penerbit ITB Press, Bandung.

Satoh, K., R. Takizawa, et al. (2004). "Two kinds of ammonia-oxidizing bacteria isolated from biologically deodorizing plants in cold district." Journal of bioscience and bioengineering 98(3): 207-210.

Sholikah, M. and W. Suyono "PR. 2013. Efektivitas kandungan unsur hara N pada pupuk kandang hasil fermentasi kotoran ayam terhadap pertumbuhan tanaman terung (Solanum melongena l)." UNESA Journal of Chemistry 2: 131-136.

Sund, J., C. Evenson, et al. (2001). "Nutrient conversions by photosynthetic bacteria in a concentrated animal feeding operation lagoon system." Journal of environmental quality 30(2): 648-655.

Tortora, G., B. Funke, et al. (2001). "Microbiology: An Introduction, Benjamin Cummings Publishing." San Francisco, USA: 88.

Wu, X., J. Xu, et al. (2013). "Determination of herbicide propisochlor in soil, water and rice by quick, easy, cheap, effective, rugged and safe (QuEChERS) method using by UPLC-ESI-MS/MS." Bulletin of the Korean Chemical Society 34(3): 917-921.

Zhu, J. (2000). "A review of microbiology in swine manure odor control." Agriculture, Ecosystems & Environment 78(2): 93-106.

O-31-3

ISOLATION OF INDIGENOUS DENITRIFYING BACTERIA FROM THE ODOROUS REGION OF AN LAYER FARM AND ITS POTENTIAL AS NITRATE REDUCING AGENT

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INTRODUCTION

Poultry production within the agricultural sector represents the high source of CH_4, NH_3, and CO_2 emissions and possibly have a huge potential for greenhouse gas (GHG) mitigation. Unpleasant odor is generally produced by the animal from the poultry farm industry, and may cause environmental problems such as ammonia emission in high concentration which can decrease the productivity of poultry and endanger of person health who is living in the farm or people community who is living nearby the poultry industries. Alteration of volatile ammonia into non-volatile form is a general biological treatment process including of nitrification and denitrification processes.

Some technical and management for mitigating of NH_3, CH_4 and CO_2 emissions from poultry operating systems have been suggested in the previous investigation (Borhan et al., 2011, Fredeen et al., 2013). Volatilization of ammonia is a critical issue due to it represents a loss of fertilizer value, and it can significantly impact the environment, becomes one of the pathways for N loss from poultry operations. Ammonia could be deposited from the atmosphere and may be beneficial to plants nutrient sources for growth, but when excess N is stored in N-sensitive ecosystems, this may impact adverse effect (Borhan et al., 2011).

High ammonia concentration may cause many environmental emission problems because of their odor, toxicity and contribution to acid rains (Zeng et al., 2012). Potential consequences regarding with high concentrations of both oxidized and reduced forms of N in the environment include: (1) vegetation or ecosystem changes due to higher concentrations of N (2) respiratory diseases caused by exposure to high ammonia (3) decreasing of water quality and eutrophication of surface water bodies resulting in harmful algal blooms (4) nitrate contamination of drinking water (5) soil acidification through nitrification and leaching (6) N saturation of forest soils and (7) climatic changes associated with increases in nitrous oxide (N_2O) (Ndegwa et al., 2008).

Several specific potential control strategies for NH_3 mitigation from poultry production facilities are include changing animal feed, renovating or redesigning barns and other facilities, cleaning the exhaust air, aerobic composting for treating manure as raw material, and improvement of fertilizer application to agriculture farming field. Among these treatments, biological additives using microorganisms has attracted big attention from people for the ease of utilization, faster action, and lower cost expenses (McCrory and Hobbs, 2001, Satoh et al., 2004). This ammonia removing treatment is based on the transformation of volatile N to non-volatile N comprising of nitrification and denitrification systems by nitrifying and denitrifying bacteria.

An important process involved in the Nitrogen cycle stated as Nitrification. In many ecosystems, ammonia-oxidizing bacteria (AOB) and archaea (AOA) have the ability to oxidize NH_3 to nitrite (NO_2). NO_2 is further oxidized to nitrate (NO_3) by nitrite oxidizing bacteria (NOB). Recently, the major grouping of AOB belongs to the subclass Beta-proteobacteria has been isolated and observed to be involved in nitrification system such as *Pseudomonas* spp. (Zhang Jibing et al., 2011), *Alcaligenes* faecalis (Joo et al., 2005), *Bacillus* methylotrophicus (Zhang Qing-Ling et al., 2012), Arthrobacter spp. (Verstraete and Alexander, 1972), and another group of bacteria such as *Nitrosomonas* spp., *Nitrosococcus* spp., *Nitrosospira* spp., *Nitrosovibrio* spp., *Nitrosolobus* spp. (Spieck et al., 2005). Besides bacteria and archaea, fungi are another kind of decomposer for which the role and development are not clear in the nitrification- denitrification system occurs in the composting process, the conventional biological to treat animal feces. It is believed that the composting self-heating pile may reach a temperature too extreme for their survival. They would thus be eliminated during the thermophilic stage and recovered when the temperature decreases (Zeng et al., 2012).

The objective of this paper is to isolate and to observe the capability of a microorganism reduce nitrate, as well as the development of nitrifying-denitrifying microorganisms, to reduce unpleasant odor from poultry farming activities.

MATERIALS AND METHODS

Media and Culture

The stock meat extract medium which consists of 1 g meat extract, 1 g microbiological peptone, and 0,5 g NaCl was made by diluted with 70 ml distilled water in beaker glass. The pH adjustment was into 7.2 with NaOH or H_2SO_4, and final volume was adjusted to 100 ml. The Stock of meat extract medium was always preserved in -20℃ . The Stock of 5% $NaNO_3$ was made by diluted of 5 g $NaNO_3$ in 100 ml ionic water and maintained in 4℃ . Furthermore, for screening ammonium-responsive microorganisms a 1/100 of stock meat extract medium with 1.5% agar added by 5% $NaNO_3$. Cultures were performed for 7 d at 30℃ . The liquid culture was carried out using 100 ml of 1/100 nutrient broth with 500 mgl^{-1} $NaNO_3$ in 250 ml-Erlenmeyer flasks and cells were grown aerobically at about 30℃ with a reciprocal shaker (120 rpm). Bacterial growth was monitored at Optical Density (OD) 600.

Screening of ammonium-responsive microorganisms

Indigenous isolates initially obtained from ammonia high emitted area of the poultry farm around Daerah Istimewa Yogyakarta. Nitrification-denitrification was suggested occur in this place. One gram samples of soil collected from various spots were suspended in 9 ml sterile distilled water and diluted appropriately. A portion of the cell suspension was spread on a 1/100 nutrient agar plate with 5% $NaNO_3$. Colonies appearing on the plate were picked and purified. Each purified colony was inoculated on an agar plate with and without 5% $NaNO_3$. Microorganisms displaying a peculiar growth on the agar with $NaNO_3$ were selected as ammonium-responsive microorganisms. Isolates were then purified by plating on 1/100 nutrient broth (0.01% yeast extract, 0.01% polypeptone, and 0.005% NaCl, pH 7.2) supplemented with 5% $NaNO_3$ and incubated at 30℃ for 48 h in aerobic condition.

Assessment of growth and ammonium reduction

To investigate the effect of $NaNO_3$ addition on growth and the ability of the strain in reduce nitrate shaking culture experiments were conducted. A portion of 100 ml 1/100 meat extract medium was made in 250 ml Erlenmeyer shaking flask, containing 5% $NaNO_3$. Periodically, 1 ml culture was prepared for measurement of cell density by spectrophotometer OD600, and another 1 ml culture was taken for nitrate analysis. NO_3 was analyzed by Brusin method.

RESULTS AND DISCUSSION

Results of isolation on nitrate reducing bacteria

As environmental conditions for the living of strain microorganisms in soil are usually nutritionally low, a portion of 1/100 nutrient agar was used for screening nitrate stressor-resistant strains. The selected media was added with 5% $NaNO_3$ used for screening "high nitrate stressor resistance-strain." This medium will screen the isolate which has not ability of a counter system with the stressor of $NaNO_3$ will not grow in this selected medium. We have isolated a soil bacterium, which located at the high odorous region of the poultry farming area. This nitrate resistant strain were screened at four different places from 2 poultry farms at around Yogyakarta City. One spot was chosen at the poultry farm of Surya Indah Maguwoharjo, and another spot was at Bangun Desa Kaliurang at Yogyakarta.

The strain which showed good growth in solid and liquid culture was submitted to this present study. The pictures of colony morphology of selected strain were described in Fig. 1.

One of the important steps in screening microorganisms from the environment is a method to purify the colonies in agar medium which contain specific substrate to obtain a single colony. Because in nature, the habitat of the strain microorganisms is living together with other strains and purification is obligatory steps in the screening of new isolate microbes. Fig. 1 shows us the colony of purified strains. As describe in Fig. 1, almost all the strain has white and light brown colors. Strain microorganisms employed in nitrification and denitrification system are usually has white or yellow color (Joo et al., 2005, Zhang Jibing et al., 2011, Zhang Qing-Ling et al., 2012). The purified strain was then preserved, and maintenance in slant agar medium containing 5% mgl^{-1} $NaNO_3$ and put in refrigerate temperature.

Table 1 shows the colony number, code of selected strains, some morphological characters including colony shape, color, colony edge, and cell shape of the strain isolated from various places at nitrate high contain areas. The largest number of the colony were obtained from the soil at the Bangun Desa Kaliurang Farm, which observed 3.2 x 10^5 CFU/mg. Furthermore, the colony number obtained from the soil around excreta dump at the same

farm was observed 1.8 x 10^5. From Surya Indah Maguwoharjo Layer Farm, soil and excreta sample were also investigated and resulted in the number of colonies 2.0 x 10^3 and 7.2 x 10^4, respectively. Moreover, colors of the strain colonies were observed dominant in white and light brown (Fig. 1). The ecology of denitrifying bacteria at deeper places was observed luck in some bacteria strains than at top soil location. The dominant bacteria employ for denitrification process are usually categorized as aerobic strain, this indicates that the denitrification system often occurs at the more atmospheric area.

Growth profiles in liquid medium

When the liquid medium was added by $NaNO_3$, there were additional nitrogen sources in the medium for growing. Four selected strains were given different response for $NaNO_3$ addition at the different concentration to the growth profiles (Fig. 2). Comparing to the control, the addition of 5% - 7.5% of $NaNO_3$ has improved the acceleration of exponential phase level of Strain TS1. Growing with the addition of 5% $NaNO_3$ showing the best growth profile of Strain TS1. However, the addition of 10% of $NaNO_3$ showed the same pattern with the control. Strain TS2, TB1 and TB2 have given a similar growth pattern in counter the high concentration of $NaNO_3$ addition. The addition of 10% $NaNO_3$ prolongs the adaptation phase of both three strains. The strains growth did not show the prohibition effect by the addition of 10% $NaNO_3$. In precise dose, it seems that additional nitrate will promote the growth of screened strains. Otherwise, for some strains, when the $NaNO_3$ added in very high concentration, it will poison the growth and alter the growth profiles.

Growth profiles in solid medium

Research in Fig. 3 was performed to know more details the ability of strains for growing on solid agar medium with supplementation of $NaNO_3$. In that figure, the comparison of colony diameter growing with and without $NaNO_3$ was compared. We tried to find the strain with 'nitrate stimulated growth' by the addition of $NaNO_3$ on agar medium. The results had shown that almost all of the strains have similar growth profile when growing on a solid agar medium with the addition of $NaNO_3$ at different concentration. To elucidate the ability of bacteria strains in counter 10% $NaNO_3$, research was continued by observing the colony diameter on agar medium of all strains after 5 d cultivation. Comparing with control, growing with 10% $NaNO_3$ was not affecting the colony diameter of all the strains.

Ability in decreasing nitrate concentration

Reduction of nitrate by individual strain in liquid medium was observed, and the percentage of nitrate reduction in detail was written in Table 2. All of the strain has the ability to denitrify nitrate at a different level. Strain TS1 reduced nitrate to 64.91% of 84.12 mgl^{-1} from initial $NaNO_3$ added in the medium. Strain TS2 oxidized 65.59% of 82.23 mgl^{-1} initial concentration in the medium and became the highest $NaNO_3$ reduction among the other strains. Reduction ability of Strain TB1 and TB2 was observed 65.01% and 63.00% from 84.16 and 81.04 mgl^{-1} initial $NaNO_3$, respectively. The culture was performed for 24 hours only. The reduction of ammonia by individual strain may continue by the prolonged of the cultivation period.

CONCLUSION

All the 4 selected strain isolated from the odorous region of the poultry farming area around Yogyakarta City can counter the stressor of $NaNO_3$ in solid and liquid medium. The strains also have able to reduce the $NaNO_3$ concentration during the cultivation period. Strain TS2 proved has the best ability in reducing $NaNO_3$ concentration in liquid medium comparing with the other isolated strain. Therefore, TS2 is a promising candidate for the extensive application on the various pollution control system especially odor reduction agent. Research dealing with a gas form of ammonia is under investigation.

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KEYWORD : bacteria isolation, denitrifying bacteria, manure, nitrate, odorous region

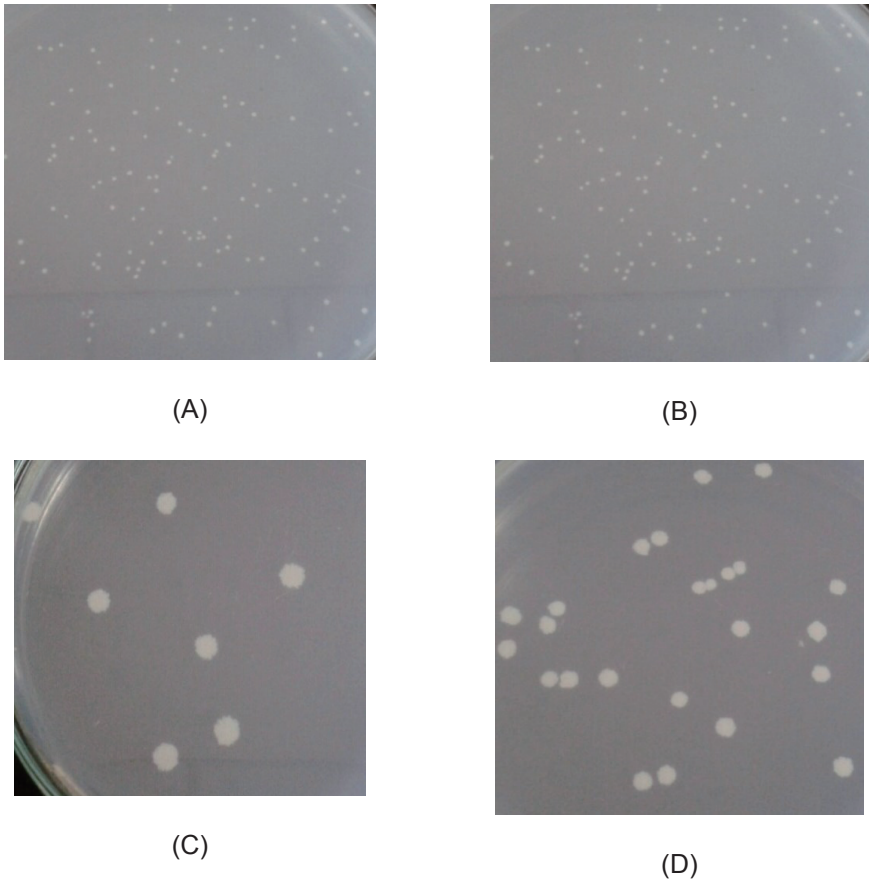

Fig. 1 Colony of selected strain. (A) Strain TS1from Surya Indah Maguwoharjo Farm; (B) Strain TS2 from Surya Indah Maguwoharjo Farm; (C) Strain TB1 from Bangun Desa Kaliurang; and (D) Strain TB2 from Bangun Desa Kaliurang

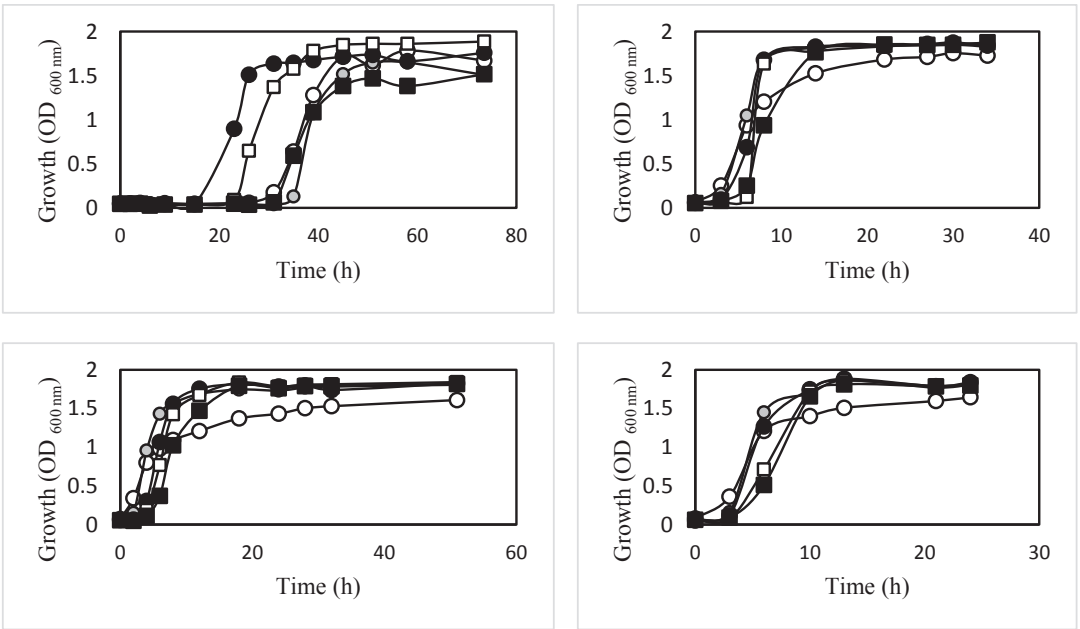

Fig. 2 Growth of isolated strains in nitrate supplemented medium at different concentration. (A) Strain TS1; (B) Strain TS2; (C) Strain TB1; (D) Strain TB2. All strains were cultivated in 1/100 dilution meat extract medium added by 0% (white circle); 2.5% (grey circle); 5% (black circle); 7.5% (white square); 10% (black square) of $NaNO_3$

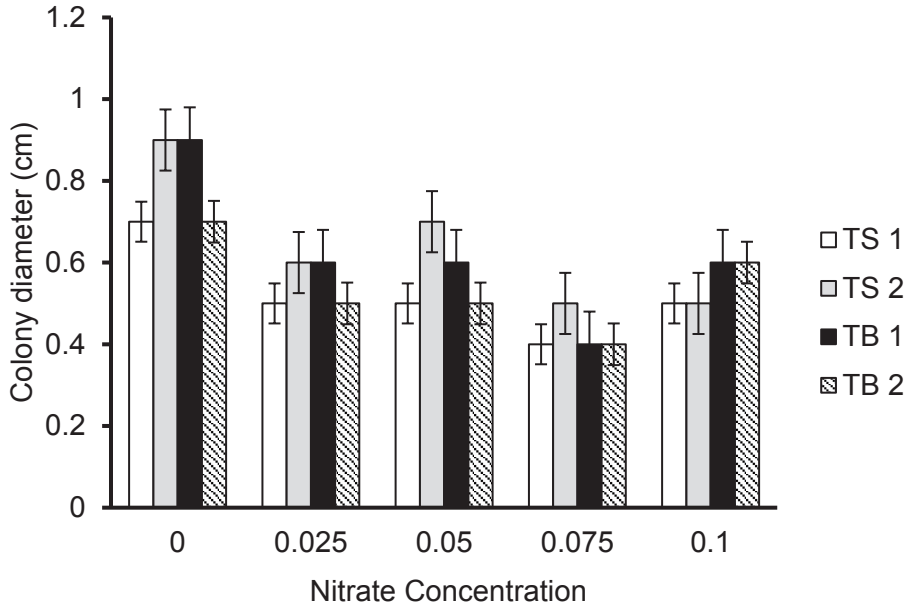

Fig 3. The growth of selected strain in solid medium with and without various NaNO3 concentrations after cultivated at 30ºC for 5 d. Medium was prepared by 1/100 dilution of meat extract stock with 1.5% agar.

Table 1. Description of selected strain (sampel location, colony number, selected strains, and morphology) appear on agar after suitable dilution from 1 g of soil sample taken from different places of dairy farming area around Yogyakarta City.

Entry	Sample Location	Colony Number	Obtained Strain	Morphology			
				Colony shape	Colony color	Colony edge	Cell shape
1	Soil around excreta dump at Surya Indah Maguwoharjo Layer Farm	2.0×10^3	TS1	Circle	White	Flat	Bacillus
2	Excreta at Surya Indah Maguwoharjo Layer Farm	7.2×10^4	TS2	Circle	White	Flat	Coccus
3	Excreta at Bangun Desa Kaliurang Farm	3.2×10^5	TB1	Circle	White	Wave	Coccus
4	Soil around excreta dump at Bangun Desa Kaliurang Farm	1.8×10^5	TB2	Circle	White	Wave	Coccus

Table 2. Nitrate decreasing ability by selected strains

Bacteria Strain	Nitrate Concentration (ppm) (hour)					Nitrate decreasing (%)
	0	4	8	12	24	
TS 1	84,12	87,39	86,95	30,71	29,52	64,91
TS 2	82,23	85,16	83,40	29,52	28,29	65,59
TB 1	84,16	83,75	86,12	32,68	29,45	65,01
TB 2	81,04	88,48	77,74	26,99	29,98	63,00

REFERENCES

Borhan M. S., S. Capareda, S. Mukhtar, W. B. Faulkner, R. McGee, C. B. Parnell, Jr. 2011. Determining seasonal greenhouse gas emissions from ground-level area sources in a dairy operation in central Texas. J Air Waste Manag Assoc. 61: 786-795.

Fredeen A., S. Juurlink, M. Main, T. Astatkie, R. C. Martin. 2013. Implications of dairy systems on enteric methane and postulated effects on total greenhouse gas emission. Animal. 7: 1875-1883.

Joo H. S., M. Hirai, M. Shoda. 2005. Nitrification and denitrification in high-strength ammonium by *Alcaligenes faecalis*. Biotechnology Letters. 27: 773-778.

Kariminiaae-Hamedaani H.-R., K. Kanda, F. Kato. 2004. Denitrification activity of the bacterium Pseudomonas sp. ASM-2-3 isolated from the Ariake Sea tideland. Journal of Bioscience and Bioengineering. 97: 39-44.

McCrory D. F., P. J. Hobbs. 2001. Additives to reduce ammonia and odor emissions from livestock wastes: a review. Journal of Environmental Quality. 30: 345-355.

Ndegwa P. M., A. N. Hristov, J. Arogo, R. E. Sheffield. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. Biosystems Engineering. 100: 453-469.

Satoh K., R. Takizawa, M. Sarai, N. Sato, R. Takahashi, T. Tokuyama. 2004. Two kinds of ammonia-oxidizing bacteria isolated from biologically deodorizing plants in cold district. Journal of Bioscience and Bioengineering. 98: 207-210.

Spieck E., E. Bock, H. P. Koops. 2005. Nitrifying bacteria, the lithoautotrophic ammonia-oxidizing bacteria, the lithoautotrophic nitriteoxidizing bacteria in Part A. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2, second ed., Springer.

Vacková L., M. Srb, R. Stloukal, J. Wanner. 2011. Comparison of denitrification at low temperature using encapsulated Paracoccus denitrificans, Pseudomonas fluorescens and mixed culture. Bioresource Technology. 102: 4661-4666.

Verstraete W., M. Alexander. 1972. Mechanism of nitrification by Arthrobacter sp. Journal of Bacteriology. 110: 962-967.

Yang X.-P., S.-M. Wang, D.-W. Zhang, L.-X. Zhou. 2011. Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying–denitrifying bacterium, Bacillus subtilis A1. Bioresource Technology. 102: 854-862.

Zeng Y., A. D. Guardia, C. Ziebal, F. J. D. Macedo, P. Dabert. 2012. Nitrification and microbiological evolution during aerobic treatment on municipal solid wastes. Bioresource Technology. 110: 144-152.

Zhang J., P. Wu, B. Hao, Z. Yu. 2011. Heterotrophic nitrification and aerobic denitrification by the bacterium *Pseudomonas stutzeri* YZN-001. Bioresource Technology. 102: 9866-9869.

Zhang Q.-L., Y. Liu, G.-M. Ai, L.-L. Miao, H.-Y. Zheng, Z.-P. Liu. 2012. The characteristics of novel heterotrophic nitrification-aerobic denitrification bacterium, Bacillus methylotrophicus strain L7. Bioresource Technology. 108: 35-44.

O-31-8

A Study on Fat Deposition from 1-6 Months of Age in Northeast Thailand Indigenous Pigs.

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Introduction

Thai indigenous pigs of Northeast Thailand, locally known as *Kadon* pigs, are small, short snout, small erected ears and black breed of pigs (Vasupen, 2007 Na-Lampang, 2012). Due to their low productivity, they have been considered unfit for modern pig production systems (Riethmuller and Chalermpao, 2002). But since they adapt well to the rural area environment and have resistance to some diseases (Serres, 1992), they can be an alternative source of income for many resource-poor smallholder farmers in rural areas of the Northeast (Rattanaronchart, 1994 Na-Lampang, 2007 Charoensook et al., 2013). The information on fat deposition of these pigs can be used to set the strategy for feeding management as to avoid the excessive deposition of fat in the pig carcasses. The present study was aimed to investigate fat deposition of Northeast Thailand indigenous pigs from 1 to 6 months old, the normal raising period.

Materials and Methods

Animals and housing

All procedures were authorized by Suranaree University of Technology Committee on Ethical Use of Animals for Research and Teaching. The present study was carried out in the Northeastern area of Thailand. A total of 60 Thai indigenous piglets reared under the environment and the feeding program of swine farm of Rajamangala University of Technology Isan (RUTI), Sakon Nakhon Campus, were randomly divided into 6 groups of 10 piglets/group (5 males and 5 females). Each group was randomly assigned to be slaughtered at 1, 2, 3, 4, 5, and 6 months of age. After weaning (at around 45 days of age), pigs (except the ones that were slaughtered at 1 months of age) were housed indoor under natural daylight condition. They were housed 5 per 4×6 square-meter pen. Each pen has a feed trough and a drinking water nozzle. They were fed 2 times per day (08:00 h and 16:00 h). The water was available ad lib.

Measurements

Body weight at slaughter of each pig was measured. All pigs were killed at RUTI slaughterhouse. Bleeding was done after a captive bolt stunning. Backfat thickness was measured at the tenth-rib and last-rib. Within 45 min postmortem, adipose samples for histochemical analysis were taken from the subcutaneous fat and perirenal fat, preserved in 10% formalin for at least 24 h and then dehydrated with alcohol. Alcohol was eliminated with xylene. Each sample was embedded in paraffin (Bancroft and Stevens, 1996) and was then cut in the microtome at 5 microns thick. The tissue was mounted on a microscope slide, stained with haemotoxylin and eosin (Humason, 1972). The stained samples were examined under a microscope and photographed to measure the fat area (an average of 25 fat cells). Fat contents of *M. longissimus*, *M. semitendinosus*, *M. rhomboideus* and *M. psoas major* were measured by Folch method (Folch et al., 1957) (using chloroform instead of hexane).

Statistical analyses

Statistical analyses were carried out by using SPSS (1998). The relationship between parameters and age groups were analyzed using non-linear regression procedure. The coefficient of determination (R^2) was used to choose the appropriate prediction equations (Linear, Quadratic, Cubic, Logarithmic, Inverse, Power, Compound, S-curve, Logistic, Growth and Exponential).

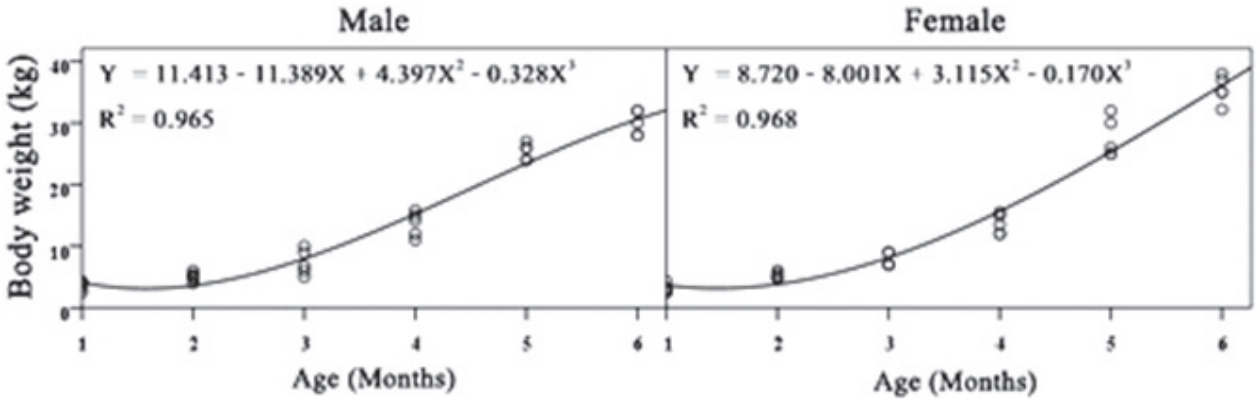
Results and discussion

Body weight

The weights of the male pigs at 1, 2, 3, 4, 5, and 6 months old were 3.58 ± 0.33, 5.04 ± 0.33, 7.36 ± 0.93, 13.60 ± 0.91, 25.40 ± 0.60 and 30.00 ± 0.89 kg., respectively. The weights of the females at the same ages were 3.14 ± 0.32, 5.22 ± 0.23, 7.80 ± 0.49, 13.60 ± 0.73, and 27.60 ± 1.44 and 35.44 ± 1.00 kg., respectively. The weights

of the male and female pigs in this study coincided with Na-Lampang (2005) and Vasupen (2007).
The cubic regression equation, with the highest R^2, is the best in predicting growth of the pigs (Figure 1). The growth curves of body weight show that during 1-3 months of age the body weight increased slowly, and after 3 months of age it increased rapidly until 6 months of age. The body weight curves found were similar to those of commercial pigs (Acker and Cunningham, 1991).

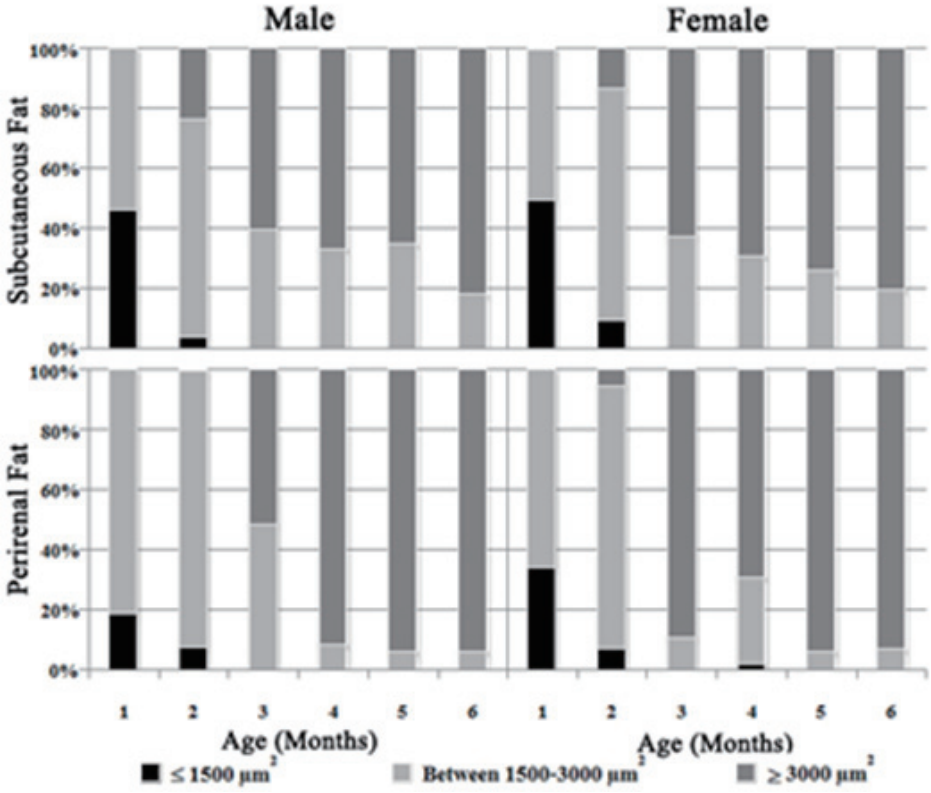
Figure 1 Body weight curves of the pigs.



Fat Deposition

The accumulation of fat in the cross-sectional area of subcutaneous fat and perirenal fat was first found when the pigs were 3 months old. From 3-6 months of age, the number of medium fat cells (1,500-3,000 μm^2) decreased while the number of large fat cells ($\geq$ 3,000 μm^2) increased (Figure 2).

Figure 2 Percentage of large, medium and small cross-sectional areas of subcutaneous fat adipocytes and perirenal fat adipocytes at different ages.



The cubic regression equation, with the highest R^2, is the best in predicting the intramuscular fat deposition and the thickness of the tenth-rib backfat and last-rib backfat (Figure 3-4).
The rate of intramuscular fat deposition of *M. longissimus*, *M. semitendinosus*, *M. rhomboideus* and *M. psoas major* were very slow during 1-4 months of age and then were very fast afterward. The thickness of the tenth-rib

backfat and last-rib backfat increased slowly during 1-3 months of age, and then increased at a higher rate from 3-6 months of age. The results are in the same study of Mersmann and brown (1973), Mersmann et al. (1975) and Mersmann et al. (1976).

Figure 3 Cubic curves for Intramuscular fat (%).

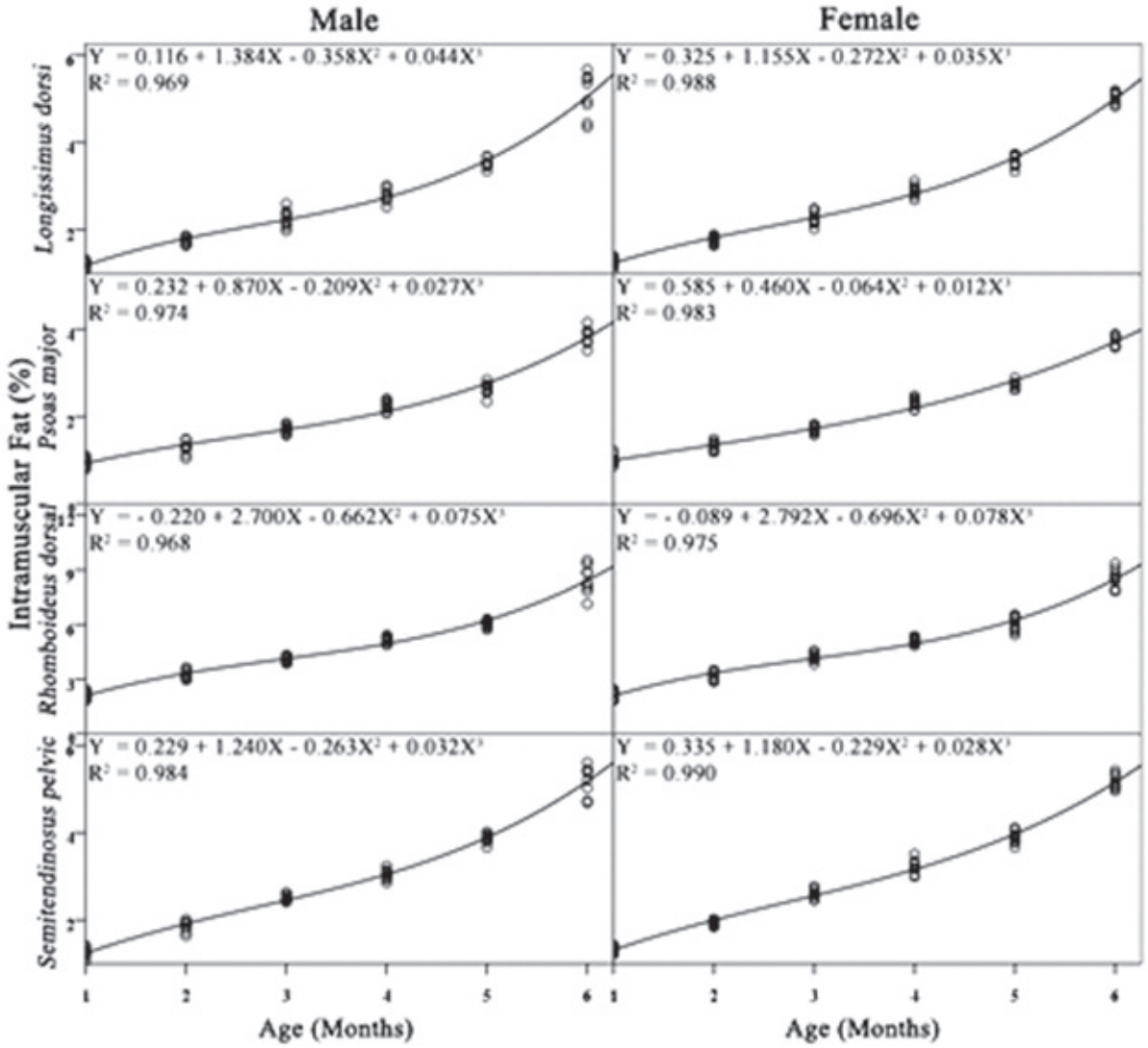
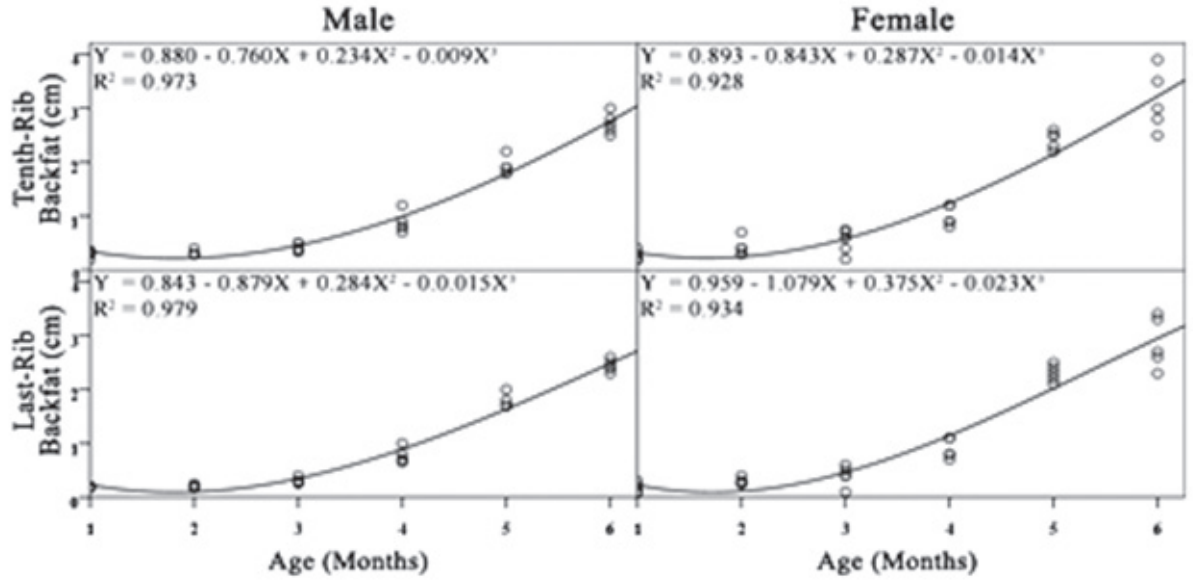


Figure 4 Cubic curves for tenth-rib backfat and last-rib backfat.



Conclusions

The cross-sectional areas of subcutaneous fat adipocytes and perirenal fat, the rate of intramuscular fat deposition, the thickness of the tenth-rib backfat, and last-rib backfat were found that the patterns of fat deposition of Northeast Thailand pigs from 1 to 6 months old were found in agreement with the commercial pigs.

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KEYWORD：Fat deposition, Indigenous pigs, Northeast Thailand

REFERENCES

Acker, D. and M. Cunningham. 1991. Animal Science and Industry. Prentice Hall Englewood Cliffs, New Jersey, Pages: 709.

Bancroft, J. D., and A. Stevens. 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill Livingstone, New York, USA., ISBN-13: 9780443047602, Pages: 766.

Charoensook, R., C. Knorr, B. Brenig, and K. Gatphayak. 2013. Thai pigs and cattle production, genetic diversity of livestock and strategies for preserving animal genetic resources. Maejo International Journal of Science and Technology 7 (1): 113-132.

Humason, G. L. 1972. Histology: Stains and staining (Microscopy): Histochemistry: Technique. W.H. Freeman, San Francisco, Pages: 569.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497-509.

Mersmann, H. J., and L. J. Brown. 1973. Adaptation of swine (*Sus domesticus*) adipose tissue to increased lipogenesis: expression of data. Znt. J. Biochem. 4: 503-510.

Mersminn, H. J., J. R. Goodman, and L. J. Brown. 1975. Development of swine adipose tissue: Morphology and chemical composition. J. Lipid Res. 16: 269-279.

Mersmann, H. J., L. J. Brown, R. D. Beuving, and M. C. Arakelian. 1976. Lipolytic activity of swine adipocytes. Am. J. Physiol. 230: 1439-1443.

Na-Lampang, P. 2005. A Study on Important Biological and Production Characteristics of Indigenous Pigs in the Northeast. Final Research Report, Suranaree University of Technology, Thailand.

Na-Lampang, P. 2007. Utilization of Thai Pigs in Sustainable Agricultural Systems in the Northeast of Thailand. Final Research Report, Suranaree University of Technology, Thailand.

Na-Lampang, P. 2012. A Study on Important Biological and Production Characteristics of Indigenous Pigs in the Northeast. Final Research Report, Suranaree University of Technology, Thailand.

Rattanaronchart, S. 1994. Present situation of Thai native pigs. Project Report, Department of Animal Science, Chiang Mai University, Thailand, Pages: 23.

Riethmuller, P. and N. Chalermpao. 2002. The Livestock Industries of Thailand. FAO Regional Office for Asia and the Pacific, Bangkok, ISBN 974-7946-25-4, Pages: 54.

Serres, H. 1992. Manual of Pig Production in Tropical Countries. Redwood Press, Melksham, London, pp: 57-67.

SPSS. 1998. SPSS for Windows. SPSS Inc., Chicargo, Illinoise, USA.

Vasupen, K. 2007. Nutritional studies in native, Thai Kadon pigs. Ph.D. Thesis, Utrecht University, Utrecht, The Netherlands.

O-32-2

Effect of fermented juice from fruit peels to use as an additive for improved quality of roughage

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INTRODUCTION

Crop and livestock production has been extensively practiced in Thailand's agricultural sector. Animal feed is commonly found to be lacking in dry season especially roughages. Farmers have been preserving forage in the form of hay or silage. Hay has lower feed intake than silage because it is unappetizing (Jinda et al., 1988). Forage preservation in silage form is kept in anaerobic condition. Silage is preserved in constant condition by the function of lactic acid bacteria and epiphytic lactic acid bacteria (Cai et al., 1994). Therefore, the making of silage has many different methods such as adding molasses, urea or fermented juice of epiphytic lactic acid bacteria (FJLB) in order to stimulate the growth of lactic acid bacteria in the fermentation process. Lactic acid bacteria in fermentation process can use to sugar in growth and lactic acid production. Jinda et al. (1988) found that fruit peels (pineapple peel, papaya peel and mango peel) have sugar which microorganisms can use for growth in the first phases of fermentation process. The pineapple peel contained 85% moisture, 8.1% fiber, pH value of 3.2 - 3.4 (Jinda et al., 1988) 15% carbohydrates and 12%total sugars. The papaya peel contained 75.13% moisture, 1.72% fiber, pH value of 4.23, 10% carbohydrates and 9.13% total sugars (Vikas, 2014). The mango peel contained 68.50% moisture, 5.40% fiber, 26.5% carbohydrates and 15% total sugars (Ajila et al., 2007). The objective of this study was to analyze the quality of fermented fruit peel of three types to see if they are suitable for improving silage.

MATERIALS AND METHODS

FJLB preparation

The FJLB was prepared using the ratio was 1 fruit peel to 2 distilled water (1:2) and the sample was blended and filtered. Then, FJLB was incubated for 0, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84 hours. The experiment was designed by completely randomized design (CRD) with three replicates and three treatments per treatment (T1: FJLB from pineapple peel, T2: FJLB from papaya peel and T3: FJLB from mango peel).

Determination of FJLB pH

Ferment juice was measured by pH meter at hours 0, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84.

Total lactic acid content of FJLB

The sample was 2 ml and 100 ml distilled water pour into Erlenmeyer flask and mixed. After that, three drops of phenolphthalein and titrated by 0.1 N NaOH. Then, the results were recorded and statistically analyzed using this formula (1) (AOAC, 1990):

Total sugar residual content of FJLB by total sugar content method

The sample, 1 ml FJLB with 3 ml sulfuric acid, was poured into a test tube then mixed by vortex mixer. Then, the sample was left at room temperature for 10-15 minutes. After that, the UV absorbance at 315 nm of the sample was measured by spectrophotometer (Ammar et al., 2013).

Microbial count on culture medium by pour plate method

This study was microbial growth on culture medium by pour plate technique. Fermented juice was serially diluted using 1 ml of sample with 9 ml of 0.85% sodium chloride. After that, the sample and culture medium were poured into a petri dish. Then, the sample was incubated at 35-37℃ for 24 hours. After that, the colonies in each culture medium were counted. Then, the results were recorded and statistically analyzed using this formula (2) (Kozaki et al., 1992):

Statistical analysis

Data was subjected to analyses of variance using proc. ANOVA. Treatment means were statistically compared using Duncan's New Multiple Range Test (SAS, 1998).

RESULTS AND DISCUSSION

Microbial count of FJLB

In this study lactic acid bacteria counts in all of the treatments were significantly different (P<0.05). The FJLB from the pineapple peel had more lactic acid bacteria than the other treatments at hour 30. Lactic acid bacteria counts were 9.22 LogN cfu/ml (Table 1). This is consistent with the study of Bureenok et al. (2006) which reported that after incubation fermented plant juice increased lactic acid bacteria more than non-fermented plant juice. Therefore, fermented plant juice is suitable to use as an additive in silage. The study of Weinberg et al. (1993) reported that the additives and lactic acid bacteria in the fermentation process can reduce fermentation time and improve silage quality.

In this study the aerobic bacteria counts in all treatments at hour 30 were not significantly different (P>0.05) (Table 1). Aerobic bacteria are found everywhere in nature and can be found on plants. This is consistent with the study of McDonald et al. (1991) which reported that the aerobic bacteria in the fermentation process is able to grow using oxygen and sugar, but aerobic bacteria in the fermentation process is unable to grow because oxygen was depleted. Consequently, aerobic bacteria did not increase but some types of microorganisms grow in acid.

In this study fungi and yeast counts of all treatments were significantly different (P<0.05). FJLB from the pineapple peel was less than the other treatments at hour 30. Fungi and yeast was 11.66 LogN cfu/ml (Table 1). This is consistent with the study of Sayan (1997) which reported fungi and yeast count increases under aerobic fermentation until oxygen is depleted. After oxygen depletion, fungi and yeast do not increase. The study of Apinya (2003) reported that some microorganisms can use acid for growth such as yeast and bacteria.

Total lactic acid content

The study found that total lactic acid content of all treatments were significantly different (P<0.05). The FJLB from the pineapple peel was greater than the other treatments at hour 36. Total lactic acid content was 0.67 % (Table 1). This result showed that lactic acid content increases following lactic acid bacteria growth. This consistent with the study of McDonald et al. (1991) which reported that lactic acid bacteria can digest sugar faster and improve quality in fermentation process (anaerobic condition). Therefore, the number of lactic acid bacteria increased.

The pH value

The study found that pH value of all treatments were statistically different (P<0.05). The pH (3.26) from the FJLB from the pineapple peel was less than the other treatments at hour 30 (Table 1). The pH value decreased following an increase in lactic acid content. The study of Nishino and Ushida (1999) reported that lactic acid content increases as pH value decreases because increasing growth of lactic acid bacteria causes an increase in lactic acid. The study of Arnat (2006) reported that pH value is related to type and number of microorganisms. Lactic acid bacteria produce lactic acid and reduce fermentation time.

Total sugar residual content

The study found that total sugar residual content of all treatments was significantly different (P<0.05). The FJLB from the pineapple peel was less than the other treatments at hour 30. Total sugar residual content was 2.96 mg/ml (Table 1). Because microorganism use sugar in the fermentation for growth, the total sugar content gradually decreased. The study of Somying et al. (2007) reported that sugar is as an energy source for growth of lactic acid bacteria. The study of Apinya (2003) reported that total sugar content decreases during fermentation because microorganism use it for growth.

Conclusion

The study fermented juice from the pineapple peel at hour 30 is suitable because:

- FJLB from pineapple peel can increase lactic acid bacteria and total lactic acid content more than the other treatments.
- FJLB from pineapple peel can decrease pH value and total sugar content more than the other treatments.
- FJLB from pineapple peel had less fungi and yeast than the other treatments.
- FJLB from pineapple peel had more aerobic bacteria than the other treatments but was not significantly different.

Therefore, this study showed that FJLB from pineapple peel at hour 30 was suitable for improving silage.

KEYWORD：FJLB, Lactic acid bacteria, Pineapple peel, Papaya peel, Mango peel

Formula (1)

$$\text{Total lactic acid content (\%)} = \frac{(N \times V_1 \times MW \times 100)}{(V_2 \times 1{,}000)}$$

When N = concentration of the standard solution sodium hydroxide (normal)

V_1 = volume of standard solution sodium hydroxide used in titration (ml)

V_2 = amount of substance sample (ml/g)

MW = molecular weight of lactic acid (90.08)

Formula (2)

$$\frac{\text{CFU}}{\text{ml}} = \frac{\left(\frac{\text{Number of Colony}}{\text{Quantity Plated}} \times \text{Dilution Factor}\right)}{(\text{ml of Sample})}$$

Table 1 Quality of fermented juice from fruit peels at log phase (30 hour)

Parameter	T1	T2	T3	SEM
Microbial count				
- Lactic acid bacteria count (Log N cfu/ml)	9.22^{a}	8.78^{b}	8.50^{c}	0.02
- Aerobic bacteria count (Log N cfu/ml)	11.60^{NS}	11.47^{NS}	11.47^{NS}	0.03
- Fungi and yeast count (Log N cfu/ml)	11.66^{b}	11.68^{b}	11.82^{a}	0.01
Total lactic acid content (%)	0.67^{a}	0.56^{b}	0.45^{c}	0.01
The pH value	3.26^{b}	3.29^{b}	4.28^{a}	0.01
Total sugar residual content (mg/ml)	2.96^{c}	4.53^{b}	26.85^{a}	0.23

^{a,b,c} Means with different superscripts differ significantly (P<0.05)

^{NS} Means with not different superscripts differ significantly (P<0.05)

SEM = Standard error of the mean

T1 = FJLB from pineapple peel, T2 = FJLB from papaya peel, T3 = FJLB from mango peel

REFERENCES

Ajila, C.M., S.G. Bhat and U.J.S.P. Rao. 2007. Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem. 102:1006-1011.

Ammar, A., A. A. Albalasmeh, and A. G. Teamrat. 2013. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydrate Polymers 97:253-261.

AOAC. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Apinya Sangsuwan. 2003. Fertilizer production from organic waste. Ph.D. Thesis. University of Kasetsart, Bangkok, Thailand.

Arnat Tanshou. 2006. Natural Agriculture Applications 2006. In: Proceedings of the Science National Technology, Pathum Thani, Thailand. pp. 300.

Bureenok, S., T. Namihira, S. Mizumachi, Y. Kawamoto and T. Nakada. 2006. The effect of epiphytic lactic acid bacteria with or without different byproduct from defatted rice bran and green tea waste on napiergrass (*Pennisetum purpureum Schumach*) silage. J. Sci. Food Agric. 86:1073-1077.

Cai, Y., S. Ohmomo and S. Kumai. 1994. Distribution and lactate fermentation characteristics of lactic acid bacteria on forage crops and grasses. J. Japan Grassl. Sci. 39:420-428.

Jinda, S., N. Ayudhya, J. Khemsawad, P. Pratchayaluk and C. Maneedun. 1988. The use of agricultural byproducts cattle. Thailand. pp. 272-284.

Kozaki, M., T. Uchimura and S. Okada. 1992. Experimental Manual of Lactic acid bacteria. Asakurashoten, Tokyo, Japan (Jpn). pp. 6-16.

McDonald, P., A.R. Henderson and S.J.E. Heron. 1991. The Biochemistry of silage 2nd Ed. Chalombe Publications, Marlow, England.

Nishino, N. and S. Uchida. 1999. Laboratory evaluation of previously fermented juice as a fermentation stimulant for lucerne. J. Sci. Food Agric. 79:1285-1288.

SAS. 1998. User's Guide: Statistics, 9.2 ed. SAS Institute. Cary, NC.

Sayan TadSri 1997. Tropical forage production and management. Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok. pp. 375.

Somying S., Y. Waiprib, A. Tongta and C. Chaiyasut. 2007. Effects of Sugar Type and Concentration on Microbial Count of *Terminalia chebula* Retz. Fermented Liquids. Agricultural Sci. J. 38(6) (Supp. l):271-274.

Vikas, O.V. 2014. Bioconversion of Papaya Peel Waste in to Vinegar using Acetobacter Aceti. IJSR- International Journal of Scientific research 3(11):2277-8179.

Weinberg, Z.G., G. Ashbell, A. Azrieli and I. Brukental. 1993. Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria and cell wall degrading enzymes. Grass Forage Sci. 48:70-78.

O-32-3

In Situ Degradation Characteristics of Indigofera zollingeriana at Different Cutting Intervals during Wet and Dry Season

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Introduction

Indigofera zollingeriana is an introduced shrubby legume that ensures supply of quality feed materials for goats. Its crude protein (CP) content is even higher than the commonly used tree legumes, *Leaucaena leucocephala* and *Gliricidia sepium* that make it an ideal forage legume supplement. Moreover, *Indigofera* is more digestible at 76.71% (Abdullah, *et al.,* 2012) than the 68.42% of *Leaucaena* and 65.42% of *Gliricidia* when used as supplement to the diet of goats or sheep (Orden *et al.,* 2000), respectively. Despite the several comparative advantages of *Indigofera* as goat feed, it has not been extensively utilized due to the limited information on its feeding value to dairy goats. Hence, this study was conducted to determine the *in situ* rumen degradation characteristics of *Indigofera* as influenced by cutting intervals during wet and dry season.

Materials and Methods

Samples of *Indigofera* leaves were harvested at 30 days (30d) and 45 days (45d) after re-growth from the forage production area of the Small Ruminant Center (SRC), Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines. The area is located at 150^0 43'N, 120^0 54'E with agro climatic condition classified as tropical monsoon type consisting of two distinct seasons, i.e., dry (December to May) and rainy (June to November). About 3g processed leaves of *Indigofera* were incubated in duplicate in the rumen of mature upgraded sheep (Merino x Native) fitted with permanent cannula for 4, 8, 16, 24, 48 and 72 hours.

Residual DM, CP and NDF values were fitted to the NEWAY© F-Curve (Chen, 1995) computer software to determine degradation characteristics based on the models of Ørskov and McDonald (1979) using $p = a + b\ (1 - e^{-ct})$ where **p** = degradation after **t** = time (**h**) **a** = soluble or highly degradable fraction **b** = slowly degradable fractions, which disappear at a constant fraction rate (**c**) **c** = degradation rate (per h). The slowly degradable fraction (**b**) was re-estimated as $B = (a + b) - A$, where **A** = actual soluble fraction (washing loss) (Ørskov and Ryle 1990).

The animals were kept individually in metabolic cages and provided free access to fresh drinking water. The animals were fed chopped Napier and *Indigofera* + 250g concentrate mix to meet daily nutrient requirement for maintenance (Kearl, 1982) that will ensure normal rumen function.

The DM of the feed and rumen undegraded samples were analyzed following the AOAC (1984) procedure, while the CP content determined by Kjeldahl method. Neutral detergent fiber was analyzed following the Goering and Van Soest (1970) technique.

ANOVA was done to determine the effect of cutting interval (30 vs 45 day re-growth) on the different degradation parameters, A, B, A+B, c and ED.

Results and Discussion

In-situ DM degradation

The *in situ* DM degradability of *Indigofera* harvested 30d and 45d re-growth at different incubation periods are shown in Figures 3 and 4. For both cutting intervals, the degradation of DM from *Indigofera* samples is more than 42% at an early stage of 4h post incubation. This is higher than the reported disappearance of *Leaucaena* (38.13%) and *Gliricidia* (34.90%) at the same incubation time (Orden *et al.,* 2015). From the 4h post rumen incubation, there was steady increase in DM degradability up to 48h and reached asymptote thereafter until 72h. After 24h incubation, more than 80% of DM from *Indigofera* (30d re-growth) was already degraded while the older *Indigofera* forage at 45d had only 68% degradability (Figure 1). On the other hand, DM degradability of *Indigofera* harvested 30d and 45d re-growth is about 10% lower during dry season, (Figure 2). Therefore, the DM fractions that disappeared from the nylon bags over time indicates degradation by microbial activity (Ørskov and McDonald, 1979). Similar observation was reported by Abdullah (2010) who concluded that *Indigofera zollingeriana* produced quality forage material with high digestibility that can be used in feeding goats. *Results*

tend to support earlier observation of Orden et. al., (2015) involving eight (8) selected leguminous shrubs and trees screened at the Small Ruminant Center that by 24 hours of incubation, majority of the DM in the forage exceeded 70% degradation.

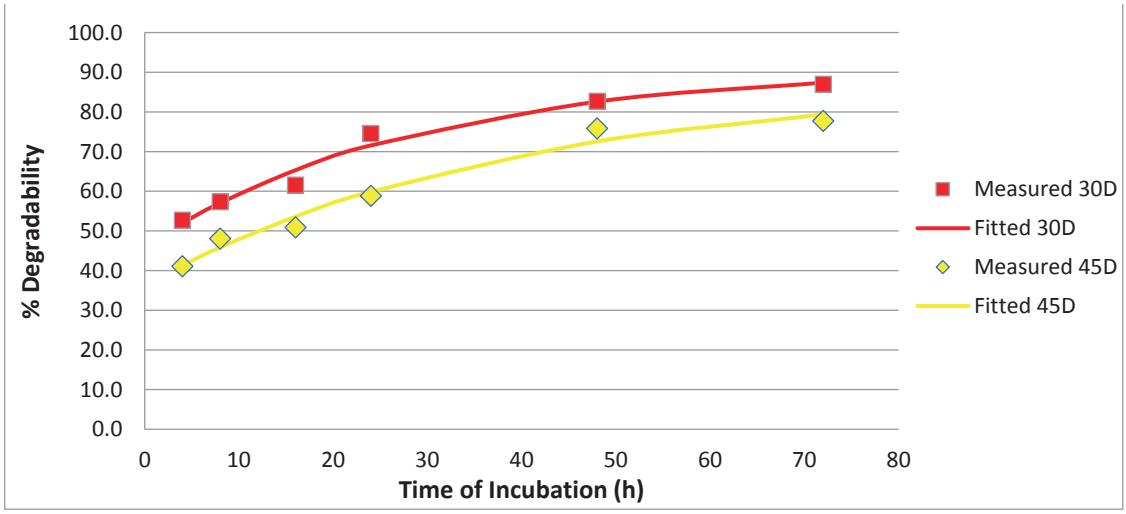

Figure 1. *In-situ* dry matter degradability at different rumen incubation periods of *Indigofera* harvested 30 and 45 days re-growth during the wet season

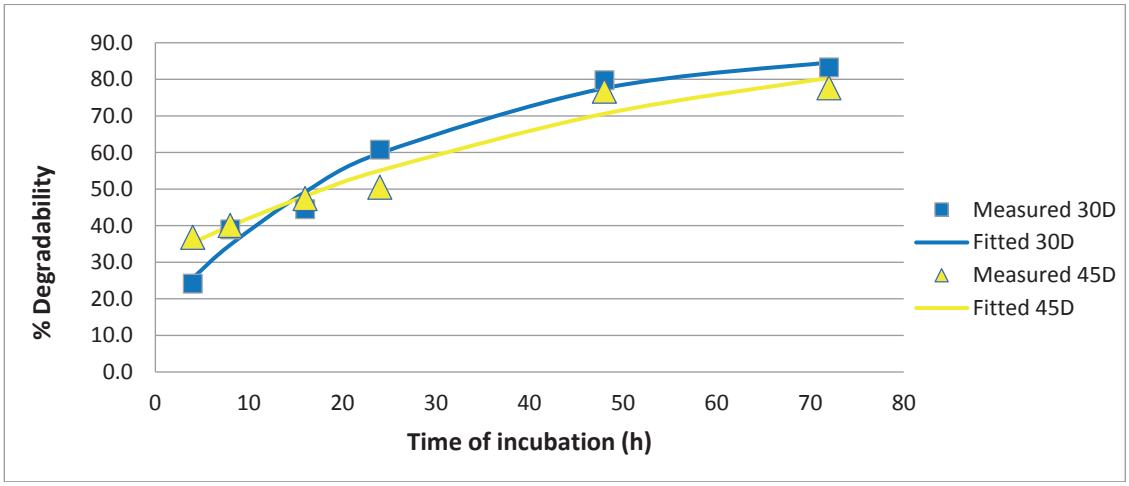

Figure 2. Dry matter degradation of *Indigofera* at different cutting intervals and rumen incubation periods during the dry season

Degradation Characteristics

Dry Matter. Table 1 shows the DM degradation characteristics of *Indigofera* harvested 30d and 45d re-growth during the wet and dry season. The highly degradable fraction (A) of *Indigofera* did not vary when harvested 30d or 45d both during wet and dry season. However the mean values A in *Indigofera* at different cutting intervals are relatively lower than those observed among traditionally used forage legumes for small ruminant feeding such as *Leaucaena leucocephala* and *Gliricidia sepium* as reported by Orden *et al.,* (2015), but comparable with the highly degradable fractions of *Flemingia macrophylla* (29.52%). Likewise, the slowly degradable fraction (B) that ranges from 56.80 to 60.04 were similar at 30d and 45d re-growth for both season. These amounts of insoluble but slowly degradable fractions in *Indigofera* are comparable with those of *Desmanthus vigatus* (50.16%), *Gliricidia sepium* (55.70%), *Leucaena leucocephala* (52.16%) *Sesbania sesban* (49%) and *Sesbania grandiflora* (55.33%) (Orden *et al.,* 2015).

Table 1. *In situ* DM degradation characteristics of *Indigofera* harvested at 30 and 45 day re-growth during the wet and dry season as defined by P= a + b (l-e^{ct})

Degradation Characteristics	Wet Season				Dry Season			
	30 day re-growth	45 day re-growth	SEM	P value	30 day re-growth	45 day re-growth	SEM	P value
A	31.50	29.84	0.48	1.00	29.40	28.65	1.23	0.100
B	58.80	59.60	2.93	0.29	60.04	59.10	2.85	0.788
A+B	90.30	89.64	3.42	0.34	89.44	87.75	1.63	0.244
c	0.035	0.026	0.01	0.49	0.038	0.029	0.03	0.001
E. D.	74.70	72.00	2.25	0.06	72.10	70.09	2.15	0.081

***A** = Highly degradable fraction; **B** = (a=b)-A; slowly degradable fraction; **A+B** = Potential Degradability: **c** = Degradation rate per hour; **ED** = Effective Degradability estimated at 0.02/h; SEM = Standard Error of Mean*

Although *Indigofera* leaves at different cutting intervals are not as soluble as *Leucaena, Gliricidia* and *Sesbania spp* as earlier reported (Orden *et al.,* 2015), more than 55%, to as high as 60.09% of its DM fractions (B) are degradable as incubation time progresses (Figure 1 and 2). Results suggest that *Indigofera* could provide highly fermentable substrate in the rumen when used as supplemental feeds for stall fed goats. Moreover, it can maintain a steady supply of degradable substrate and elevate NH_3-N and volatile fatty production in the rumen that could increase efficiency of microbial N supply (Orden *et al.,* 2000).

The estimated degradation rate (c) was at 0.035/h (30d) and 0.026/h (45d) for wet season and 0.038/h (30d) and 0.029/h (45d) in dry season. These values indicate that particle turnover in the rumen is within the measured c of *Leacaena leucocephala*, *Gliricidia sepium* and *Desmodium cineria* (Orden *et al.,* 2015).

Similar observations was reported by Abdullah (2010) who concluded that *Indigofera zollingeriana* produced quality forage material with high digestibility that can be used in feeding goats. Results tend to support earlier observations of Orden *et al.,* (2015) involving selected leguminous shrubs and tree species evaluated and screened at the SRC-CLSU, Philippines

Crude Protein. The *in situ* degradation characteristics of CP and NDF in *Indigofera* harvested at different cutting intervals are presented in Tables 2 and 3, respectively. The highly degradable CP fraction (A) is 29.40% and 31.47% while the slowly degradable fraction (B) is more than 60% at 30d and 45d old, respectively for both wet and dry season. Age at cutting did not affect the amount of soluble and slowly degradable fraction (A+B) of CP in *Indigofera* that resulted to more than 90% potential degradability. Although these values are comparable with the degradability of *Indigofera teysmanii* reported by Kier *et al.,* (1997), most of the CP in *Indigofera zollengiriana* are insoluble which is contrary to the highly soluble characteristics of CP in *Leaucaena* and *Gliricidia* (Orden *et al*, 2000 Orden *et al.,* 2015). Result suggests *Indigofera* could provide insoluble but degradable CP at an estimated degradation rate of more than 0.02/hour. This would indicate a fair amount of degradable protein to sustain the activity of the rumen microbial population. With less CP fractions that are rumen-soluble, there will be lower amount of NH_3 that will be lost when readily available carbohydrates are limited in the basal diet. Moreover, with higher proportion of insoluble but degradable CP present, *Indigofera,* could be a potential source of by-pass protein that is more beneficial among high producing livestock. Bypassing the rumen could dramatically alter the site of CP digestion in the digestive system that result to better utilization and mechanism for absorption of available CP from forage legume material (Kibon and Ørskov, 1993).

Table 2. *In situ* CP degradation characteristics of *Indigofera* harvested at 30 and 45 day re-growth during the wet and dry season as defined by P= a + b (l-e^{ct})

Degradation Characteristics	Wet Season				Dry Season			
	30 day re-growth	45 day re-growth	SEM	P value	30 day re-growth	45 day re-growth	SEM	P value
A	29.47	31.47	1.58	0.06	28.06	31.95	2.11	0.10
B	61.20	60.75	3.65	0.14	62.97	60.80	5.06	0.30
A+B	90.67	92.23	2.51	0.34	91.03	92.75	3.12	0.20
c	0.04	0.02	0.01	0.49	0.05	0.03	0.02	0.31
E. D.	77.80	75.03	2.35	0.01	78.60	76.43	7.54	0.01

***A** = Highly degradable fraction; **B** = (a=b)-A; slowly degradable fraction; **A+B** = Potential Degradability. **c** = Degradation rate per hour; **ED** = Effective Degradability estimated at 0.02/h; SEM = Standard Error of Mean*

Neutral Detergent Fiber. Similarly, the degradation characteristics (A, B and A+B) of fiber fractions of *Indigofera* express as NDF was not influenced (p>0.05) by age at cutting (Table 3). The 28.66% (30d) and 26.37% (45d) degradability of A during wet season, and 25.31% (30d) and 23.85% (45d) for dry season were lower than the average value of utilizable NDF of 35.07% reported by Tarigan (2009) and those reported (NDF 49-57%) values by Abdulla (2012). However, the B values for NDF showed that its fiber fractions are degradable at an average c value of 0.045/h during wet season and 0.35/h in dry season (Table 3). The amount of degradable B fractions of 60.28% (30d) and 59.94%(45d) in wet season and 59.025% (30d) and 58.19% (45d) during dry season were found to be similar across cutting interval. It is interesting to note that these B values are within the range of the previous findings for *Desmanthus virgatus, Leuacena leucocephala, Gliricidia sepium and Sesbania sesban* (Orden *et al.,* 2015). With almost 60% of NDF in *Indigofera* that are degradable, it means that potential degradability (A+B) for fiber is more than 85% at different cutting intervals. Results suggest that *Indigofera* is a good source of fermentable fiber that is comparable to the earlier observation of Hassen *et al,* (2008) that 49-57% of NDF and 32-38% of ADF fractions are in *Indigofera zollengiriana* can be utilized by ruminants.

Table 3. *In situ* NDF degradation characteristics of *Indigofera* harvested at 30 and 45 day re-growth during the wet and dry season as defined by P= a + b (l-e^{ct})

Degradation Characteristics	Wet Season				Dry Season			
	30 day re-growth	45 day re-growth	SEM	P value	30 day re-growth	45 day re-growth	SEM	P value
A	28.66	26.37	1.78	0.41	25.31	22.85	1.89	0.41
B	60.28	59.94	2.63	0.10	59.25	58.19	2.41	0.72
A+B	88.94	86.31	2.01	0.06	84.56	81.04	2.38	0.24
c	0.05	0.04	0.01	0.42	0.04	0.03	0.01	0.12
E. D.	70.17	68.10	4.89	0.18	68.90	65.03	0.92	0.08

***A** = Highly degradable fraction; **B** = (a=b)-A; slowly degradable fraction; **A+B** = Potential Degradability: **c** = Degradation rate per hour; **ED** = Effective Degradability estimated at 0.02/h; SEM = Standard Error of Mean*

Conclusion and Implication

The degradability of *Indigofera zollengiriana* harvested at 30d and 40d of age showed that it is not only a protein-rich forage material but a potential source of soluble and degradable fiber to supply the needed fermentable sugars necessary for normal rumen function. High producing animals require a steady supply of both NH_3 and volatile fatty acids for efficient microbial protein synthesis. In this regard, *Indigofera zollengiriana* can provide the

essential substrate for normal rumen function required by high producing animals.

KEYWORD : Indigofera, rumen, degradability, in situ, regrowth

REFERENCES

Abdullah, L., 2010. Herbage production and quality of shrub Indigofera treated by different concentration of foliar fertiliser. *Med. Pet*. 33 (3): 169–175.

Abdullah, L., D. Apriastuti, T.A.P. Apdini. 2012. Use of *Indigofera zollingeriana* as a forage protein source in dairy goat rations. Proceedings of the 1st Asia Dairy Goat Conference, Kuala Lumpur, Malaysia April 2012 pp. 70-72.

AOAC (Association of Official Analytical Chemists). 1984. Official methods of analysis. 14th edition. AOAC, Washington, DC, USA.

Goering, H.K. and P.J. VAN SOEST. 1970. Forage Fibre Analysis. Agriculture Handbook 379. Agric. Res. Service, United States Department of Agriculture, Washington, DC, USA.

Hassen, A., N.F.G. Rethman, W.A.Z. Apostolides and W.A. Van Niekerk 2008. Forage production and potential nutritive value of 24 shrubby Indigofera accessions under field conditions in South Africa. *Trop. Grasslands* 42: 96–103.

Kearl, A. J. 1982. Nutrient requirement of farm animals. Reston, Virginia, Reston Publishing Co., Inc.

Keir, B, N.V. Lai, T.R. Preston and E.R. Ørskov. 1997: Nutritive value of leaves from tropical trees and shrubs: 1. In vitro gas production and in sacco rumen degradability. Livestock Research for Rural Development. Volume 9, Article #34. Retrieved February 13, 2016, from http://www.lrrd.org/lrrd9/4/bren941.htm

Kibon, A. and E.R. Ørskov. 1993. The use of degradation characteristics of browse plants to predict intake and digestibility by goats. Brit. Soc. of Anim. Prod. 57: 247-251

Orden, E.A., K. Yamaki, E.M. Cruz, M.E.M. Orden, S.A. Abdulrazak, T. Ichinohe and T. Fujihara. 2000. Leucaena leucocephala and Gliricidia sepium supplementation in sheep fed with ammonia treated rice straw: Effects on intake, digestibility, microbial protein yield and live-weight changes. Asian-Australasian J. Ani. Sci. Vol. 13(12):1659-1666.

Orden, E.A, E.M. Cruz, M.E.M. Orden and T. Fujihara. 2015. Mineral Profile of Forages and its Influence on Goat Nutrition. Published by Central Luzon State University and Dept. of Agriculture-Bureau of Agricultural Research. ISBN 978-971-705-355-4,

Ørskov, E.R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to role of passage. J. Agric. Sci. 92:499-503.

Ørskov, E.R. and M. Ryle. 1985. Evaluation of crop residues and agro-industrial by-products using the nylon bag method. In: guidelines for research on crop residues. ILCA-FAO Publication. Pp 126-133.

Tarigan, A., 2009. Productivity and utilization of *Indigofera sp*. as goat's feed obtained from different interval and intensity of cutting. Thesis. Bogor Agricultural University, Indonesia.

O-32-7 The effect of supplementation ginger (Z. Officinale) powder on feed consumption, milk production and components of goat

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Introduction

In the rural condition, herbs were commonly used by farmers. Ginger (*Zingiber Officinale*) has been used to overcome many kind of health problem, such as bloat and cold in goat. According to Wenk (2003) the beneficial effects of herbs in farm animals included to increase feed intake, secretion of digestive secretions, immune stimulation, and anti-bacterial. Bunyapraphatsara (2012) reported that herbs product either in extract or powder could be used alternatively as feed aditive or antibiotic to increase productivity. Anonymous (2009) reported there were many disease which frequently attack dairy goat in the rural condition, such as scabies, mastitis, bloat and worm.

This study was carried out to observe the effect of supplementation of dried ginger in goat feed on milk production and composition. There was probably an indirect effect of active component in ginger such as antibacterial, antioxidant, vitamin C and anti bloat on feed consumption, milk production and milk components and therefore assured the utilization of ginger for medication and supplementation of goat feed.

Material and Methods:

The study used ten lactating Etawah Crossed bred goats weighed around 37 to 45 kg, dried ginger, Callyandra calothyrsus, grass and concentrates. Dried ginger was prepared by sun drying then grinded in small mass. The goats were divided into Control and Supplemented groups. All goats were given 4% of body weight (BW)/day of feed dry matter (DM), consisted of forage (70%) and concentrate (30%). The goat in Supplemented group was given dried ginger, 2% of concentrate, equal to 9 to 11 g/day. Feeding trial was conducted during 40 consecutive days to measure feed and nutrient consumption, milk production and milk composition. Dry matter, crude protein of feed and milk were determined followed AOAC (2005), milk fat was determined with Babcock test. The active component of ginger was analysed by observation of zone inhibition on *Staphylococcus aureus*, anti-oxidant activity and vitamin C content.

Result

The result showed that the content of dry matter, organic matter, crude protein, crude fiber, and ash in ginger powder were 37.13%, 66.35%, 5.82%, 9.73%, and 27.65%, respectively. Ginger has anti-oxidant and vitamin C content respectively were 16.09% and 2.19 g/100 mg. The examination on inhibition zone on microbial activity showed no inhibition zone of *Staphylococcus aureus* or *Escheria coli.* This indicated that ginger did not have antimicrobial activity, however the result of nutrient analysis showed that, it high in mineral content and crude protein. Based on the above data, ginger could be used as feed aditive than as protection of disease. In-vitro digestibility on dry matter and organic matter of dried ginger as 59.77 and 60. 19%, repectively, indicated that the herbs as highly digested in the rumen. Total volatile fatty acid production as 7.65 mm/l consisted of asetat, propionat and butirat were 6.39, 0 and 1.26 mm/l. Production of rumen ammonia was 90.92 mg/100 g. In vitro result in this study showed that dried ginger was potentially increase protein digestion and produced more asetat and butirate than propionat, therefore probably increase milk fat content.

According to Bhowmik *et al.* (2008) cited from Saeed and Tariq (2006) Ginger (*Zingiber officinale*) has antibacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant properties. The specific characteristic of Ginger according to Anonymus (2012) was to stimulates to releasing adrenalin which play a role in vasodilatation and increased blood flow. Ginger also contains protease and lipase which is important to digest protein and fat. This herbs was probably useful to keep the body warm, improve digestion process and therefore could protect goat from bloat.

Tabel 1. The effect of dried ginger supplementation on nutrient consumption, yield and components of goat milk
Variables

Treatments	Control	Supplemented
Consumption of		
Dry matter (g/day)	1470.00 ± 17	1747.25 ± 177.45
Crude protein (g/day)	290.00 ± 28.48	316.25 ± 38.98
Energy (kg TDN/day)	0.92 ± 0.09	1.01 ± 0.10
Crude fiber (g/day)	309.51 ± 65.28	360.54 ± 41.18
Milk yield and composition		
Milk yield (g/day)	412.5[a]	634.8[b]
Total solid (%)	14.58	19.69
Fat (%)	4.96	7.08

a,b in the same row is significant $P < 0.05$

There was no significant effect of supplementation of dry ginger powder on the consumption of dry matter, crude protein, energy and crude fiber by goats, although the values tend to be higher in supplemented feed. The effect of supplementation was significant on milk production and there was also tendency to be high in total solid and fat content of milk. This effect was probably due to high digestibility of dry ginger that was supplemented in the concentrate. That was an indirect effect of dry ginger supplementation which probably increased digestibility of feed in the rumen and subsequently gave a positive effect on milk production and components. The digestibility of ginger in this study was considerably high, so that possibility to support rumen fermentation and growth of rumen microbes. According to Leng (1999) high degradable protein provide more nitrogen required for growth of rumen bacteria. There was probably indirect effect on goat health status which probably increased efficiency of nutrient utilization and absorption. The level of hemoglobin, erytrocytes and leukocytes in both groups were 10.18 to 10.12 g/100 ml 16.84 to 15.59 x 10^6/µl 13.960 to 16.600/µl, respectively, indicated that the goat in both groups have normal blood profil. El-Far *et al*. (2014) showed the benefit of *Zingiber Officinale* as supplement to decrease egg number of gastrointestinal nematodes in ewes.

Conclusion Dried ginger (*Zingiber Officinale*) contained high mineral, high digestibility of dry matter and digestibility of organic matter, rumen VFA and NH3 but did not have anti microbial activity. The herb was pottentially to be used as feed supplements. The effect supplementation dried ginger in the concentrate of goat feed significantly increase milk production but no significant different on milk composition and nutrein consumption.

KEYWORD : ginger powder, feed consumption, milk production, composition, goat

REFERENCES

A.O.A.C. 2005. Association of Official Analytical Chemist, Official Methods of Analysis 15th ed. Washington, DC.

Bhowmik, S., Chowdhury, S.D., Kabir., M.H., Ali, M. A. 2008. Chemical composition of some medical plant product of indigenous origin The Bangladesh Vet 25, 23-29

Bunyapraphatsara, N. 2012. How to develop feed additives from herbs. Proceedings of the 15th AAAP. pp 26-30. Bangkok, Thailand

El-Far., A.H., Eman, K,, Moharan, M.S. 2014. Antioxidant and Antinematodal Effect of Nigela sativa and Zingiber Officinale Supplementation in Ewes. Int.J.Pharm. Sci. Rev. Res.26:222-227

Wenk, C. 2003. Herbs and botanicals as feed additives in monogastric animals. Asian-Australasian J. Anim. Sci., 16, 282-289

O-32-9

EVALUATION OF COMPLETE FEEDS FOR RAISING DUAL- PURPOSE SHAMI GOATS UNDER MALAYSIA ENVIRONMENT

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INTRODUCTION

The small ruminant industry currently contributes about 10% of the total requirement for meat in Malaysia. Lately, the interest on meat goat farming is reducing amongst local smallholders for the economic reasons. Some of smallholders in the state of Kelantan, have shifted to dairy goat farming. Apart from Saanen, Shami is another breed of interest to smallholders. Shami is a newly imported breed from Cyprus and it's high prolificacy and high milk production are well established (Mavrogenis *et. al.,* 2006). Issues related to poor performance of exotic goat breeds in Malaysia are mainly related to feed resources, breed and environmental factors. In many cases, feeds given by the smallholders are inferior in terms of nutritive values and in general not meeting the animal's daily nutrient requirements. Nutrient imbalances and deficiencies will impair growth and reproductive performances, regardless of the breeds of the animals (Wan Zahari and Devendra, 1985).

Shami, also known as the Damascus goat is a native breed of Syria and other Near East countries. It is a dual purpose breed i.e. for milk and meat production. This is one of the improved breeds that the Technical Consultation of FAO/UNEB on Animal Genetic Resources, Conservation and Management agreed should be given a high priority due to its quality (Mavrogenis *et. al.,* 2006). Their nutrient requirements are higher than those of meat goats. Hence, provision of local shrubs, palm kernel cake (PKC) and o ther agricultural by-products as the main ingredients in their daily rations as commonly practiced by local smallholders could be detrimental to their growth and production over a long term period. Knowledge on nutrition and feeding requirement are vital in ensuring their production potential in Malaysia. Salleh *et. al*. (2012) stressed the need of research to determine the relationship between body linear measurements with body weight in goats under different management and feeding systems.

OBJECTIVE

Presently, information on the nutrient requirements for Shami goats that were raised specifically under tropical environment is not available based on literature research. The reports gathered are more towards feeding under subtropical climate, covering Mediterranean and semi-arid type of environments (Constantinou, 1981 Hadjipanayiotou, 1987 Keskin and Bicer, 2002 Mavrogenis *et. al.,* 2006). The aim of this trial was to develop complete feeds for Shami goats at four different physiological stages under Malaysia environment.

METHODOLOGY

Four groups of the Shami goats, owned by a private farm (Makmur Dairy Sdn. Bhd., 33km, Segamat-Kuantan Highway, 26900 Bandar Tun Razak, Pahang, Malaysia) were selected for this study. This farm is focusing more on dairy milk production, utilizing Jersey as the main producer. Shami goats were introduced to this farm in early 2013 and at the time of the trial, < 200 heads were available, regardless of sex, age and physiological stages. Routine rations were given to these animals before the implementation of the trial.

The animals selected consisted of four different stages / groups i.e. Kid (n: 14, 8 males and 6 females), grower (n: 38, 22 males and 16 females), doe (n: 23) and buck (n: 5). Estimated age of the animals for the respective groups were < 12, 12-63, >72 and >96 weeks. The animals were separately placed in groups and they were fed with pelleted-formulated rations over 110 days feeding period. The rations were pelletized at a feed mill in Pasir Gudang, Johore, Malaysia. Data collected include feed intake, live weight changes, plasma mineral concentrations, body condition score (BCS) and feed conversion efficiency (FCE). Performance of kid (before weaning) and growing groups were evaluated based on rate of growth and BCS. The results from each group were then compared to those in the control groups which were housed separately. The control groups were fed routine rations which consisted of Napier grass *(Pennisetum purpureum)* (30%) and a commercial feed (70%). Palm kernel cake (PKC) was the main ingredient in the commercial feed. Proximate and mineral analyses on feed samples were carried out

at the Faculty of Veterinary Medicine, UMK. Statistical analysis was carried out by a SPSS program.
The formulated rations developed consisted of Guinea grass *(Panicum maximum),* maize, decanter cake, PKC, soya bean meal (SBM) and crude palm oil (CPO) **(Table 1).** Two dietary ratios of grass (G): concentrate (C) (30%G: 70%C and 70%G: 30%C) were evaluated on the growers (male and females) and does. The latter ration (70%G: 30%C) was similar in terms of the G: C ratio to the routine ration, but differing in nutritive values. Kids were fed 100 % pelleted ration which also contained Guinea grass *(Panicum maximum)* at 10% inclusion level. Limitation of Shami bucks in this farm, only allowed evaluation on 30%G: 70%C ration.

The crude protein (CP) and metabolic energy (ME) of the diets for kids, growers, does and bucks were 22% and 10 MJ/kg, 15% and 9.5 MJ/kg, 14% and 9.5 MJ/kg, and 14% and 9.0 MJ/kg respectively. The respective values for the routine rations used in the control groups (for growers, does and bucks) ranged from 12% - 14% and 6.5 - 8.5 MJ/kg.

Table 1: Ration Formulations for the Feeding Trial (% inclusion level)

Ingredient	Kid	Grower	Doe	Buck
Guinea grass	10	30	11	11
Maize	5	16.5	10	11.5
Rice bran / decanter cake	49	15	45	38
Palm kernel cake (PKC)	-	23	10	17
Soya bean meal (SBM)	30	10.3	11	10.5
Molasses	2.5	-	7	6
Urea	-	1.25	-	-
Crude palm oil (CPO)	-	-	-	-
Di-calcium phosphate (DCP)	-	-	0.5	0.3
Sodium chloride (NaCl)	1	1	0.6	0.8
Calcium carbonate ($CaCO_3$)	2	2	2	1.5
Sodium bicarbontae (Na_2CO_3)	-	-	1	0.8
Ammonium chloride (NH_4Cl)	0.5	0.5	1	1.5
Magnesium oxide (MgO)	-	-	0.2	0.3
Mineral-vitamin mixture	-	0.5	0.5	0.5
Zeolite	-	-	0.2	0.3

RESULTS AND DISCUSSION

Regardless of the sex, the mean initial live weight of the kids (before weaning), male growers, female growers, does and bucks were 9.2kg, 27.7kg, 24.8kg, 51.2kg and 56.4kg respectively. Over 110 days feeding period, the mean live weight gain (LWG) achieved for male kids, female kids, male grower (30%G:70%C), male grower (70%G:30%C), female grower (30%G:70%C), female grower (70%G:30%C), does (30%G:70%C), does (70%G:30%C) and bucks (30%G:70%C) were 98.9, 69.7, 82.7, 82.6, 69.3, 62.5, 21.5, - 4.55 and 94.5 g/day respectively. In general, the LWG achieved were considered 20 - 35% higher than those of the control groups. Body condition of all groups fed formulated diets were markedly improved, especially the male and female growers with the BCS of 3.5 - 4.0 as compared to 2.5 - 3.0 in those consuming routine rations. Improved LWG is associated to improved appetite, increased dry matter intake (DMI) and more importantly, enhanced dietary CP and ME concentration**.** It is evident that in general, the formulated diets were meeting the daily requirements for the respective physiological stages of dairy goats (NRC, 2007) with the CP and ME concentrations of 22%: 10 MJ/kg 15% : 9.5 MJ/kg 14%: 9.5MJ/kg and 14%: 9.0 MJ/kg for kid, grower, doe and buck groups respectively **(Table 2).** The daily feed intake between groups is shown in **Table 3.**

Table 2: Nutritive values of the rations+

Group	CP (%)	ME (MJ/kg)	Ca	P	TDN
Male kid	22	10	0.6	0.3	69
Female kid	22	10	0.6	0.3	69
Male grower	15	9.5	0.4	0.2	65
Female grower	15	9.5	0.4	0.2	65
Doe	14	9.5	0.6	0.3	65
Buck	14	9.0	0.4	0.2	60

Notes: CP Crude protein, ME: Metabolizable energy TDN: Total digestible nutrient

Table 3: Daily Feed Intake between Groups

Group	Mean LW+	Grass (kg)**	Pellet (kg)**	Estimated DMI based on mean LW (%)
Male kid	8.6	-	3	4.5
Female kid	9.8	-	3	4.5
Male grower (30%G:70%C)	27.4	8.6	8.1	3.5
Male grower (70% G: 30%C)	27.9	20.1	3.5	3.5
Female grower (30%G: 70% C)	24.0	5.3	4.9	3.5
Female grower (70%G: 30%C)	25.5	12.3	2.1	3.5
Doe (30% G: 70% C)	51.3	18.4	17.1	4.5
Doe (70% G: 30% C)	51.0	39.4	6.7	4.5
Buck (30% G:70% C)	56.4	6.3	6	4.0

Notes: + Mean live-weight data used for the estimation of dry matter intake (DMI)
** The weight of grass (G) and concentrate (C) were based on fresh basis

Surprisingly, body weight of does receiving 70%G: 30%C was markedly depressed (reduced by 4.55 kg per day) as compared to those receiving 30%G: 70%C (increment of 21.5 g per day). The reason for reduced weight is unclear and merits further investigation. The formulation ration for bucks (30%G: 70%C) was well consumed with the LWG of 94.5 g/day **(Table 4).**

Table 4: Live weight changes between groups

Group	N	Initial LW	Mean LWG	Range of LWG
Male kid	8	11.1	98.9	63.6 – 136.4
Female kid	8	9.83	69.7	45.3 – 99.9
Male grower (30%G:70%C)	11	27.4	82.7	36.4 – 154.5
Male grower (70% G: 30%C)	11	27.9	82.6	27.3 – 127.3
Female grower (30%G: 70% C)	8	24.0	69.3	27.3 – 118.2
Female grower (70%G: 30%C)	8	25.5	62.5	45.5 – 90.9
Doe (30% G: 70% C)	11	51.3	21.5	(-) 90.9 – 190.9
Doe (70% G: 30% C)	11	51.0	(-) 4.55	(-) 118.2 -163.6
Buck (30% G:70% C)	5	56.4	94.5	(-) 45.5 – 172.7

Notes: + LW: Live weight LWG: Live weight gain G: Grass C: Concentrate

It is interesting to note that Shami goat kids rearing on milk replacer that contained 22% protein and 21% fat increased farm profitability on the basis of milk price and the body weight of kids (Keskin and Bicer, 2002). Feeding Shami goat kids during the finishing period with high levels of energy improved the total weight gain and total feed conversion (Abdelrahman, 2009). Additionally, nutrient digestibility and balance studies should be carried out on the respective groups to further understand the benefit of formulated rations. Previous study

revealed that the digestibility trial showed higher organic matter, crude fiber and nitrogen-free extract digestibility's in Shami X Baladi crossbred in comparison with Jamnapari X Baladi crossbred. Crude protein digestibility gave similar values (Abdel-Rahman and Kaschab, 1998).

Plasma Ca, P, Mg, Cu and Mn concentrations in most of the animals, regardless of the groups were within normal acceptable limits. However, 20% of female growers and does fed on 70%G: 30%C (comparable to animals in the control groups that received routine rations) were low in Ca and P. Before the implementation of the trial, plasma Ca, P and Mg concentrations in each group were found lower than the normal levels of 10mg/100ml, 6.0 mg/100ml and 2.0 mg/100 ml respectively (McDowell, 1976 Wan Zahari and Abdul Wahid., 1985). The body condition of does receiving 30%G: 70%C were better than those on 70%G: 30%C with the BCS of 4.0 and 3.0 respectively. This could be due to increased demand of energy in doe for milk production which can be met by enhancing ME content of the ration. In this regard, supplementation of by-pass fat could be considered. Supplementing fat to does rations at 3 or 4% during their *postpartum* period can improve their milk production and milk fat content, as well as body weight changes of does, weaning weight and average daily gain of suckling kids, without any effect on their milk protein content (Al-Dabbas and Azmi, 2011). Supplementing lactating goat diet with soya bean and sunflower oils did not improve milk production, milk composition, or growth rate and weaning weight of their kids, but improved their energy corrected milk and total conjugated linoleic acid (CLA) content (Titi, *et. al*., 2011). The responses of early lactating Shami goats to varying amounts of dietary supplementation of calcium salts of fatty acids has been reported (Titi, 2011).

CONCLUSION

In conclusion, the present formulated rations, especially with ratio of 30%G: 70%C were suitable in enhancing growth performances of growing male and female Shami goats in Malaysia. Pelleted ration, containing 10% Guinea grass can be used as a creep feed to increase growth performance of Shami goat kids. Under the condition of trial, male kids achieved higher growth rate (98.9 g/day) than the female kids (69.7g/day). The growth rates of male growing Shami goats were found higher than females, regardless of the ratio of grass to concentrate. Dietary ratio of 30%G: 70%C is more suitable for does in milk production. Further studies are required on the feeding management of Shami goats in view of increased interest on this breed for milk and meat production.

KEYWORD : Complete feed, Goat, Growth performance, Pelleted diets, Shami

REFERENCE

Abdelrahman, M. (2009). General performance of growing Shami kids fed high energy and protected methionine. Asian Journal of Animal and Veterinary Advances. 4 (21): 52-59.

Abdel-Rahman, K.M. and El-Kaschab (1998). Nutritional performance of Damascus and Jamnapari crossbred growing male goats, Small Ruminant Research, 27(3): 279-281.

Al-Dabbas F.M. and Azmi, H. (2011). The effect of fat supplementation in Shami goat diets on milk production and composition, does body weight and growth performance of their suckling kids. Pakistan Journal of Biological Sciences, 14:725-728.

Constantinou, A. (1981). Damascus goats in Cyprus. World Animal Review, 40:17-22.

Hadipanayiotou, M. (1987). Studies on the response of lactating Damascus goats to dietary protein. Journal of Animal Physiology and Animal Nutrition, 57:41-52.

Keskin, M. and Bicer, O. (2002). Effects of milk replacer on kid growth and farm profitability in the Shami goat. Turkish Journal of Animal Science, 26:1133-1136.

Mavrogenis, A.P., Antoniades, N.Y. and Hooper, R.W. (2006). The Damascus (Shami) goat of Cyprus. Animal Genetic Resources Information, no. 38, 57-65.

McDowell, L.R. (1976). Mineral deficiencies and toxicities and their effect on beef production in developing countries. In Beef Cattle Production (Editor: A J Smith), Univ. Edinburgh, 216-241.

National Research Council (NRC) (2007). Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids. The National Academic Press.

Salleh, S.I., Ernie-Muneerah, M.A., Hafiz, A.R., Raymond, A.K., Zawawi, I., Kamarulrizal, M.I. Hafizal; A.M., Safaruddin, M.D., Abu Hassan, M.A., and Kamaruddin, I. (2010). Bioprospecting of Cyprus Shami goats for dairy production in Malaysia. 2nd National Agro biodiversity Conservation and Sustainable Utilization, 139-141.

Titi, H.H. (2011). Effects of varying levels of protected fat on performance of Shami goats during early and mid-lactation. Turkish Journal. Vet. Anim. Sci., 35(2):67-74.

Titi, H.H., Hasan, Y.I., Al-Ismail, K., Zakaria, H. Tabbaa, M.J., Abdullah, A.Y. and Obeidat, B.S. (2011). Response of Shami goats and kids to variable levels of soya bean or sunflower oils in diets. Journal of Animal and Feed Sciences, 20, 493-508 .

Wan Zahari, M. and Abdul Wahid, S. (1985). Mineral concentration in blood plasma and body tissues of local crossbred goats. MARDI Res. Bull., 13:3: 333-340.

Wan Zahari, M. and Devendra, C. (1985). The mineral profile of indigenous swamp 101:88

O-33-1 In vitro digestibility of fermented rice straw supplemented with cassava tuber and leaves using ruminal fluid of Bali cattle

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INTRODUCTION

Digestibility of high fiber feed depends on cellulolytic microbes. In order to maximize the degradation of fibrous feed, the growth of ruminal microbes is needed to be improved. One effort to increase the microbe population in the rumen is by manipulating the adequacy of nutrients for their growth.

Microbes in the rumen require energy and protein for their growth and development. A synchronized protein-to-energy ratio indicates optimal fermentation efficiency. Cattle fed only fermented rice straw may be able to provide energy from structural carbohydrates of the rice straw. However, since fermented rice straw also contains rapidly degraded N derived from urea that used in fermentation process, feedstuffs that contain rapidly degraded carbohydrates (non-structural carbohydrates) such as cassava tuber can be used. On the other hand, to synchronize the slowly degraded carbohydrate of rice straw, slow degraded protein source feedstuffs such as cassava leaves can be added. With a good balance of energy and protein availability in the rumen, an increase of microbial population as well as increasing volatile fatty acids (VFA) production in the rumen can be expected.

Cassava as a local food commodity in Indonesia is abundant throughout the year. Cassava tuber is known as a good carbohydrate source that is degraded rapidly, while the leaves contain quite high crude protein (CP) which is ranged 20 - 36% (Askar, 1996). The rapidly degraded carbohydrates in cassava tuber and slowly degraded CP in cassava leaves are a good combination as additional feed for cattle fed fermented rice straw. Based on these, a research that focused on improving the *in vitro* digestibility of fermented rice straw basal feed by supplemented with cassava tubers and leaves was established.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Feed Technology, Department of Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. The experimental design used in the study was a 2×2 factorial experimental design, with 2 levels of cassava tuber (without or with 5% cassava tuber) and 2 levels of cassava leaves (without or with 5% cassava leaves). Fresh cassava (*Manihot utilisima*) tuber and leaves were collected from Gunungkidul area, while fresh rice straw was gathered from Sleman area, Yogyakarta. Fresh cassava tuber and leaves were dried under direct sunlight, and then ground using hammer mill with 1 mm screen. The probiotic used for rice straw fermentation was Starbio® at a dose of 0.3% of dry matter (DM) of rice straw with probiotic-to- urea ratio was 1: 2 (1 g probiotic + 2 g urea/kg DM). After mixed with probiotic and urea mixture, rice straw was stored in the room temperature for 3 weeks.

The chemical compositions of samples were analyzed by proximate analysis according to (AOAC, 2005). The chemical compositions of fermented rice straw, cassava tuber and leaves are presented in Table 1. The *in vitro* digestibility analysis was carried out using two-stage in vitro method (Tilley and Terry, 1963). The data were analyzed by one-way analysis of variance (ANOVA) using SPSS ver. 22 (IBM, USA). Comparisons of means for treatments were done by contrast test with Duncan's new multiple range tests (Gomez and Gomez, 1984) when the effects of treatments ($P \leq 0.05$) were detected.

RESULTS AND DISCUSSSION

The results of supplementing cassava leaves and tuber in fermented rice straw on *in vitro* digestibility of DM, organic matter (OM), and CP are presented in Table 2.

Dry matter and OM digestibilities (DMD and OMD) of fermented rice straw were increased ($P < 0.05$) due to cassava tubers supplementation, while no significant effect was detected on CP digestibility (Table 2). Cassava contains non-structural (62.5%) and structural carbohydrates, which includes 2.69% cellulose, 0.36% hemicellulose, and 0.02% lignin (Arnata, 2009). Therefore, by adding cassava tuber in the diet would increase the soluble carbohydrates availability in feed. Since soluble carbohydrates is rapidly fermented in rumen, the volatile

fatty acids (VFA) and adenosine triphosphate (ATP) production from this fermentation may increase, thus more energy is available for microbial growth which lead to greater microbes population in the rumen. Furthermore, the increasing soluble carbohydrates from cassava tuber matched with rapidly degraded N of urea contained in the fermented rice straw, thus the rumen microbes population might be increased significantly. With greater microbe population in the rumen, feed can be degraded more efficient which ended with greater DMD and OMD.

Compared with controls, the digestibilities of DM, OM, and CP of fermented rice straw were increased ($P < 0.05$) due to cassava leaves supplementation (Table 2). The digestibility improvement of fermented rice straw due to cassava leaves supplementation were caused by increasing amount of protein available in the feed. In this study, CP content of dried cassava leaves was 30.6%, which was greater than the other reports (Inthapanya et al., 2012 Kiyothong and Wanapat, 2003). Furthermore, the cassava leaves supplementation (as slow degraded protein source) also matched with structural carbohydrates of fermented rice straw, which also leads to increasing population of rumen microbes and then resulted in greater *in vitro* digestibility of the diet.

A positive interactions on DM, OM, and CP digestibilities due to cassava tuber and leaves supplementation were noticed ($P<0.05$ Table 2) with the best effects was shown in the T1 × L1 (34.0, 45.2, and 34.5%, respectively). These results was achieved due to the soluble carbohydrates of cassava tuber matched with the urea from fermented rice straw while the slowly degraded protein of cassava leaves matched with the structural carbohydrate of fermented rice straw. These combinations created a good balance between energy and protein availability in the rumen for microorganisms growth. Previous researcher reported that adding rapidly degraded carbohydrates and protein feedstuffs improved growth of rumen microbes (Kurniawati, 2007).

CONCLUSION

Supplementing cassava tubers and leaves on fermented rice straw in this study showed positive effects on the *in vitro* digestibility of fermented rice straw with the best combination was at 5% cassava tuber and 5% cassava leaves.

KEYWORD : In vitro digestibility, Fermented rice straw, Cassava tuber, Cassava leaves, Supplementation

Table 1. Chemical compositions of fermented rice straw, cassava tuber and leaves (%)

Feedstuffs	Dry matter	Organic matter	Crude protein	Crude fiber	Ether extract
Fermented rice straw	92.9	79.7	8.20	32.4	1.70
Cassava tuber	86.1	97.0	3.11	2.24	0.63
Cassava leaves	87.0	93.4	30.6	24.2	4.87

Table 2. *In vitro* digestibility of fermented rice straw supplemented with cassava leaves and tuber

Leaves (% as fed)[2]	Tuber (% as fed)[1]		Means
	T0	T1	
Dry matter digestibility (%)			
L0	22.8^{p}	27.4^{q}	25.1^{x}
L1	30.5^{r}	34.0^{s}	32.3^{y}
Means	26.7^{a}	30.7^{b}	
Organic matter digestibility (%)			
L0	36.0^{p}	41.1^{q}	38.6^{x}
L1	44.1^{qr}	45.2^{r}	44.7^{y}
Means	40.1^{a}	43.2^{b}	
Crude protein digestibility (%)			
L0	25.5^{p}	26.5^{p}	26.0^{x}
L1	33.1^{q}	34.5^{q}	33.8^{y}
Means	29.3	30.5	

^{a,b} Means in the same row within same group with different superscripts differ at P<0.05.
^{x,y} Means in the same column within same group with different superscripts differ at P<0.05.
^{p,q,r,s} Means in the same row and column within same group with different superscripts differ at P<0.05.
[1]T0= fermented rice straw without cassava tuber; T1= fermented rice straw with 5% cassava tuber.
[2]L0= fermented rice straw without cassava leaves; L1= fermented rice straw with 5% cassava leaves.

REFERENCE

AOAC. 2005. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Arnata, I. W. 2009. Teknologi bioproses pembuatan bioetanol dari ubi kayu (*Manihot utilisima*) menggunakan kultur campuran *Trichoderma viride, Aspergillus niger* dan *Saccharomyces cerevisiae*, Institut Pertanian Bogor, Bogor.

Askar, S. 1996. Daun singkong dan pemanfaatannya terutama sebagai pakan tambahan. Wartazoa 5: 21-25.

Gomez, K. A., and A. A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, New York.

Inthapanya, S., T. R. Preston, D. N. Khang, and R. A. Leng. 2012. Effect of method of processing of cassava leaves on protein solubility and methane production in an in vitro incubation using cassava root as source of energy. Livest. Res. Rural Dev. 24.

Kiyothong, K., and M. Wanapat. 2003. Cassava hay and Stylo 184 hay to replace concentrates in diets for lactating dairy cows. Livest. Res. Rural Dev. 15.

Kurniawati, A. 2007. Teknik produksi gas *in vitro* untuk evaluasi pakan ternak: Volume produksi gas dan kecernaan bahan pakan. Jurnal Ilmiah Aplikasi Isotop dan Radiasi 3: 40-49.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18: 104-111.

O-33-2

Rumen contents from slaughter house as alternative feed for replacing forage in ruminant diets

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INTRODUCTION

To anticipate the problem of forage shortage during dry season in Indonesia, the usage of unconventional feedstuffs such as agricultural crop residues (Utomo, 2015) and livestock waste as feed is one of the strategies that can be applied. This strategy may optimize the use of natural resources for the benefit of livestock production in concern with environment sustainability (Anonymous, 1996). One of livestock waste that can be used as a feed substitute for forage basal feed is rumen contents from slaughter houses, which until now was discarded or only used as organic fertilizer. The increase of slaughtered cattle to meet the needs of meat was followed by raising amount of rumen contents, which causing pollution problems in urban areas. Each cattle slaughtered would produce about 24.5 kg of fresh rumen contents or 3.8 kg in dry matter, since they contain 15.5% dry matter (Witherow and Lammers, 1976). The use of rumen contents from a slaughter house as feed for cattle have been reported by Messermith (1973) which used rumen content in the ration up to 15% and produced similar average daily gain (ADG), feed consumption, feed efficiency, and feed conversion compared to those fed with control ration.

Ensilage is one of the ways to prevent spoilage, maintain nutritional value, and in this case can eliminate the typical odor of rumen contents. Ensilage is a preservation method performed by fermentation process. The main products in the ensilage are lactic acids which act as preservatives. To increase the dry matter of rumen contents for ideal ensiling (35% DM), dried cassava pomace has been added, while molasses was added to increase the water soluble carbohydrate content.

This study was done to determine the effect of using rumen contents silage as a substitute for basal feed (forage) in beef cattle ration on production performance and carcass percentage of Ongole crossbred cattle.

MATERIAL AND METHODS

By following completely randomized experimental design, 16 Ongole crossbred cattle aged about 18 months with average initial weight 308.1 ± 46.8 kg were divided into four treatments of basal feed and located in individual stalls. Dietary treatments offered was: Treatment A (100% Napier grass (*Pennisetum purpureum*), as control), Treatment B (67% grass + 33% rumen contents silage), Treatment C (33% grass + 67% rumen contents silage), and Treatment D (100% rumen contents silage, without grass). Each treatment consisted of four cattle as replication. All treatments were applied to cattle for three months. Commercial concentrate with 13.2% crude protein (CP) and 2.04 Mcal/kg metabolic energy (ME) was also offered in addition to basal feed. The amount and nutrient content of the diets were calculated based on the cattle's need for maintenance and production (Kearl, 1982).

Silage was made from a mixture of rumen contents with dried cassava pomace (71.9 : 28.1), and added with molasses (4%) and *Lactobacillus plantarum* (0.01%). Fresh rumen contents were obtained from slaughter houses in Malang, East Java dried cassava pomace from cassava processing factory in District Kandangan, Kediri, East Java molasses from a sugar factory in Kebun Agung, Malang, East Java *Lactobacillus plantarum* from the University Centre of Universitas Gadjah Mada, Yogyakarta. Silage fed to cattle after finishing the ensilage process for three weeks. The research was conducted at Indonesian Beef Cattle Research Station in Grati, Pasuruan, East Java, Indonesia. Chemical composition was analyzed at Laboratory of Feed Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Chemical composition analysis of feed ingredients used as fed was done on Napier grass, rumen contents silage, and commercial concentrates. The variables measured were feed consumption (feed intake, FI), average daily gain (ADG), feed conversion, and carcass percentage.

RESULTS AND DISCUSSION

Chemical composition of feed

The proximate analysis of feed ingredients (dry matter/DM, CP, ether extract/EE, crude fiber/CF, ash, and nitrogen free extract/NFE) and total digestible nutrients (TDN) are listed in Table 1.

The CP and CF contents of Napier grass were relatively low. According Utomo (2012), CP content of Napier grass at 60 day-old is about 8.3% with CF content about 33.5% and TDN about 50%. Although the CP and CF contents of Napier grass was lower than those of rumen contents silage, the TDN content of rumen contents silage was greater than that of Napier grass. This may happen due to dried cassava pomace and molasses were added in the ensilage process of rumen contents. Crude protein, CF, and NFE contents of dried cassava pomace were 0.90, 16.5, and 60.5%, while molasses' were 3.40, 18.1, and 54.5%, respectively (Isnandar et al., 2010). Total digestible nutrients of commercial concentrate was low due to its determination using a regression formula of Harris et al. (1972) cit. Utomo (2012), which is actually fit more for single feed.

Feed intake, weight gain, feed conversion, and carcass percentage

The data of FI, ADG, feed conversion, and carcass percentage of Ongole cattle crossbred were fed dietary treatments are listed in Table 2.

The results showed a declining trend of FI as the increasing of rumen contents silage used in the diet ($P<0.05$). Interestingly, although the FI showed a decreasing trend, the ADG of cattle fed diets contain rumen contents silage were greater ($P<0.05$) than those with control diet. This data imply that using rumen contents silage up to 100% (Treatment D) as substitution for Napier grass positively affect the cattle performance, especially the ADG. Furthermore, substitute Napier grass with rumen contents silage (Treatment D) resulted in the best feed conversion (8.09 Table 2). Previous researchers reported that feed conversion Ongole crossbred cattle was 11.8 (Nusi et al., 2011) and 22.6 (Carfalho et al., 2010). The feed conversion of treatment D in this study was much lower compared to that of previous researchers. Since feed conversion depends on the quality of feed was given, this may imply that rumen contents silage can be categorized as good quality feed.

A noticeable increase of carcass percentage (58.6%) was on Treatment C, which 33% of Napier grass and 67% of rumen contents silage were used as basal diet for the cattle. Carcass percentage in this study was greater that reported by previous researchers. Nusi et al. (2011) in their research reported that carcass percentage of Ongole crossbred cattle was 51.3%, while Carfalho et al. (2010) found that the carcass percentage was 49.4%. Greater carcass percentage obtained showed a better animal productivity, which is in concomitant with the increase of ADG.

CONCLUSION

The use of rumen contents silage as substitute for forage (Napier grass) up to 100% decreased FI, increased ADG, and resulted in better feed conversion. However, using rumen contents silage up to 67% resulted with the greatest carcass percentage. Thus, it can be concluded that the most optimum use of rumen contents silage is up to 67% in the diet.

IN MEMORIAM

In memoriam to our colleagues Uum Umiyasih who participated in this research, but she have been summoned to Allah. Hopefully her sins are forgiven and got the best place with Allah, Aamiin.

KEYWORD : Rumen contents silage, Ongole steers, Average daily gain, Carcass percentage

Table 1. Chemical composition of feed ingredients used for feed (% DM)

Feed ingredient	DM	CP	EE	CF	Ash	NFE	TDN[1]
Napier grass	28.8	6.70	2.43	24.5	13.6	53.1	59.6
Rumen contents silage	38.0	4.63	2.13	13.9	7.05	72.3	70.4
Commercial concentrate	84.3	15.7	3.03	15.5	12.6	52.7	59.0

Source: Laboratory of Feed Technology, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

[1] Calculated based on formula in Harris et al. (1972) cit. Utomo (2012).

Table 2. Feed intake, average daily gain, feed conversion, and carcass percentage of Ongole crossbred cattle were fed diet treatments based on rumen contents silage

Items	Treatments			
	A	B	C	D
Feed intake (kg)	$12.3^{a} \pm 2.42$	$11.0^{ab} \pm 2.14$	$10.3^{ab} \pm 1.57$	$8.54^{b} \pm 0.42$
ADG (kg)	$0.72^{a} \pm 0.09$	$1.09^{b} \pm 0.21$	$1.10^{b} \pm 0.39$	$1.09^{b} \pm 0.25$
Feed conversion	$17.5^{a} \pm 5.14$	$10.9^{ab} \pm 1.65$	$9.94^{ab} \pm 2.42$	$8.09^{b} \pm 1.40$
Carcass percentage (%)	$53.3^{a} \pm 2.76$	$56.0^{ab} \pm 0.75$	$58.6^{b} \pm 1.48$	$56.1^{ab} \pm 3.08$

^{a,b} Means with different superscripts in the same row are significantly different ($P<0.05$).
A = 100% Napier grass, B = 67% Napier grass + 33% rumen contents silage, C = 33% Napier grass + 67% rumen contents silage, D = 100% rumen contents silage.

REFERENCE

Anonymous. 1996. Buku Statistik Peternakan. Direktorat Jenderal Peternakan Departemen Pertanian R.I. Jakarta.

Isnandar, Ristianto Utomo, Siti Chuzaemi, Endang Sutariningsih, and Lies Mira Yusiati. 2010. The role of lactic bacteria on silage duration process and rumen content silage quality. Proceeding ISTAP held at Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta.

Carvalho, M.C., Soeparno, and N. Ngadiyono. 2010. Growth and carcass production of Ongole crossbred cattle and Simmental Ongole crossbred reared in a feedlot system. Bulletin of Animal Sci. 34 (1): 38 – 46.

Kearl, L.C. 1982. Nutrient Requirement of Ruminants in Developing Countries. IFI. Utah Agriculture Exp. Station. Utah State University. Logan, Utah.

Messersmith, T.L. 1973. Evaluation of Dried Paunch Feed as Roughages Source in Ruminant Finishing Rations. Master Thesis. Department of Animal Science, University of Nebraska.

Nusi, N., Ristianto Utomo, and Soeparno. 2011. Effect of utilization of corn cob in complete feed and undegraded protein supplementation on gain and meat quality of Ongole crossbred cattle. Bulletin of Animal Sci. 35 (3): 173 – 181.

Utomo, R. 2012. Evaluasi Pakan dengan Metode Noninvasif. Cetakan Pertama. PT. Citra Aji Parama. Yogyakarta.

Utomo, R. 2012. Bahan Pakan Berserat Untuk Sapi. Cetakan Pertama. PT. Citra Aji Parama, Yogyakarta.

Utomo, R. 2015. Konservasi Hijauan Pakan dan Peningkatan Kualitas Bahan Pakan Berserat Tinggi. Cetakan Pertama. Gadjah Mada University Press. Yogyakarta.

Witherow, J.L. and S. Lammers. 1976. Paunch and Viscera Handling. Pp.37-66 in Workshop (1973) On in-Plant Waste Reduction In The Meat Industry. Compiled by: J.L.Witherow On J.F.Sci. Environ. Prot. Technol. Ser. EPA-600/2-76-214.

O-33-3

Influences of Parts of Frond Ensilage and Pelleting Methods on Chemical Physical and Degradability of Pellet Ensiled Oil Palm Frond

Ongarge Insung

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Abstract

Fresh oil palm fronds (OPF) were cut, chopped for ensilage either by using total frond or the distal part. Four different ensilage methods were applied 1) common ensilage without additives 2) ensilage with molasses 3) ensilage with molasses together with inoculants 4) ensilage with molasses together with PentoZymeò. The ensiled OPF were used for pH measurement and for pelleting through two different die sizes and two binder levels. The pellet ensiled OPF were dried at 65℃ using for the bulk density measurement and for evaluation of the dry matter degradability. It was found that the parts of frond and the ensilage methods have no effect on the pH value ($P>0.05$) and on the bulk density ($P>0.05$). However, the use of additives at different levels provided the different pH value ($P<0.01$). Moreover, the part of fronds, ensilage methods and the die size had no effect on the ruminal degradation parameters ($P>0.05$), only the binder levels that had the effect on the 'b' ($P<0.05$), the 'c' ($P<0.05$), the lag time ($P<0.05$), the 'ed3' ($P<0.01$) and the potential degradability ($P<0.01$). This research result implies that ensilage of OPF by using total frond provides a higher dry matter content without any adverse effects on the chemical and the physical properties and the nutrient availability.

INTRODUCTION

Oil palm (*Elaeis guinensis*) is an important crop of Thailand as well as other countries in the tropical region. In Thailand the government proposed the target to increase the oil palm plantation area about 64,000 hectares annually to reach the plantation area of about 1.6 million hectares in the year 2029 (DOAE Thailand, 2005). The fresh oil palm fruit in the production year 2016 was about 10,944,884 metric tons for the production of the oil palm for 1,700,000 metric tons. There are several by-products from oil palm production, including oil palm trunk (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), palm kernel cake (PKC), palm oil mill effluent (POME) and palm press fiber (PPF). Among these by-products, OPF in particular has been given emphasis lately as it has great potential to be utilized as a roughage source or as a component in complete feed for ruminants (Wan Zahari, et al., 2003). In Thailand, it is well established that the oil palm tree at 15 years old provide OPF with one frond has its fresh weight for 14.88 kgs. Therefore, annually each oil palm tree can provide the fresh OPF about 372 Kgs (Aimoeb, 2008). On this ground, it can estimate that about 81,840 million metric tons OPF can be produced from 1.6 million hectares of all plantation area in the year 2029. This is an enormous amount of the OPF that can be used for many purposes including for ruminants feed resources. The use of OPF for ruminant feed, is however,needed the development of the OPF before using.

The use of OPF as fresh form has some adverse effects in term of water loss and rapidly dried and hard shortly after harvesting. Therefore ensilage OPF for ruminant feed had been proposed for some years. The use of pelleted ensiled OPF is a new coming technology that had been demonstrated earlier in Malaysia (Dahlan, et al., 2000). The properties and qualities of ensiled pelleted OPF are varied depends on many factors both in the ensilage process and the pelleting process. The objective of this research work was to determine the influences of the use of different parts of frond, the ensilage and pelleting methods of the OPF on the chemical, physical properties and on the ruminal degradability of pelleted ensiled OPF.

MATERIALS AND METHODS

The research consists of three consecutive experiments. The first experiment was to evaluate the effect of using of different part of frond and ensilage methods on the pH value of the ensiled OPF. Fresh OPF were cut, chopped for ensilage in the 200 kgs capacity plastic bin either by using total frond or using only the distal part of frond by cut off the proximal part at the first leaflet position. Four different ensilage methods were applied 1) common ensilage without additives 2) ensilage with molasses as additive 3) ensilage with molasses as additive together with inoculants 4) ensilage with molasses as additive together with the commercial, multienzyme preparation PentoZyme[ò]. The ensiled OPF samples were taken after 21 days for pH measurement by using the pH meter

(ORION® pH-/ISE meter model 95-12).

The second experiment coped with the evaluation of the bulk density of pelleted ensiled OPF obtained from the first trial for pelleting through 2 pore sizes of the die (8 and 10 mm) and using 2 levels (400 and 500 g/kg) of rice brand as the pelleting binder. The pelleted OPF samples were used for measurement the bulk density measured by using the water replacement method.

The third trial dealt with the evaluation of the pelleted ensiled OPF obtained from the second trial for the ruminal degradation parameters study by using the nylon bag technique, introduced by Ørskov and McDonald (1979). The pelleted ensiled OPF samples obtained from the second trial were used for incubation in the rumen for 96 hours. The data were calculated and the ruminal degradation parameters were generated by using the Neway excel, an application program, written by Chen (1995). The data obtained was analyzed using the analysis of variance (ANOVA) procedures (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The effect of using of different part of frond and ensilage methods on the pH value of the ensiled OPFThe mean values of the pH of ensiled OPF were presented in Table1. The pH of all ensilage combinations were ranked from 3.58 to 3.63 which were classified as a good silage quality (McDonald, et al., 1991). It is well accepted that the most important factors affecting the pH of ensiled grass silage is that the moisture content of the raw material using for ensilage. The higher moisture content of the grass used for ensilage provided a lower pH value of the silage. When the unwilted grass and the wilted grass were used for ensilage the pH value of the silage were 4.0 and 4.4, respectively (McDonald, et al., 1991). The pH value obtained from this research work revealed that the use of additive provided the silage that have a lower pH ($P>0.05$) than that of the control group. However, the pH of all groups were not significant different across treatments ($P>0.05$).

The effect of using of different part of frond, ensilage methods, the pore size of the die and the levels of binder on the bulk density of the ensiled OPF.The mean values of the bulk density of the ensiled OPF either of the fresh or the dried weight were shown in Table2. It was clearly indicated that the bulk density of the pelleted ensiled OPF either using total frond or using only the distal part cutting at the first leaflet position were not significant different either the fresh weight or the dried weight ($P>0.05$). This might mean that the proximal part that was cut off with having their amount about 33.65% of the total frond (Insung et al., 2014) had property in term of the bulk density not much different with the other part of the frond. The use of only the distal part of frond for ensilage and pelleting, therefore, had the bulk density not much different with another one that using the total frond. However, the bulk density of the pelleted ensiled OPF by using the different ensilage methods ($P<0.05$), the different pore size of the die's diameter ($P<0.01$), and the binder level ($P<0.01$). For this reason, it is well established that the ingredient which had the lower bulk density, had the higher porosity (KoÇ et al., 2008). The bulk density of the fresh pelleted ensiled OPF obtained from this research work, therefore, higher than that of the dry pelleted ensiled OPF. The feature of pelleted ensiled OPF found from this research work can be used for explanation of the property of pelleted ensiled OPF in which the pelleted ensiled OPF which is has the lower bulk density has the higher porosity. Because the dry pelleted ensiled OPF has more porosity than that of the fresh pelleted ensiled OPF, the bulk density of the dry pelleted ensiled OPF, therefore, lower in every factor. Moreover the greater pore size of the die and the higher binder levels provided the pelleted ensiled OPF with the higher the bulk density ($P<0.01$) (Table 2).

The effect of parts of frond ensilage and pelleting methods on the ruminal degradability of ensiled OPF. The mean values of the ruminal degradation parameters of pelleted ensiled OPF using different part of frond, ensilage method, dies diameter size and levels of binder were shown in Table 3. It is clearly indicated that almost all of the ruminal degradation parameters, including the a, b, c, ed1, ed2, ed3, lag time and the potential degradability of all factors were not significant different ($P>0.05$) in every level for factor part of frond, ensilage method and the die diameter size. Only for the factor the binder level that provided the differences for the 'b' ($P<0.05$) and the POTDG (a+b) parameters ($P<0.01$) as well as the effective degradation at 0.08 fraction/hour passage rate (ed3) which were significant different ($P<0.01$) across the binder level. It is well accept that the rate and extent of rumen digestion are influenced by the complementarity of the release of nitrogen and energy compounds that has an effect on the ruminal microbial population (Rotger et al., 2006). The higher level of rice brand, a feedstuff that has a higher water soluble carbohydrate which is used as the binder, therefore, provide the higher the degradability of the not water soluble but fermentable (the 'b' value)($P<0.05$) and the potential degradability (POTDG) ($P<0.01$) of the ensiled pelleted OPF.

CONCLUSIONS

Ensilage the OPF by using different parts of frond and ensilage methods did not caused the ensilage products to have the differences in the pH value. The ensilage methods, the die diameter size and the levels of the pelleted binder influence the bulk density of the pelleted ensiled OPF. Most of the ruminal degradation parameters of the pelleted ensiled OPF were not affected by the part of fronds, the ensilage methods and the die diameter size but depend mostly on the binder levels used for the pelleting process.

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KEYWORD : Pellet ensiled oil palm fronds, Ruminal dry matter degradation parameters, Bulk density

Table 1: Mean values of the pH of ensiled OPF using different part of frond and ensilage methods

Factors/Item	**pH**
1.Parts of frond (A)	
Total frond	3.62(±0.07)
Proximal cut off	3.58(±0.06)
2.Ensilage methods (B)	
Common ensilage	3.63(±0.10)
Ensiled with molasses as additive	3.58(±0.04)
Ensiled with molasses and inoculums	3.61(±0.04)
Ensiled with PentoZyme®	3.58(±0.06)
Interaction effects	
AB	0.09(±0.06)

Table 2: Means value of the bulk density (g/cc^3) of pelleted ensiled OPF using different parts of frond, ensilage method, dies diameter size and level of binder.

Factors/Item	The bulk density	
	Fresh weight	Dried Weight
Part of frond (A)		
Total fronds	0.82(±0.09)	0.64(±0.08)
Proximal cut off	0.82(±0.08)	0.65(±0.08)
Ensilage method (B)		
Normal ensilage	0.80^{b}(±0.08)	0.63^{B}(±0.07)
Ensiled with molasses as additive	0.84^{a}(±0.06)	0.62^{B}(±0.04)
Ensiled with molasses and inoculums	0.83^{ab}(±0.06)	0.61^{B}(±0.05)
Ensiled with Pentozyme®	0.80^{b}(±0.11)	0.73^{A}(±0.10)
Die's diameter size (C): mm		
8	0.77^{B}(±0.07)	0.61^{B}(±0.06)
10	0.86^{A}(±0.05)	0.69^{A}(±0.08)
Level of Binder (D) (g/kg)		
400	0.80^{B}(±0.08)	0.63^{B}(±0.09)
500	0.83^{A}(±0.08)	0.67^{A}(±0.07)
Interaction effects		
AB	0.07	0.83
AC	0.34	0.55
AD	0.12	0.06
BC	0.07	0.07
BD	0.15	0.34
CD	0.56	0.39
ABC	0.09	0.08
ABD	0.06	0.07
ACD	0.79	0.60
BCD	0.23	0.16
ABCD	0.13	0.20

$^{A\ B\ C\ D}$ = Means with standard deviation in the same column under the same factors with different super script differ significantly (P<0.01)

$^{a\ b\ c\ d}$ = Means with standard deviation in the same column under the same factors with different super script differ significantly (P<0.05)

Table 3: Ruminal dry matter degradation parameters of pelleted ensiled OPF using different part of frond, ensilage method, die diameter size and level of binder (%)

Parameter	Part of frond (A)		P>F	SEM	Ensilage method (B)				P>F	SEM	Die diameter size (C)		P>F	SEM	Level of Binder (D)		P>F	SEM	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	BCD	ABCD
	Total	Proximal cut off			NORM	MOLL	INNOC	PENTO			8 mm	10 mm			400g	500g													
a	56.91	55.78	0.75	0.13	54.01	56.42	57.31	56.15	0.31	0.18	57.24	54.73	0.06	0.03	56.19	55.78	0.75	0.04	0.22	0.70	0.58	0.07	0.13	0.25	0.70	0.17	0.23	0.13	0.64
b	19.36	19.61	0.86	0.15	20.08	19.70	18.99	19.18	0.95	0.21	18.66	20.32	0.25	0.03	17.80^{b}	21.18^{a}	0.02	0.04	0.58	0.81	0.92	0.44	0.11	0.81	0.58	0.90	0.11	0.08	0.10
c	0.04	0.05	0.21	0.00	0.05	0.04	0.04	0.05	0.51	0.03	0.05	0.05	0.15	0.01	0.05	0.05	0.40	0.01	0.36	0.51	0.81	0.07	0.45	0.09	0.75	0.46	0.54	0.18	0.92
ed1	69.11	69.64	0.30	0.06	68.76	69.36	69.68	69.72	0.52	0.07	69.83	68.92	0.08	0.01	69.21	69.55	0.51	0.02	0.83	0.43	0.52	0.97	0.61	0.53	0.50	0.23	0.75	0.78	0.72
ed2	65.39	65.95	0.27	0.36	65.35	65.60	65.92	65.81	0.77	0.07	66.11	65.23	0.11	0.01	66.14	65.20	0.09	0.02	0.80	0.22	0.43	0.76	0.63	0.18	0.81	0.34	0.71	0.81	0.87
ed3	63.53	63.94	0.42	0.05	63.33	63.78	64.08	63.73	0.76	0.07	64.12	63.34	0.12	0.01	64.15^{A}	63.05^{B}	<0.01	0.01	0.74	0.16	0.35	0.75	0.51	0.16	0.89	0.32	0.48	0.81	0.88
Lag time	0.11	0.01	0.21	0.05	0.01	0.01	0.21	0.01	0.44	0.60	0.11	0.01	0.57	0.01	0.01	0.11	0.17	0.01	0.78	0.53	0.68	0.28	0.51	0.80	0.53	0.24	0.90	0.76	0.30
POTDG	75.55	75.39	0.85	0.08	74.09	76.12	76.36	75.33	0.21	75.90	75.90	75.05	0.31	0.02	73.99^{B}	76.96^{A}	<0.01	0.02	0.78	0.86	0.57	0.57	0.39	0.18	0.25	0.12	0.35	0.84	0.14

Degradation constants derived from the Ørskov and McDonald (1979) equation $P = a + b(1-\exp(-ct))$ where P is degradability at time 't', 'a', the rapidly soluble fraction, 'b', the insoluble but fermentable fraction, 'c', the degradation rate of the 'b' fraction. Effective degradation in the rumen at 0.02, 0.05 and 0.08 fraction/h passage rate is represented by ed1, ed2 and ed3, respectively and is calculated by using the Neway Excel: a curve fitting programs for processing feed degradability data written by Chen (1995).

POTDG: Potential degradability (a+b).

NORM: Normal ensilage; MOLL: Ensiled with molasses as additive; INNOC: Ensiled with molasses and inoculums; PENTO: Ensiled with Pentozyme®

$^{A, B}$ Mean in the same row under the same factors not having at least a common superscript differ significantly (P<0.01).

$^{a, b}$ Mean in the same row under the same factors not having at least a common superscript differ significantly (P<0.05).

SEM: Standard error of the means

REFERENCES

Aim-oeb, J. 2008. Potential of oil palm fronds for ruminants feed. Master of Science thesis. Kasetsart University, Thailand. 133 pp.

Chen, X. B. 1995. Neway Excel, An Excel Applications Program for processing feed degradability data User Manual.

Dahlan, I., M. Islam, M. A. Rajion. 2000. Nutrient Intake and Digestibility of Fresh, Ensiled and Pelleted Oil Palm (Elaeis guineensis) Frond by Goats. Asian Australas. J. Anim. Sci. 2000;13 (10): 1407-1413.

Department of agricultural extensions of Thailand. 2005. Meeting report. Available Source: http://eoffice.doae.go.th:8080/CDMS2/viewimg.php?tid=6569&pageno=3

Insung, O., A. Songsang, J. Noola-aong. 2014. Development of the use of oil palm fronds for goats feed. Research report of the Rajamangala University of Technology Srivijaya. 35 pp

Koç, B., Eren, I., Ertekin, F. K. 2008. Modelling bulk density, porosity and shrinkage of quince during drying: The effect of drying method. Journal of Food Engineering, Volume 85, Issue 3, April 2008 Pages 340-349.

McDonald, P.; A. R. Henderson and S.J. E. Heron. 1991. The biochemistry of silage. Chalcombe pubication, England. 340 pp.

Ørskov, E. R. and I. McDonald.1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agric. Sci. (Cambridge). 92:499-503.

Rotger A., A. Ferret, S. Calsamiglia and X. Manteca. (2006): Effects of nonstructural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism *in vivo* and *in vitro* with high-concentrate beef cattle diets. Journal of Animal Science, 84, 1188–1196.

Steel, R.G.D. and J. H. Torrie. 1981. Principle and Procedures of Statistics: A Biometrical Approach 2nd. ed. McGraw-Hill Book Company, Singapore. 633 pp.

Wan Zahari, M. O. Abu Hassan1,H. K. Wong and J. B. Liang. 2003. Utilization of Oil Palm Frond - Based Diets for Beef and Dairy Production in Malaysia. *Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 625-634.*

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Voluntary feed intake, rumen fermentation and microbial protein synthesis of beef cattle fed fermented cassava starch residue

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INTRODUCTION

During the last 15 years, the use of grain and tuber in animal feed rations has gained more importance for intensive animal production. Rising prices of carbohydrate sources for cattle such as corn meal, cassava chip and rice bran has resulted in a search for cheaper feedstuffs to reduce diet cost. Several industrial by-products have been found as substitutes accordingly, some principles must be considered in utilization of those alternative feedstuffs such as nutrient content and their variability, spoilage and subsequent mold growth, and toxicity. Cassava by-products have widespread use in the nutrition of many livestock especially in ruminants. Cassava starch residue (CSR) is a source of non-forage fiber which has potential for cattle such as beef and lactating dairy cattle while also controlling feed costs (Bradford and Mullins, 2012). However, there are limitations to the use of CSR as occasioned by their low protein and high fiber (the non-starch polysaccharides) content. Therefore, intensive efforts have been made to reduce these constraints to CSR utilization. Nutritive value of CSR may be increased by further additions of supplements followed by microbial fermentation (Aro *et al.,* 2008 Khampa *et al.,* 2009). Protein content in cassava pulp was increased by fermentation with *Saccharomyces cerevisiae* (Khampa *et al.,* 2009), or a combination of fungi and bacteria (Aro *et al.*, 2008). Disruption of the fibrous structure of CSR, allowing fermentation by micro-organisms, has been made by enzymatic treatment (Sriroth *et al.,* 2000). Our previous study (Pilajun and Wanapat, unpublished) found that addition of urea and molasses to fermentation of CSR with yeast (*Saccharomyces cerevisiae*) or with effective microorganisms (EM) increased crude protein and potential of *in vitro* gas production whereas, exogenous enzyme supplementation was ineffective in enhancing fermentation characteristics. Therefore, supplementation with fermented cassava starch residue could improve rumen fermentation, microbial protein synthesis, and utilization of nitrogen by beef cattle.

MATERIALS AND METHODS

The experiment was conducted under the control and advice of the Office of Experimental Field and Central Laboratory, Faculty of Agriculture, Ubon Ratchathani University, Warinchamrap, Ubon Ratchathani, Thailand, 34190.

Experimental design and treatment

Four Thai-native x Lowline Angus crossbred beef cattle with initial body weight (BW) of 130±30 kg were randomly assigned to a 4 × 4 Latin square design. Fermented CSR with four types of supplement including non-supplement (the control, NSFC), effective microorganism (EM Kyusei®) (EMFC), yeast (*Saccharomyces cerevisiae*) (YFC), and yeast with exogenous enzyme (A-Zyme F1, ASAH®) (YEFC), were used as a main carbohydrate source in concentrate. Urea (3.3% DM) and molasses (4.2% DM) were added to fermentation of CSR in last three groups, according to the method of Khampa et al. (2009). Rice straw was fed as roughage diet *ad libitum* allowing for 10% of refusals. Concentrate diets were formulated prior to blending with fermented CSR, concentrate 1 blended with NSFC while concentrate 2 blended with others, at feeding time, and then fed to cattle at 0.75% of BW. Ingredient and chemical composition of experimental diets are showing in Table 1. All cattle were kept in individual pens, and clean fresh water and mineral blocks were available at all times. The experiment took place over 4 periods, and each lasted 21 d. During the last 7 d of each period, cattle were moved to metabolism crates for total urine and fecal collection with feed restricted to 90% of the previous voluntary feed intake of straw.

Data collection and sample analysis

Voluntary feed intake of roughage was recorded daily in the morning. Feeds were sampled and fecal samples were collected at the morning of the last 5 d of each period. Feeds, refusals and fecal samples were dried at 60℃

and ground (1mm screen) and then analyzed for DM (Method #930.15), ash (Method #923.03), and CP (Method #976.06) using standard methods of AOAC (1995), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Goering and Van Soest (1970). At the end of each period, 200 ml of rumen fluid were collected before morning feeding by using a stomach tube connected with a vacuum pump. Rumen fluid sample was then filtered through 4 layers of cheesecloth before being divided into 2 portions 1 portion was used for NH_3-N analysis with 5 mL of 1 *M* H_2SO_4 added to 45 mL of rumen fluid. The mixture was centrifuged at 16,000×*g* for 15 min, and the supernatant was stored at -20℃ before NH_3-N analysis using the Kjeltech Auto 1030 Analyzer and VFA analysis using Waters Alliance 2695 HPLC System (Massachusetts, USA). The chromatographic conditions were optimized: column Symmetry C_{18}, mobile phase consisting of H_2SO_4 0.005 mol/L, flow rate of 1.0 mL/min and temperature of 55℃ . The VFAs were analyzed by photodiode array (Waters® model 2996, USA) at 210 nm. The second portion of rumen fluid was fixed with 10% formalin solution in sterilized 0.85% saline solution. The total direct count of protozoa and fungal zoospores was made by the methods of Galyean (1989) based on the use of a hemocytometer. Urine sample was analyzed for total nitrogen for calculation of nitrogen balance. For estimation of microbial protein synthesis, urinary purine derivative (PD) excretion was analyzed by Waters Alliance 2695 HPLC System (Massachusetts, USA). The separation was carried out at 25 ºC temperature using a Symmetry C18 column (5 µm, 15 x 0.46 cm), mobile phase consisting of 0.05 M $(NH_4)_2HPO_4$ with pH 7.78, and flow rate of 1.0 mL/min. The PD was detected by photodiode array (Waters® model 2996, USA) at 218 nm. The supply of microbial N (MN) was estimated by the urinary excretion of PD according to the equations of Chen and Gomes (1995) as follows:

$Y = 0.85X + (0.385\ BW^{0.75})$

$MN\ (g/d) = 70X / (0.116x0.83x1000) = 0.727X$

where X and Y are, respectively, the absorption and excretion of PD in mmol/d. The N content of purines was 70 mg/mmol, the ratio of purine N to total N in mixed rumen microbes was 0.116, mean endogenous contribution of urinary purine derivative excretion was 0.385 mmol/kg $BW^{0.75}$ (Verbic et al., 1990), digestibility of microbial purines in the intestines was estimated at 83% (Chen and Gomes, 1995), and recovery of absorbed purines as urinary purine derivatives was assumed to be 85% (Verbic et al., 1990). Efficiency of microbial protein synthesis (EMPS) was calculated using the following formula:

EMPS = microbial N (g/d) / DOMR

where DOMR (digested OM in the rumen, kg/d) = DOMI (digestible OM intake) x 0.65 (ARC, 1990).

Statistical analysis

All 4 × 4 Latin square design data were statistical analyzed using the SAS (1996) GLM procedure according to the model:

$Yijk = \mu + Ti + Aj + Pk + \varepsilon\ ijk$

where *Yijk* = observation from beef *j*, receiving diet *i*, in period *k* μ = the overall mean, *Ti* = the effect of the treatment *i*, *Aj* = the effect of animal *j*, *Pk* = the effect of period *k*, and εijk = residual effect. Results were presented as mean values with the standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test (DMRT) with P.

RESULTS AND DISCUSSION

Crude protein content of non-supplement fermented CSR was 2.0% DM while other fermented CSR ranged between 11.0-12.0% DM (Table 1), according to urea and molasses addition. However, proportion of fiber (NDF, ADF) in fermented CSR was not affected by various sources of supplement. These results agree with previous studies which found that protein content was increased according to supplementation of urea and molasses while enzyme treated did not affect the chemical composition of fermented CSR (Pilajun and Wanapat, unpublished). Rehman et al. (2014) found that enzyme did not affect the nutrient profile of wheat straw because of alkaline pH due to rapid production of ammonia in the silo. However, a dose of 3 L of enzyme per ton of fibrous feed product improved the DM, NDF and ADF degradation of rice straw, distillers dried grains with soluble and their mixture (Gado et al., 2013). Therefore, dose of exogenous enzyme and ensiling condition used for fermentation of CSR could be further investigated.

Voluntary feed intake and digestibility

Dietary feed intakes and digestibility of beef cattle was similar between treatments (Table 2). The result agrees with Kaewwongsa et al. (2011) who found that although crude protein content of fermented cassava

pulp was increased with level of yeast, *in vitro* dry matter disappearance was not altered. In terms of effective microorganism utilization, significant improvement in nutritive value and dry matter degradability of agricultural residues ensiled with the use of EM as inoculants has been found (Yesuf, 2010). Lack of EM effects in this study could be explained by an already high level of degradable fraction of fermented CSR (76% DM) as reported by Pilajun and Wanapat (unpublished). There is limited data related to exogenous enzyme applied to cassava starch residue and used as ruminant feed however, enzyme treated cassava pulp produced a high yield of free sugar (Virunanon et al., 2013) as well as disruption of the fibrous structure of pulp (Sriroth et al., 2000). On the other hand, the nutritive value and fermentation, and *in vitro* dry matter and aNDF digestibility of silage can be improved by treating it with fibrolytic enzymes (Xu et al., 2011). Moreover, exogenous fibrolytic enzymes can improve cell wall digestion and the efficiency of feed utilization of ruminants by altering fiber cell wall structures (Beauchemin et al., 2003).

Rumen fermentation and microbial population

Although concentrations of ammonia nitrogen and volatile fatty acid in rumen fluid of beef cattle were variable, there were not significant differences between treatments (Table 3). YFC fed group trended to be higher in ammonia concentration (P<0.08) when compared with other treatments. These may have resulted from high content of NPN in yeast fermented CSR (60.0% of CP, Pilajun and Wanapat, unpublished). Availability of soluble N source in rumen fluid allowed microbes to utilize and release ammonia N into rumen contents. Trend to a higher propionic acid proportion in the rumen of cattle fed with EMFC than YFC fed group may indicate that several group of microbes in EM may have degraded CSR and released more soluble carbohydrate than yeast fermentation. Filamentous fungi from EM could secrete more amylase to degrade carbohydrate molecule and release free sugar than mono-cellular fungi or yeast (Saranraj and Stella, 2013). Moreover, Shigechi et al. (2004) found that *Saccharomyces cerevisiae* yeast cannot produce enzyme amylase and utilize starchy materials especially when growing under anaerobic conditions. However, the similar volatile fatty acid concentration between treatments indicates that degradation of CSR by ruminal microorganism was not impacted by type of CSR fermentation. Protozoal population in the rumen of beef cattle fed with YEFC was higher than the control (P<0.05) but not different from EMFC and YFC fed groups while number of fungal zoospores in rumen fluid was not affected by treatment. The number of protozoa in the rumen increased with increasing available starch and sugar in rumen fluid (Newbold et al., 2015) therefore, fermented CSR with yeast and exogenous enzyme may produce a high fraction of free sugar.

Nitrogen balance and microbial protein synthesis

The control had higher nitrogen intake than other groups (P<0.05), and trended to higher in nitrogen absorbed than YFC fed group (P<0.06). However, nitrogen retained was not different when compared with other groups (Table 4). Different nitrogen intake but similar total dry matter intake among groups could result from nitrogen content in feedstuff in concentrate particularly soybean meal and palm kernel meal. However, nitrogen absorbed was similar between treatments although the control trended to higher than YEFC fed group (P<0.06). Nitrogen retention of cattle fed with the control trended lower than YEFC fed group, 38.9 and 46.3% N intake respectively. This may be an effect of the source of nitrogen contained in feed ingested. Cattle in the control group were offered urea directly from concentrate while other groups may have accessed remaining urea in fermented CSR. Our previous study (Pilajun and Wanapat, unpublished) found that non-protein nitrogen content in yeast, EM, and yeast plus fibrolytic enzyme fermented CSR was 50.4, 59.5 and 60.4% respectively. The assimilation of NPN by rumen microbes had low effectiveness when higher than microbial requirement. However, the present study found ammonia nitrogen concentration in the rumen was in the normal range (15.0-30.0 mg/dL) as reported by Wanapat and Pimpa (1999). It indicated that levels and proportion of NPN in feed especially in fermented cassava starch residue did not exceed the requirement of rumen microorganisms.

Microbial protein synthesis and efficiency of microbial protein synthesis in cattle fed with YEFC was significantly higher than EMFC and YFC groups (P<0.05) but similar to the control. These indicate that exogenous enzymes supplementation to increase quality of fermented CSR also increases the fermentation process and microbial growth in the rumen. Exogenous enzymes may demolish structure of fiber contained in CSR allowing yeast and rumen microbes to degrade and utilize it as their energy source. Sriroth *et al.* (2000) reported that disruption of the fibrous structure of CSR, allowing fermentation by micro-organisms, has resulted from enzymatic treatment. Moreover, fermentation of CSR with microbes improved both quantity and quality of protein fraction (Aro *et*

al., 2008 Khampa *et al.,* 2009) which raised its usefulness as an animal feed. True protein content of CSR was increased 3.4% DM when fermented with yeast plus exogenous enzymes (Pilajun and Wanapat, unpublished). Besides, synchronizing of energy and nitrogen available in the rumen is also important for microbial utilization rather than other factors (Seo et al., 2013). Therefore, supplementation of exogenous enzyme to fermentation of CSR with yeast (*Saccharomyces cerevisiae*) could provide an appropriate level of available starch and free sugar, and a degradable nitrogen source for rumen microbes to synthesize their own protein, subsequently available to the ruminant host.

Conclusion, fermentation of CSR with yeast (*Saccharomyces cerevisiae*) and fibrolytic enzymes improves their nutritional properties particularly enhancing of rumen fermentation, nitrogen utilization, and microbial protein synthesis.

Conflict of Interest Statement

There are no conflicts of interest in this study.

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KEYWORD : Saccharomyces cerevisiae, Effective microorganism, Exogenous enzyme, Cassava starch residue, Rumen fermentation

Table 1. Ingredients and chemical composition of experimental diet (%DM)

Items	Concentrate 1	Concentrate 2	NSFC	EMFC	YFC	YEFC	Rice straw
Ingredient composition							
Corn meal	22.2	32.5					
Rice bran	11.0	12.0					
Soybean meal	31.0	18.0					
Palm kernel meal	19.0	23.0					
Fish meal	10.0	10.0					
Urea	2.0	-					
Molasses	2.0	2.0					
Palm oil	1.0	1.0					
Salt	0.5	0.5					
Sulfur	0.3	-					
Mineral mixture	1.0	1.0					
Chemical composition							
Dry matter	91.0	90.9	15.2	14.4	14.5	15.5	91.4
Organic matter	93.0	93.6	96.9	96.9	97.1	96.3	84.4
Crude protein	30.9	21.0	2.13	14.4	12.5	13.5	4.18
Neutral detergent fiber	21.9	23.7	41.8	42.7	44.8	42.6	70.4
Acid detergent fiber	13.3	13.4	15.2	15.6	16.5	16.9	46.8

FC: fermented cassava starch residue, NSFC: non-supplement FC, EMFC: effective microorganisms FC, YFC: yeast (*Saccharomyces cerevisiae*) FC, YEFC: yeast and exogenous enzyme FC

Table 2. Voluntary feed intake and nutrient digestibility of beef cattle fed with fermented cassava starch residue (FC)

Items	NSFC[1]	EMFC	YFC	YEFC	SEM	P-value
Total feed intake						
kg/d	3.01	3.02	3.03	2.98	0.06	0.86
% BW	2.38	2.39	2.41	2.38	0.03	0.93
g/kg BW^{75}	79.7	80.1	80.6	79.5	0.94	0.92
Roughage intake						
kg/d	2.08	2.05	2.04	2.01	0.05	0.73
% BW	1.64	1.62	1.62	1.61	0.03	0.86
g/kg BW^{75}	55.1	54.3	54.2	53.7	0.99	0.82
Concentrate intake, kg/d	0.62	0.62	0.63	0.62	0.01	0.40
FC intake, kg/d	0.34	0.36	0.37	0.35	0.02	0.36
Nutrient digestibility, % DM						
Dry matter	61.7	63.7	62.8	64.7	1.10	0.33
Organic matter	60.7	63.8	62.4	64.6	1.24	0.22
Crude protein	69.3	69.2	64.3	70.5	2.02	0.24
Neutral detergent fiber	58.3	60.3	60.3	61.9	1.16	0.56
Acid detergent fiber	60.7	63.4	64.8	65.5	2.19	0.48

[1] NSFC: non-supplement FC, EMFC: effective microorganisms FC, YFC: yeast (*Saccharomyces cerevisiae*) FC, YEFC: yeast and exogenous enzyme FC

SEM: standard error of the mean

Table 3. Fermentation and microbial population in the rumen of beef cattle fed with fermented cassava starch residue (FC)

Items	NSFC[1]	EMFC	YFC	YEFC	SEM	P-value
Ammonia nitrogen, mg/dL	15.7	15.3	21.9	13.5	2.74	0.08
Total volatile fatty acid (TVFA), mmol/L	106.4	91.3	110.9	88.9	9.28	0.36
Acetic acid (C2), % TVFA	59.4	54.3	63.4	62.7	3.26	0.20
Propionic acid (C3), % TVFA	33.9	38.7	28.6	32.4	3.20	0.07
Butyric acid (C4), % TVFA	6.66	6.96	8.01	4.95	0.71	0.18
C2:C3 ratio	2.23	1.75	2.70	2.57	0.46	0.15
Protozoa, log cell/mL	3.5^{b}	4.2^{ab}	4.1^{ab}	6.2^{a}	0.64	0.04
Fungal zoospore, log cell/mL	4.7	5.3	4.9	5.5	1.20	0.72

[1] NSFC: non-supplement FC, EMFC: effective microorganisms FC, YFC: yeast (*Saccharomyces cerevisiae*) FC, YEFC: yeast and exogenous enzyme FC

SEM: standard error of the mean

Table 4. Nitrogen utilization and microbial protein synthesis of beef cattle fed with fermented cassava starch residue (FC)

Items	NSFC[1]	EMFC	YFC	YEFC	SEM	P-value
Nitrogen utilization						
Nitrogen intake, g/d	45.8[a]	42.6[b]	42.1[b]	41.6[b]	0.82	0.03
Nitrogen absorbed, g/d	31.6	29.5	26.9	29.3	0.67	0.06
Nitrogen retained, g/d	17.8	19.2	18.2	19.4	1.22	0.74
Nitrogen retention, % N intake	38.9	45.2	43.6	46.3	2.71	0.32
Microbial protein synthesis[2]						
PD excretion, mmol/d	62.7[ab]	50.2[b]	56.3[b]	78.0[a]	5.06	0.02
PD absorption, mmol/d	56.6[b]	42.0[b]	49.2[b]	74.8[a]	5.76	0.01
Microbial N supply, g N/d	41.2[ab]	30.5[b]	35.8[b]	54.3[a]	4.33	0.02
Efficiency of microbial protein synthesis, g N/kg DOMR[3]	39.4[ab]	27.8[b]	33.2[b]	50.2[a]	7.71	0.02

[1] NSFC: non-supplement FC, EMFC: effective microorganisms FC, YFC: yeast (*Saccharomyces cerevisiae*) FC, YEFC: yeast and exogenous enzyme FC

[2] PD: purine derivatives (Chen and Gomes, 1995)

[3] DOMR: digested OM in the rumen (650 g/kg DM of organic matter digestible in total tract) according to ARC (1990)

SEM: standard error of the mean

REFERENCES

Agriculture Research Council. 1990. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux International, Agricultural Research Council, Wallingford, UK., pp. 59-71.

AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemistry. 16th Edn., AOAC International, Washington, USA., Pages: 1141.

Aro, S. O., V. A. Aletor, O. O. Tewe, A. N. Fajemisin, B. Usifo and A. B. Falowo. 2008. Preliminary investigation on the nutrients, anti-nutrients and mineral composition of microbially fermented cassava starch residues. Proc. 33rd Annual Conf., Nigerian Society for Animal Production (NSAP), Ayetoro, Ogun State, Nigeria: pp 248-251.

Beauchemin, K. A., D. Colombatto, D. P. Morgavi and W. Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81 (E. Suppl. 2): E37–E47.

Bradford, B. J. and C. R. Mullins. 2012. Invited review: Strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. J. Dairy Sci. 95: 4735-46.

Chen, X. B. and M. J. Gomes. 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivative–an overview of the technique details. Occasional Publication 1992, International Feed Resources Unit, Rowett Research Institute; Aberdeen, UK.

Gado, H. M., A. Z. M. Salem, L. M. Camacho, M. M. Y. Elghandour and M. C. Salazar. 2013. Influence of exogenous enzymes on in vitro ruminal degradation of ensiled rice straw with DDGS. Anim. Nutr. Feed Tech. 13: 569-574.

Galyean, M. 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science, New Mexico state University, U.S.A. 193 pp.

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some application). Agric. Handbook No. 379, ARS, USDA, Washington, DC.

Kaewwongsa, W., S. Traiyakun, C. Yuangklang, C. Wachirapakorn and P. Paengkoum. 2011. Protein enrichment of cassava pulp fermentation by Saccharomyces cerevisiae. J. Anim. Vet. Adv. 10: 2434-2440.

Khampa, S., P. Chaowarat, R. Singhalert, R. Pilajun and M. Wanapat. 2009. Supplementation of yeast fermented cassava chip as a replacement concentrate on rumen fermentation efficiency and digestibility of nutrients in heifer. J. Anim. Vet. Adv. 8: 1091-1095.

Newbold, C.J., G.d.l. Fuente, A. Belanche, E. Ramos-Morales and N.R. McEwan. The role of ciliate protozoa in the rumen. Front. Microbiol. 6: 1313.

Raman, A., S. T. Baum, R. J. Colman, J. W. Kemnitz, R. Weindruch and D. A. Schoeller. 2007. Metabolizable energy intake during long-term calorie restriction in rhesus monkeys. Exp. Gerontol. 42: 988-994.

Rehman, A. U. M. U. Nisa, A. Shazad, M. Sarwar, O. A. Khan and M. Sharif. 2014. J. Anim. Plant Sci. 24(Suppl. 1): 36-39.

Saranraj, P. and D. Stella. 2013. Fungal Amylase - A Review. Int. J. Microbiol. Res. 4: 203-211.

SAS, User's Guide: Statistic, Version 5. Edition. 1996. SAS. Inst Cary, NC., U.S.A.

Seo, J. K., M. H. Kim, J. Y. Yang, H. J. Kim, C. H. Lee, K. H. Kim and J. K. Ha. 2013. Effects of synchronicity of carbohydrate and protein degradation on rumen fermentation characteristics and microbial protein synthesis. Asian-Aust. J. Anim. Sci. 26: 358-365.

Shigechi, H., J. Koh, Y. Fujita, T. Matsumoto and Y. Bito. 2004. Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplayingglucoamylase and alpha-amylase. Appl. Environ. Microbiol. 70: 5037-5040.

Sriroth K., R. Chollakup, S. Chotineeranat, K. Piyachomkwan and C. G. Oates. 2000. Processing of cassava waste for improved biomass utilization. J. Biores. Technol. 71: 63-69.

Verbic, J., X. B. Chen, N. A. MacLeod and E. R. Orskov. 1990. Excretion of purine derivatives by ruminants: effects of microbial nucleic acids infusion on purine derivative excretion by steers. J. Agric. Sci. (Camb). 114: 243-248.

Virunanon, C., C. Ouephanit, V. Burapatana and W. Chulalaksananukul. 2012. Cassava pulp enzymatic hydrolysis process as a preliminary step in bio-alcohols production from waste starchy resources. J. Cleaner Prod. 39: 273–279.

Wanapat, M. and O. Pimpa. 1999. Effect of ruminal NH3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian-Aust. J. Anim. Sci. 12: 904-907.

Xu, Ch., H. Wang, F. Yang and Zh. Yu. 2011. Effect of an inoculant and enzymes on fermentation quality and

nutritive value of erect milkvetch (Astragalus adsurgens Pall.) silages. J. Anim. Feed Sci. 2011: 449–460.
Yesuf, Y. K. 2010. Chemical composition and in vitro digestibility of coffee pulp and coffee husk ensiled with grass (Hyperchennia hirta) hay and effective microorganisms (EM). The thesis for the degree of master of science, College of Agriculture, and Veterinary Medicine, Jimma University, Ethiopia.

O-33-5

The influence of sowing date and trellising on the flowering of some promising herbaceous legumes for eastern Indonesia

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Objective

Cattle production can markedly improve the livelihoods of small farmers in eastern Indonesia through the production of a high-value ready-saleable product (feeder cattle, beef meat and hides) while utilising (and contributing to) the resources available (land, labour and farming expertise) in many small-holder systems (Nulik et al., 2013). However, cattle require large amounts of high-quality (digestible energy and protein) feed to achieve optimum animal growth and reproductive performance (Nulik and Bamualim, 1998 Bamualim and Wirdahayati, 2002 Mullik and Jelantik, 2003) and this can limit the benefit of growing cattle to farmers.

The availability of high quality feed limits cattle growth rates in seasonally dry areas of eastern Indonesia. Naturally occurring or planted grasses comprise the key component of the diet, but production and feed quality is seasonal, both declining in the dry season. As a result, animals will often grow poorly, or lose weight, at this time of the year without supplementary feeding: up to 60% of weight gained during the wet season to early dry (January to May) may be lost during the dry season to early wet (May to December) (Nulik and Bamualim, 1998 Bamualim and Wirdahayati, 2002 Mullik and Jelantik, 2003).

A range of supplementary feeding (e.g. high quality forages such *Gliricidia sepium* and *Lannea* species for protein and 'palm pith' for energy (Nulik and Bamualim, 1998 Bamualim and Wirdahayati, 2002 Copland et al., 2011)) and animal management strategies (e.g. sale, restricted calving period) can be used to overcome feed deficits, but these can be expensive or not easily adopted by smallholder farmers.

In an Indonesia-Australia research partnership, a number of herbaceous legumes, particularly *Centrosema pascuorum*, *Clitoria ternatea* and *Lablab purpureus*, were identified as having a role in overcoming seasonal feed deficits when incorporated into maize- and rice-based smallholder mixed farming systems (Dalgliesh et al., 2013). The legumes, grown either as an intercrop (maize only) or in rotation (maize and rice), efficiently utilise soil available water and can be fed either directly as green forage or conserved as hay to livestock (cattle and goats) (Nulik et al., 2013).

Adoption of the legumes by farmers relies on the availability of inexpensive, high-quality seeds for sowing. Within-region or local production of seed is desirable because imported seed is expensive and can be difficult to source. The potential to grow the key legumes for seed in eastern Indonesia was previously identified in non-replicated studies, and preliminary guidelines were developed to aid adoption (Nulik et al., 2013). Seed production was inconsistent, however. To better understand regional limitations to seed production and refine seed production practices, replicated studies were conducted using a range of sowing dates at sites of varying latitude and elevation in eastern Indonesia and north-eastern Australia.

Methodology

The experimental program was conducted over three years, initially in north-eastern Australia (north-eastern Queensland) using spaced plants to develop experimental principles for the subsequent research in eastern Indonesia using small-plot studies (Table 1). Replication was used at all sites. Five legumes were studied at one site in Australia (~17° S) and four legumes at four sites in Indonesia (~10° S). The sites included a range of elevation (~40-900 m asl) and soil types (red and black clay soils of differing origin). The sowing date treatments spanned November to August at the Australian site and February to August (with one late sowing at one site during December) in Indonesia. Fertiliser and irrigation were only applied at the Australian site and not at the Indonesian sites as for current practice.

Measures were standardised across the sites where possible using 0.7 x 0.7 m quadrats placed either around individual plants (Australia) or onto permanently marked positions within plots (two per plot). The measurements included: plant populations (4 weeks after sowing) number of nodes (usually weekly) with expanded inflorescences number of mature pods and, at some sites and following drying and threshing pods, the number and weight of

seeds (only the flowering data are presented here). Each crop cycle was monitored for 6-8 months after sowing. Total daily rainfall and mean daily air temperature were recorded using data from on-site data loggers.

At the conclusion of the November-sown growth cycle at the Australian site (6 June 2014), the individual plants of legumes which had previously shown a substantial flowering response to trellising were cut to 5 cm, all pods removed from the plants and counted and both components dried at 70℃ until constant weight and subsequently re-weighed. Simple statistics (means and standard errors) and Fischer's least significant difference procedure (P=0.05) were used to compare means of the variables.

Table 1. Experiment site characteristics and key treatments.

Indice		Walkamin	Kupang	Soe	Tobu	Ende
Region		Queensland	West Timor	West Timor	West Timor	Flores
Latitude	°S	17.1	10.0	10.0	10.0	9.9
Elevation	m asl	630	50	900	850	400
Soil type		Acidic red clay (ferrosol)	Alkaline black clay (vertosol)		Alkaline black clay (vertosol)	
Legumes[1]		CP1, CP2, CT, LP, MB, VP	CP1, CT, LP, VL		CP1, CT, LP, VL	
Sowing times		25 Nov. 2013	27 Mar. 2015	4 Feb. 2015	11 Feb. 2015	30 Mar. 2015
		7 April 2014	27 May 2015	9 Apr. 2015	02 Apr. 2015	15 May 2015
		29 Aug. 2014	27 Aug. 2015	6 Jun. 2015	08 Jun. 2015	30 June 2015
						15 Dec, 2015
Design		Individual plants	Small plots: 3 x 1.2 m		Small plots: 2 x 1.2 m	
Replicates		30 plants/ species, 3 blocks	4		3	
Plant spacing		70 x 70 cm	40 cm rows x 10 cm within rows		40 cm rows x 10 cm within rows	
Trellis+/-		All legumes	CT	CT	CT	CT
Basal fertiliser	kg/ha	17.6 P, 22 S, 50 K	none	none	none	none
Irrigation		dry season	none	none	none	none

[1] CP1 = *Centrosema pascuorum* 'Bundey'; CP2 = *C. pascuorum* 'Cavalcade'; CT = *Clitoria ternatea* 'Milgarra'; LP= *Lablab purpureus* 'Highworth'; MB = *Macroptilium bracteatum* 'Juanita'; VL = *Vigna luteola*' Dalrymple', VP = *Vignaparkeri* 'Shaw'.

Results

The effect of location, sowing date and trellising on the onset of flowering

Sowing time influenced the period from sowing to the onset of flowering for all of the legumes studied as vigorously-growing, spaced plants in north Queensland (~17 ° S)(Table 2). However, the magnitude of the effect varied considerably between the four species: *Centrosema pascuorum* 'Bundey' and 'Cavalcade' and 'Highworth' *Lablab purpureus* had flowering patterns indicative of strong 'short-day' responses for flowering (*i.e.* flowering earlier when sown closer to the onset of short days) whereas 'Milgarra' *Clitoria ternatea* and 'Juanita' *Macroptilium bracteatum* flowered readily regardless of sowing date, but the period to the onset of flowering was delayed at cooler periods of the year ('day-neutral' response). 'Bundey' *C.Pascuorum* flowered approximately 3 weeks later than 'Cavalcade'. These responses are consistent with previous reports for these species grown in northern Australia (Cook *et al*., 2005) and indicate there is more flexibility for sowing time when considering seed crops of 'Milgarra' and 'Juanita' compared to 'Highworth', 'Bundey' and 'Cavalcade'. 'Shaw' *Vigna parkeri* produced no or few inflorescences after all sowing dates.

Daylength had a lesser effect on the time to onset of flowering when the legumes were grown in small plots at lower latitudes (~10 ° S) in Indonesia and onset to flowering was considerably earlier in 'day-neutral' 'Milgarra' than when grown in the upland north Queensland environment (Table 4). The 'short-day' flowering response in 'Bundey' *C. pascuorum* remained strong in eastern Indonesia, whereas sowing time between February and June had relatively little effect on the period to onset of flowering in 'Highworth' *L.purpureus*. 'Dalrymple' *V. luteola* showed a strong short-day response for flowering. Upland and lowland sites appeared suitable for seed production, although the onset of flowering tended to be delayed in upland compared to lowland sites.

The effect of trellising on flowering

Simple pole trellises increased the number of reproductive nodes produced in the 6 (summer) to 8 (winter)

months after sowing by 48 to 91% in spaced 'Milgarra' *C ternatea* plants grown in north Queensland (Table 3), but had no measurable effect in the *C. pascuorum, L. purpureus* and *M. bracteatum* cultivars (data not presented). The effect was strong at all sowing times, but greatest when the plants were grown over the hot spring and summer periods under irrigation. Trellis effects on flowering continued through to pod and seed production with the November sown plants grown with trellises having 53% greater pod weight (from 51% more reproductive nodes) than plants grown without trellises (data not presented).

The use of trellises on 'Milgarra' plots grown in eastern Indonesia also increased the number of reproductive nodes, but the responses were less (no response up to 64% increase) than observed in north Queensland (Table 4). The Indonesian grown plants were, however, considerably smaller and less actively covered the trellises than the north Queensland plants having been grown without fertiliser or irrigation to supplement rainfall (the plants grown at the upland site at Tobu grew the most vigorously and had the greatest response to flowering). The presence of pole trellises had no effect on the period from sowing to flowering for all legumes studied in north Queensland or Indonesia.

Conclusions

The onset of flowering of the *pascuorum, L. purpureus* and *V. luteola* cultivars studied is likely influenced by short-days in eastern Indonesia, but the response in 'Highworth' *L. purpureus* is weaker than in north Queensland. Shorter-duration seed crops can be achieved by sowing in the six months before the shortest day, which is usually coincident with favourable growing conditions.The flowering of 'Milgarra' *ternatea* and 'Juanita' *M. bracteatum* cultivars studied appear less influenced by daylength. Seed crops can be attempted all year provided conditions are suitable for vigorous growth.The onset of flowering tends to be later in cooler upland environments in eastern Indonesia than warmer coastal areas. Earlier sowing may benefit seed production in these cooler environments.

Table 2. Effect of time of sowing on the time to flowering of spaced plants of selected forage legumes grown in north Queensland.

Crop cycle		Mean[1] days from sowing to flowering			
Period of crop cycle	**Days[2] (GDD)[3]**	**Bundey (CP1) *Cavalcade*(CP2)**	**Milgarra (CT)**	**Highworth (LP)**	**Juanita (MB)**
25 Nov 2013 - 6 June 2014	193 *(4575)*	148.1 c *122.2* b	67.9 a	124.0 b	93.3 a
7 April 2014- 10 Dec 2014	247 (5180)	64.1 a	184.2 c	86.7 a	117.0 b
29 Aug. 2014 - 10 Mar. 2015	193 (4692)	DNF	99.0 b	DNF	98.7 a
F-statistic		<0.001	<0.001	<0.001	<0.001

1 Means with the same letter are not significantly different (P=0.05): DNF = did not flower

2 Days over the monitoring cycle (days)

3 Growing degree days: total daily mean temperature (°C)

Table 3. The influence of pole trellises on the time to flowering and total number of flowering nodes for spaced *Clitoria ternatea* 'Milgarra' plants grown in north Queensland.

Trellis treatment	Mean[1] days from sowing to flowering (DF) and total flowering nodes per plant (FN)					
	25 Nov 2013 - 6 June 2014 (193 days/4575 GDD[2])		**7 April 2014- 10 Dec 2014** (247 days/5180 GDD)		**29 Aug. 2014 - 10 Mar. 2015** (193 days/4692 GDD)	
	DF	**FN**	**DF**	**FN**	**DF**	**FN**
Without trellis	67.1	289.7 b	192.1 b	62.7	99.3	105.1
With trellis	68.7	439.5 a	180.3 a	92.9	99.8	201.2
F-statistic	0.391	0.012	0.03	0.082	0.719	0.004
Trellis effect (%)		+51.7		+48.2		+91.4

1 Means with the same letter are not significantly different (P=0.05):

2 Growing degree days: total daily mean temperature (°C)

Table 4. The influence of location, sowing date and simple pole trellises (*Clitoria ternatea* only) on the flowering of selected forage legumes grown in eastern Indonesia.

Site	Sowing date (2015)	Mean (standard error) days from sowing to flowering					Trellis effect on total CT flowers (%)
		Bundey (CP1)	Milgarra (CT) No trellis	Milgarra (CT) with trellis	Highworth (LP)	Dalrymple (VL)	
Kupang	27 Mar.		42.4 (1.09)	42.0 (3.07)	47.0 (0.66)		-6.4
	20 Apr.[1]	77.4 (1.65)				100.0 (12.1)	
	27 May	44.4 (3.24)	45.8 (0.65)	46.0 (1.83)	50.9 (5.65)	78.5 (0.61)	+15.6
	27 Aug.	None by 1 Jan.	49.1 (4.50)	49.6 (3.90)	None by 1 Jan.	None by 1 Jan.	+12.2
Soe	4 Feb.	142.1 (1.76)	69.5 (1.73)	65.5 (1.91)	66.4 (1.50)	164.7 (2.42)	UD[2]
	9 April	104.5 (0.94)	108.6 (9.18)	75.5 (1.15)	77.3 (1.80)	92.2 (1.30)	UD
	6 June	Cattle damage	Cattle damage	Cattle damage	Cattle damage	Cattle damage	UD
Tobu	11 Feb.	90.3 (12.9)	69.5 (2.06)	59.8 (1.71)	75.0 (1.29)	163.3 (1.93)	+63.5
	2 April	84.0 (0.92)	62.3 (1.25)	55.0 (1.58)	63.8 (1.11)	132.8 (1.38)	+29.0
	8 June	65.5 (0.96)	59.3 (1.49)	53.3 (1.11)	61.3 (1.11)	93.3 (1.75)	+58.2
Ende	30 Mar.	54.3 (3.20)	75.1 (2.12)	78.3 (4.91)	Poor growth	Poor growth	+16.0
	15 May	35.1 (0.48)	96.0 (8.64)	105.0 (25.1)	Poor growth	Poor growth	-5.9
	30 June	55.3 (2.47)	Poor growth	Poor growth	Poor growth	Poor growth	UD
	15 Dec.	113.8 (1.91)	113.8 (1.91)	62.5 (2.82)	Insect	Insect	+15.8

[1] Re-sow due to insufficient plant population when sown on 27 March

[2] UD = unreliable data due to poor growth or damage after the onset of flowering

KEYWORD：herbaceous legumes, flowerring, sowing date, trellising, Eastern Indonesia

REFERENCES

Bamualim, A.M. and Wirdahayati, R.B. (2002) Peternakan Lahan Kerin di Nusa Tenggara (Dryland Animal Husbandry in Nusa Tenggara). BPTP Nusa Tenggara Timur (The East Nusa Tenggara Assessment Institute for Agriculture Technology).

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. and Schultze-Kraft, R. (2005) Tropical Forages: an interactive selection tool., [CD-ROM and web-site: www.tropical forages.info], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia.

Copland, R., Jelantik, G.,Mullik, M., Belli, H.L.L., Sogen, J. and Nalley A. (2011) Evaluating strategies to improve calf survival in West Timor villages. LPS/2006/005, Final Report. ACIAR, GPO Box 1571, Canberra ACT 2601, Australia.

Dalgliesh, N., Nulik, J., Cox, K. and Poulton, P. (2013) Integrating forage legumes into the maize cropping systems of West Timor. *Final Report for ACIAR project LPS/2006/003.*

Mullik, M. and Jelantik, I. G. (2003) Bali cattle productivity in West Timor. Paper presented in Final ACIAR Project Meeting (AS2/2000/157). University of Queensland, Brisbane, Australia.

Nulik, J. and Bamualim, M. (1998) Pakan Ruminansia Besar di Nusa Tenggara (Fodder for Large Ruminants in Nusa Tanggara). Balai Pengkajian Teknologi Pertanian Naibonat Bekerja Sama dengan (In Collaboration with) Eastern Islands Veterinary Services Project.

Nulik, J., Dalgliesh, N., Cox, K. and Gabb, S. (2013) Integrating herbaceous legumes into crop and livestock systems in eastern Indonesia. ACIAR Monograph no. 154. Canberra.

O-33-6

BIOCONVERSION OF COCOA POD HUSK THROUGH FERMENTATION WITH MOL RUMENT CONTENT AS RUMINANT FEED

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INTRODUCTION

Cocoa pod has enough potential as alternative feedstuffs to substitute grass, because the production of cocoa is numerous and concentrated in specific areas. An increased cocoa plantations will annually produce cocoa pod waste more. In 2013, an extensive cocoa plantation in Bengkulu Province has reached 13,517 hectares with a production of 6,159 tons/year (Dinas Perkebunan, 2014), and produce a cocoa pod waste as much as 4,619.25 tons.

Cocoa pod waste has not been used, and cause environmental pollution. In addition, pod cocoa is usually allowed to pile up around the cocoa plant, which is a very good place for the development of *Phytoptora palmifora* causing black pod disease. The usage of cocoa pod as animal feedstuffs will provide a solution to address the problem of environmental pollution and conserve the environment. It also can break the chain of disease transmission for cocoa plants.

Cocoa pod has a good nutritional value for animal feedstuffs particularly ruminants. However, a high lignin content (31.51%) and theobromine, which is toxic become a limiting factor in its use as animal feedstuffs. To optimize the usage of cocoa pod as feedstuffs, they should be processed. There are some processing techniques which has beed proven to improve the value of the byproduct (Akbar et al., 2005 Astuti, 2014 Nurhaita et al., 2007, 2008, 2010, 2011, 2014 Neli et al., 2013, 2014 Zain, et.al., 2006). in order to improve the digestibility and eliminate toxins contained in the cocoa pod, the cocoa pod could be fermented.

Fermentation is the process of processing the material with the help of microbes capable of breaking down complex components into simpler forms, such as cellulose and hemicellulose to glucose (Winarno, et.al. 1980). Fermentation waste fiber materials ranging frequent lately, because in addition to more easily and cost are also safer and more environmentally friendly than the use of chemicals. The feedstuffs that undergo fermentation usually have better nutritional value of its origin, caused by microorganisms that break down the components of the complex into simple substances that are so easy to digest. In addition, fermentation can also increase the crude protein feed ingredients, improve palatability because it produces a fragrant smell and eliminate toxins, microorganisms can also synthesize certain vitamins such as riboflavin, vitamin B 12, pro-vitamin A and other growth factors. Thus, the fermentation treatment is expected to improve the quality of waste palm fronds for the better.

Fermentation is the process of processing feedstuffs by using microbes which degrade the complex components into simpler forms, such as cellulose and hemicellulose to glucose (Winarno, et.al. 1980). Recently, fermentation of byproduct which is rich in fibers is used, because this method is easier, lower cost, safer and more environmentally friendly than the use of chemicals. The feedstuffs that undergo fermentation usually have better nutritional value than its origin, because the microorganisms convert the complex components into simple substances that are eazier to digest. In addition, fermentation can also increase the crude protein, improve palatability, because it produces a fragrant smell and eliminate toxins. Furthermore, microorganisms can also synthesize certain vitamins such as riboflavin, vitamin B 12, pro-vitamin A and other growth factors. Thus the fermentation treatment is expected to improve the quality of by product.

Fermentation with local microorganisms is one alternative and lately often used. Local microorganisms in the form of a solution are fermented from a variety of waste materials. The local microorganism solution contains bacteria and fungi which could degrade organic materials. The most benefit of local microorganism is no cost for local microorganism which can be made from the waste of fruits and vegetables, livestock waste, slaughterhouse waste or household waste. In addition, the manufacturing process is easy and applicable. In this study, local microorganism is derived from rumen contents. Astuti, et al (2016) showed that there are 8 thermophilic bacteria isolated from rumen contents including gram-positive bacteria. Fermented cocoa pod with rumen contents is expected to improve the quality of the cocoa pod and eliminate toxic compounds.

MATERIALS AND METHODS

Local microoragnism manufacture of rumen contents

Local microorganism was made from rumen contens as a source of microorgnisms and enriched with coconut water and palm sugar as a source of energy. The materials used were 10 liters of coconut water was filtered, 2 kg of palm sugar that has been dissolved and 2 kg of rumen contents. All the ingredients put into a container and then closed. The container was perforated to insert the tube. Another container was filled with water. Connect the first container with a small tube into the second container to to drain the gas formed, incubated for 10 days.

Fermentation of cocoa pod

Cocoa pod was chopped into 3-5 cm size, dried under the sun until its moisture content of 60%. Cocoa pod was then added rice bran as much as 10% and 1% sugar and local microorganisms according to each treatment and mixed. the cocoa pod was then put into a plastic bag, tied and incubated for 7 days. After 7 days of incubation, they were then opened, and measured pH, mushrooms, texture and smell. After that, they were then dried under the sun, and ground into flour. The results of this fermentation were then analyzed their nutrient content.

The experimental treatments

The present study used completely randomized design with 5 treatments of 4 replications each.The dose of MOL to ferment pod cocoa was as follows A: 0 ml/kg of substrate, B: 3 ml/kg of substrate C: 6 ml/kg substrate, D: 9 ml/kg of substrate, and E: 12 ml/kg of substrate. The content of fiber fractions was measured by the method of Goering and Van Soest (1970), whereas in vitro digestibility was measured using the DAISY ^{II} method as described by ANKOM Technology Corporation

RESULTS AND DISCUSSION

The content of fiber fractions

The content of fiber fractions of fermented cocoa pod is presented in Table 1.

Table 1. The content of fiber fraction of fermented cocoa pod

Treatment	Parameters (%)				
	NDF	ADF	Hemicellulose	Cellulose	Lignin
A	70.66[a]	60.96[a]	9.71[a]	26.65	34.31
B	69.18[a]	60.23[a]	8.95[a]	26.14	34.09
C	68.44[a]	59.79[a]	8.65[a]	25.23	33.82
D	68.16[a]	59.39[a]	8.77[a]	26.66	32.73
E	61.75[b]	55.17[b]	6.57[b]	23.66	31.51
SE	0.34	0.22	0.27	0.44	0.56

The different superscripts in the same column indicate significantly different (P <0.05)

The unfermented cocoa pod had 71.06% NDF, 60.90% ADF, 10.17% hemicellulose, 25.04% cellulose and 35.29% lignin. The content of fermented fiber fraction tends to lower than the unfermented cocoa pod.

Experimental results showed that the dosage of MOL significantly (P <0.05) affected the contents of NDF, ADF and hemicellulose, but not significantly (P> 0.05) affected the content cellulose and lignin. The content of NDF, ADF and hemicellulose in E group treatment was significantly lower than other treatments.

The reduction in the fraction of the content of fiber in fermented cocoa pod occurred due to the microorganisms converted the complex molecules into simpler compounds. In this case, the bacteria contained in MOL converted the NDF, ADF into hemicellulose and cellulose, finally into glucose. This indicates that the MOL of rumen contents contains bacteria that have cellulolytic activity which can break down the components of fiber fraction.

Fraction fibers which are composed of NDF, ADF hemicellulose, and cellulose is a component of plant cell walls that can be digested by ruminants and is a major source of ruminant feed. However, the lignin binds the hemicellulose and cellulose to form lignohemicellulose and lignocellulose. This cause hemicellulose and cellulose could not be degraded by rumen microbes. Cell wall structure of cocoa pod have lignified further and its lignin has crystallized making it difficult to digest.

The reduction in NDF, ADF and hemicellulose contents of fermented cocoa pod reached 13.10% (71.06% vs 61.75), 9:41% (60.9% vs 55.15%) and 35.40% (10.17% vs 55.17%), respectively when compared with the unfermented cocoa pod. The largest decrease occurred in the treatment E, whereas in other treatment groups

did not siginificantly differ from the unfermented cocoa pod. This suggests that a dose of MOL for fermentation should be higher, which means it contains more bacteria to degraded fiber components more.

The digestiblity of dry matter and NDF

The digestibility of dry matter and NDF of fermented cacao pod are presented in Table 2.

Table 2. The digestibility of dry matter and NDF of fermented cacao pod (%)

Treatment	Parameters	
	Dry matter	NDF
A	42.97[a]	16.14[a]
B	42.65[a]	17.09[a]
C	42.65[a]	18.55[a]
D	42.44[a]	19.55[a]
E	55.89[b]	35.67[b]
SE	0.74	1.09

The different superscripts in the same column indicate significantly different (P <0.05)

Experimental results showed that the treatments significantly affected (P<0.05) the dry matter and NDF digestibilities. It was shown that E had higher dry matter and NDF digestibilities than A, B, C, and D (P<0.05), whereas A, B and C and D did not differ significantly (P>0.05) to each other. Dry matter and NDF digestibilities in E was increased at a level of 31.69% and 121.0%, respectively. This phenomenon may closely relate to a decrease in the content of fiber fractions (NDF, ADF and hemicellulose) as a result of the activity of microorganisms, which convert the complex molecules into simple molecules during the fermentation process.

Digestibility is a reflection of the quality of the feedstuffs, which describes what percentage of substances that are digested and what percent is excreted through feces. Substances contained in the feedstuffs are not entirely available to the body, part of them will be excreted through the feces. The higher digestibility of feedstuffs will result the higher value of feedstuffs for livestock.

The values of dry matter and NDF digestibilities of fermented cacao pod were low, ranging from 42% -55% and 16.14% -35.67%, respectively.

CONCLUSION

In conclusion, fermentation by MOL at a dosage of 12 ml/kg substrate reduced fiber fraction component and increased digestibilities of dry matter and NDF.

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KEYWORD : cocoa pod husk, fermentation, local mikroorgasnisme (MOL), fiber fraction component, in-vitro digestibility

REFERENCES

Akbar, S.A. , N Jamarun, R Saladin dan Mardiati, 2005. Pengaruh fermentasi dan defaunasi tandan kosong sawit terhadap kandungan gizi, kecernaan dan karakteristik cairan rumen in-vitro. Jurnal Ilmiah Ilmu-ilmu Peternakan Vol VIII no 2 : 132-144

Astuti,A., Y. Amir., Irdawati dan U. Santoso. 2016. Nutritional Improvement of Palm Oli Fronds for Ruminant Feedstuffs Using a Local Biotechnological Approach. Pakistan Jornal of Nutrition Vol 15 No 5. P 450-454

Dinas Perkebunan Propinsi Bengkulu, 2014.

Goering, H. K. and P.J. Van Soest. 1970. Forage Fiber Analysis. Apparatus, Reagent, Procedures and Some Applications. Handbook. ARS-USDA, Washington, D.C.

Nurhaita, N. Jamarun, R. Saladin, L Warly dan Mardiati Z. 2007. Efek beberapa metoda pengolahan limbah daun

kelapa sawit terhadap kandungan gizi dan kecernaan secara in-vitro. J. Ilmu-Ilmu Pertanian Indonesia Ed. Khusus Dies natalis ke 26 Unib. No 2. P: 139-144

Nurhaita, 2008. Evaluasi dan Pemanfaatan Daun Kelapa Sawit dalam Ransum Ternak Ruminansia. Disertasi Program Pascasarjana Universitas Andalas, Padang

Nurhaita, N. Jamarun, L. Warly dan M. Zain. Nurhaita, 2010. Kecernaan ransum domba berbasis daun sawit teramoniasi yang disuplementasi sulfur, fosfor, dan daun ubi kayu. J. Media Peternakan Vol 33 No. 3 P: 144 -149

Nurhaita, N. Definiati, R. Zurina dan Edi, E. 2011. Nilai gizi dan kecernaan pelepah sawit fermentasi (evaluasi secara in vitro). Prosiding Seminar Nasional Peternakan "Prospek dan Potensi Sumber Daya Ternak Lokal dalam Menunjang Ketahanan Pangan Hewani". Fakultas Peternakan UNSOED Purwokerto, 15 Oktober 2011

Nurhaita, W. Rita, N. Definiati dan R. Zurina, 2012. Fermentasi Bagase Tebu Dengan Neurospora sitophila dan Pengaruhnya Terhadap Nilai Gizi dan Kecernaan Secara in Vitro. Jur. Embrio Vol 5 No 1 P:1-7

Nurhaita, W Rita, Ruswendi dan Robiyanto. 2014. Optimizing Rumen Bioprocess through Supplementation of Microbe Precursor Nutrient in Ammoniation of Palm Oil Frond-Base Cattle Ration. Journal of Advanced Agricultural Technologies Vol. 1, No. 1,P : 10-13

Stell, R. G. and J. H. Torrie. 1991. Prinsip dan Prosedur Statistik, Suatu Pendekatan Biometrik. Edisi 2. Alih Bahasa B. Sumantri. PT. Gramedia Pustaka Utama, Jakarta.

Van Soest, P. J. 2006. Rice straw the role of silica and treatment to improve quality. J. Anim. Feed Sci and Technology. Vol. 130 : 137 - 171

Winarno, F. G., S. Fardiaz and D. Fardiaz. 1980. Pengantar Teknologi Pangan. PT. Gramedia. Jakarta.

Zain. M., Jamarun, Suryahadi dan Nurhaita. 2006. Fermentabilitas dan kecernaan in-vitro serbuk sabut kelapa yang difermentasi dengan mikrob rumen. Jurnal Ilmiah Ilmu-ilmu Peternakan. Vol. IX No 1: 39 - 49

Zain, M., N. Jamarun, and Nurhaita. 2010. Effect of sulfur suplementation on in vitro fermentability and degadability of ammoniated rice straw. Pakistan Journal of Nutrition 9 (5) : 413-415

Winarno, F. G., S. Fardiaz and D. Fardiaz. 1980. Pengantar Teknologi Pangan. PT. Gramedia. Jakarta.

O-33-7

Nutrients Quality of Fermented Complete Feed Based on By-Product of Sago (Metroxylon sp.) and Cassava (Mannihot esculenta Cranz)

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INTRODUCTION

Sago (*Metroxylon* sp.) was grown in the tropics and well-adapted on peat and swampy soil (Melling et al., 2005). The largest supply of sago comes from the South East Asia, particularly Indonesia and Malaysia. The trunk of sago tree may reach 3 to 5 cm in diameter at 24 months and may grow until 20 m tall (Bintoro et al., 2010). The main product of sago is the starch, which is extracted from the spongy center of sago's trunk and produced sago dregs as the by-product. Another by-product from sago is the leaves. In the past, sago leaves were commonly used to make roof, but nowadays people do not use sago roof anymore, thus the leaves were throw away and considered as waste.

Cassava (*Mannihot esculenta* Cranz) is another main agricultural product in Indonesia. Cassava mainly planted for its tuber, and left the leaves as by-product. Cassava tuber is a good energy source, while the leaves contain high crude protein (CP) which is ranged from 20 to 36% (Askar, 1996). Leaves of sago and cassava may be used as fiber sources in ruminant feeding, while sago dregs and cassava tuber can used as energy source.

In this study, the fermented complete feeds were formulated based on sago and cassava by-products. The purpose of this study was to formulate good fermented complete feeds made from sago and cassava by-products, thus the by-products of sago will be able to be used as feed for ruminants.

MATERIALS AND METHODS

Materials used in this research were: sago leaves, sago trunk, sago dregs, cassava tuber, and cassava leaves. All materials were dried directly under the sunlight until dry and the weight was constant. The materials were mixed into four complete feed based on their nutrient composition (Table 1) as follow: AS (sago leaves + sago dregs + cassava leaves), BS (sago leaves + grated sago trunk + cassava leaves), KP (sago leaves + cassava tuber + cassava leaves), and ASKP (sago leaves + sago dregs + cassava tuber + cassava leaves). All complete feeds were conditioned to reach water content of 65% (Sapienza and Bolsen, 1993), wrapped in airtight polyethylene plastic, and then stored for 21 days (Jaelani et al., 2014).

In the end of fermentation stage, all fermented complete feeds were analyzed for DM, CP, CF, EE, and ash (AOAC, 2005). The pHs of fermented complete feeds were tested by mixing samples with distilled water in 1: 10 ratio (Nahm, 1992). Ammonia determination of fermented complete feeds was done as explained by Chaney and Marbach (1962).

All data were analyzed using analysis of variance by following completely randomized design using SPSS ver. 16 software. If the results were significantly different, the analysis continued with Duncan's new multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The pH, NH_3 concentrations, and nutrient compositions of fermented complete feed formulated based on sago and cassava by-products are presented in Table 2 and 3.

The pH of fermented complete feed in this study was ranged from 4.76 to 5.37 (Table 2). According to McDonald et al. (1984), the optimal pH for silage was ranged from 3.8 to 4.4. In this study, the relatively high pH is due to that the fermented materials were complete feed, instead of silage. Therefore, pH of fermented complete feed cannot as low as pH of silage due to fermented complete feed consisted of soluble and structural carbohydrates as well as protein source that formulated to meet 12 - 13% of CP content. Furthermore, the addition of cassava leaves as protein source resulted in high buffering capacity of the feed, which caused the pH could not decrease to below 4.4 as that in the ensilage process. Previous researchers reported that feed with protein content more than 10% resulted in higher pH than the optimal pH for fermentation due to its high buffering capacity (McDonald, 1995 Angthong et al., 2007).

The pH of the AS and ASKP were higher than those on the BS and KP (5.37 and 5.03 vs. 4.76 and 4.90,

respectively P<0.05 Table 2). The high pH of the AS and ASKP was due to the addition of sago dregs in the AS as well as the mixtures sago dregs and cassava tuber in the ASKP did not provide enough rapidly degraded carbohydrates for microbes, thus the fermentation process did not optimum that lead to low lactic acids and acetic acids production, and resulted with the high pH of the AS and ASKP. Jaster and Moore (1988) reported that when fermentation process was not going well, it resulted in generating low amount of organic acids, thus the pH at the end of fermentation were higher.

Compared to the BS and KP, NH_3 concentrations of the AS and ASKP were greater (13.4 and 14.0 mg/dL vs. 35.6 and 48.3 mg/dL, respectively P<0.05 Table 2). The great NH_3 concentrations of the AS and ASKP indicate that a secondary fermentation has been occurred (Chamberlain and Wilkinson, 1996), which is related to the high pH of those fermented complete feeds (5.37 and 5.03, respectively, Table 2). The high pH caused proteolytic activity of microbes that convert proteins of complete feed into amino acids, and the N of amino acid formed into NH_3. Protein in feed might be used by microbes to form N of amino acids in feed, which caused NH_3 formation. Proteolysis process would occur during the fermentation if the optimum pH could not be reached (Sun et al., 2009 Kung et al., 2010). Low pH in the silage inhibits bacteria to perform proteolysis protease activity will take place optimally when the fermentation pH reached 4 - 7 (Slottner and Bertilsson, 2006).

Crude protein content of the KP was greater than that of the ASKP (12.4 vs. 11.0%, respectively P<0.05 Table 3), but did not differ with the AS and BS. The low CP content of the ASKP was due to the high rate of feed protein hydrolysis that occurred during fermentation process. The hydrolyzed feed protein formed NH_3, which reflected by greater NH_3 concentration of the ASKP compared to the KP (48.3 vs. 14.0 mg/dL, respectively, P<0.05 Table 2).

Crude fiber content of all fermented complete feeds did not differ, and ranged from 20.8 to 23.0% (Table 3). High fiber of sago leaves and cassava leaves are difficult to be digested and utilized by livestock, thus the ruminal degradation would be slow. Based on the CF content, the nutrient quality of fermented complete feed in this study was relatively low. Lubis (1992) reported that high CF content can limit the digestion of OM, thus the feed digestibility would be low.

Total digestible nutrients of the BS and KP were greater than those of the AS and ASKP (67.5 and 66.0% vs. 64.1 and 63.3%, respectively P<0.05 Table 3). The lower TDN of the AS and ASKP was due to the pH of those fermented complete feeds could not go down well. Higher pH in both treatments caused the activity of starch digester bacteria could not be inhibited and caused sugar were hydrolyzed.

CONCLUSION

The results of this study showed that the BS and KP had the lowest pH and NH_3, and also had greater CP and TDN contents than the other fermented complete feeds. Thus, it can be concluded that grated sago trunk and cassava tuber addition as energy source increase the nutrient quality of fermented complete feed.

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KEYWORD : Sago dregs, Sago trunk, Cassava tuber, Cassava leaves, Fermented complete feed

Table 1. Nutrient composition (%) of sago leaves, sago dregs, grated sago trunk, cassava tuber, and cassava leaves

Items	Dry matter	Crude protein	Crude fiber	Ether extract	Ash	TDN[1]
Sago leaves	60.7	9.11	36.0	5.53	7.29	50.1
Sago dregs	35.2	0.76	13.1	0.27	4.53	85.6
Grated sago trunk	41.0	1.49	10.4	1.08	4.17	77.2
Cassava tuber	30.8	1.83	3.40	3.22	3.74	82.4
Cassava leaves	30.9	24.5	18.8	4.70	5.89	65.3

[1] Calculated based on formula in Harris et al. (1972) cit. Utomo (2012).

Table 2. pH and NH_3 concentrations (mg/dL) of fermented complete feed based on sago and cassava by-products

Treatments	pH	NH_3
AS	5.37^{c}	35.6^{b}
BS	4.76^{a}	13.4^{a}
KP	4.90^{a}	14.0^{a}
ASKP	5.03^{b}	48.3^{b}

^{a,b,c} Means in the same column with different superscripts differ at $P<0.05$.

Table 3. Nutrient compositions (%) of fermented complete feed

Treatments	Dry matter	Crude protein	Crude fiber	Ether extract	Ash	TDN[1]
AS	58.2	12.1^{ab}	23.0	3.96	5.71	64.1^{a}
BS	58.0	12.0^{ab}	21.1	4.05	5.25	67.5^{b}
KP	58.6	12.4^{b}	20.8	4.11	4.55	66.0^{b}
ASKP	57.4	11.0^{a}	21.4	4.42	5.51	63.2^{a}

^{a,b} Means in the same column with different superscripts differ at $P<0.05$.
[1] Calculated based on formula in Harris et al. (1972) cit. Utomo (2012).

REFERENCES

Angthong, W., S. Promma, and B.C. Isarkul. 2007. Beta-carotene, mimosine and quality of silage leucaena kept at different duration. Kasetsart J. Nat. Sci. 41: 282-287.

AOAC. 2005. Official Methods of Analysis. 18th ed. AOAC International, Gaithersburg, MD.

Askar, S. 1996. Daun singkong dan pemanfaatannya terutama sebagai pakan tambahan. Wartazoa 5: 21-25.

Bintoro. M.H., Y.J. Purwanto, dan S. Amarilis. 2010. Sagu Di Lahan Gambut. IPB Press. Bogor.

Chamberlain, A.T. and J.M. Wilkinson. 1996. Feeding the dairy cow. Chalcombe Publication. UK.

Chaney A.L. and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8: 130-132.

Jaelani, A., A. Gunawan, and I. Asriani. 2014. Effect of storage silage old leaves of palm oil crude protein and fiber content. J. Zira'ah. 39: 8-16.

Jaster, E.H. and K.J. Moore. 1988. Fermentation characteristics and feeding value of enzyme-treated alfalfa haylage. J. Dairy Sci. 71: 705-711.

Kung, L. Jr., E.C. Stough, E.E. McDonell, R.J. Schmidt, M.W. Hofherr, L.J. Reich, and C.M. Klingerman. 2010. The effect of wide swathing on wilting times and nutritive value of alfalfa haylage. J. Dairy Sci. 93: 1770-1773.

Lubis, D.A. 1992. Ilmu Makanan Ternak. Cetakan kedua. PT Pembangunan. Jakarta.

McDonald, P., R.A. Edwards, J. Greenhalgh, and C.A. Morgan. 1995. Animal Nutrition. 5th ed. Longman Scientific & Technical, England.

Melling, L., R. Hatano, and K.J. Goh. 2005. Methane fluxes from three ecosystems intropical peatland of Sarawak, Malaysia. Soil Biol. Biochem. 37: 1445-1453.

Nahm, K. H. 1992. Practical Guide to Feed, Forage and Water Analysis. Yoo Han Publishing Inc., Seoul.

Sapienza, D.A. and K.K. Bolsen. 1993. Technology Silage: Cultivation, Manufacture and Administration to Livestock. Pioner-Hi-Bred International, Inc. Kansas State University. Kansas.

Slottner, D. and J. Bertilsson. 2006. Effect of protein degradation during ensiling technology on ensilage. Anim. Feed Sci.Technol. 127: 101-111.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. (With special Reference to the Biological Sciences.) McGraw-Hill Book Company, New York, Toronto, London.

Sun,T., X. Yu, S.L. Li, Y.X. Dong, and H.T.Zhang. 2009. Responses of dairy cows to supplemental highly digestible rumen undegradable protein and rumen-protected forms of methionine. Asian-Aust. J. Anim.Sci. 22: 659-666.

Utomo, R. 2012. Bahan Pakan Berserat Untuk Sapi. Cetakan Pertama. PT. Citra Aji Parama, Yogyakarta.

O-33-8

Improving Rice Straw Quality by Treated with Monosodium Glutamate by-Product (MSGB) for Ruminant Diets

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Introduction

Utilization of industrial by-products for livestock feed is urgently required to raise the international competitiveness of livestock production. Monosodium glutamate (MSG) is used in the food industry as a flavor enhancer. The by-products from a MSG factory are rich in amino acids particularly glutamic acid and nitrogen source. In addition, MSG by-product (MSGB) has been studies in various animals such as swine diet and cattle (Kananurak et al., 1987 and Keaokliang et al., 2012). Moreover, Padunglerk et al. (2016) has been studied using of MSGB in cow diet on performance of lactating dairy cows and it can replacement for soybean at 20-60% in the feed for dairy cows presented on negative effects on their performances.

Rice straw is a major by-product produced in many regions of East and South-East Asia, and it is routinely utilized as a main roughage resource for ruminants. Rice straw has very low nutritive values with low crude protein content and metabolic energy for ruminants. Furthermore, rice straw has low digestibility by rumen microbes (Van Soest, 2006). Enormous advances have been achieved to overcome the problems using physical, chemical and biological treatments. However, commercial application of these treatments is limited to improve nutritive quality of rice straw. Due to the MSGB is rich in crude protein and rice straw has low crude protein content. Thus the objective of this study was to improving rice straw quality by treated with MSGB for ruminant diets.

Materials and methods

Experiment I, experimental units were randomly assigned in (3 x 3) +1 factorial arrangement in completely randomized design with three replications. Factor A is ratio of RS to MSGB (1:1, 1:1.5 and 1:2), factor B is fermentation times (0, 7 and 14 days). Control is 4 % urea treated rice straw (UTR) at 14 days. Rice straw was chopped into lengths of 2-3 cm then mixed with 4% urea and MSGB as followed by factor A and kept in plastic bag under anaerobic condition at room temperature for 0, 7 and 14 days, respectively. At the end of fermentation time, all samples were collected and dried immediately in an air-forced oven at 60 ℃ ground and analyzed using the method of AOAC (1995) for DM, OM, CP and ash. NDF, ADF and ADL in samples were estimated according to Van Soest et al. (1991). Cellulose and Hemicellulose were calculated.

Experiment II, experimental units were randomly assigned in completely randomized design with six replications in *in sacco* technique. Experimental treatments are UTR and MSGB treated rice straw at 1:2 ratio at 0, 7 and 14 days, respectively. Dry matter digestibility of all samples were investigated with the *in sacco* nylon bag technique at 0, 24, 48 and 72 hours in two rumen fistulated beef cattle. All data were analyzed by SAS (1996).

Results and discussion

Chemical composition of UTR and MSGB treated rice straw in several of fermentation times are shown in Table 1. All parameters were significantly different among UTR and other treatments ($P < 0.001$). Urea treated rice straw contained lowest ($P < 0.05$) in DM, OM and CP and highest ($P < 0.05$) in hemicellulose and cellulose. Organic matter and CP of MSGB treated rice straw was significantly different due to the effect of RS to MSGB ratio. It was shown that OM and CP was increased when increasing the ratio of MSGB from 1:1, 1:1.5 and 1:2, respectively. While, proportion of hemicellulose was shown lowest in 1:2 of RS to MSGB ratio. The proportion of cellulose was significantly different among treatment due to the effect of RS to MSGB ratio ($P < 0.001$). It was found that proportion of cellulose was decreased when increasing the ratio of MSGB from 1:1, 1:1.5 and 1:2, respectively.

In *sacco* dry matter digestibility of UTR and MSGB treated rice straw ratio 1: 2 at 0, 24, 36 and 72 hours are shown in Table 2. Dry matter digestibility at 0, 24, 48, 72 hours were significantly different among treatments ($P < 0.05$), which UTR treatment was significantly different compared with other treatments ($P < 0.001$). Dry matter digestibilities at 0, 24, 48 and 72 hours of UTR were the lowest value. At 7 days fermentation of rice straw with

MSGB has significantly highest (P < 0.05) in DM digestibility at 0 and 72 hours. While, DM digestibility at 72 hours of 7 and 14 days were not significantly among treatment (P = 0.551).

In this study, improving the quality of rice straw by treated with MSGB can increase organic matter and crude protein content. While, fermented time (0, 7 and 14 days) factor tended to be increased (P = 0.062) crude protein content. Because of monosodium glutamate by-product was used in this study contained 846 g/kg of organic matter and 460 g/kg of crude protein leading to increase organic matter and crude protein content in rice straw. Moreover, Padunglerk et al. (2016) was reported that MSGB contained 91.8 g/kg of total amino acids and glutamic acid was the most common amino acid (47.9 g/kg on a DM basis) contained in MSGB. At 0 and 72 hours, in *sacco* DM digestibility of all RS:MSGB treatments were significantly higher than UTR treatment. It could explain by MSGB physical properties which it characteristics like slurry and water soluble. Moreover, Padunglerk et al. (2016) was found that replacement soybean meal with MSGB at 20 - 60 % tended to be increased NDF digestibility. In addition, Wuisman et al. (2006) reported that rumen degradability of DM and NDF of the whole plant in situ was also significantly increased by phenylalanine fermentation by-product.

Conclusion

Under this study it could be concluded that that MSGB can improve qualities of rice straw in terms of increase crude protein content and increase digestibility when compare with 4% urea treated rice straw.

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KEYWORD : Monosodium Glutamate by-Product, Rice Straw, Roughage, Digestibility, Ruminant

Table 1. Chemical compositions of UTR and MSGB treated rice straw in several of fermentation time (DM basis).

Items		DM	OM	CP	Ash	Hemicellulose	Cellulose
UTR		44.3^{f}	85.3	9.6	16.1	22.8^{a}	39.3
RS:MSGB	0 day	62.1^{a}	86.5	26.4	16.4	18.0^{bc}	29.1
(1:1)	7 days	64.2^{a}	85.6	26.4	16.6	18.2^{b}	28.1
	14 days	59.4^{b}	87.0	28.8	16.4	17.2^{bcd}	29.0
RS:MSGB	0 day	58.1^{b}	86.7	33.2	16.3	16.0^{d}	26.4
(1:1.5)	7 days	57.7^{bc}	86.3	33.3	15.9	15.6^{d}	26.7
	14 days	55.6^{cd}	86.9	34.4	15.8	16.4^{cd}	24.7
RS:MSGB	0 day	57.5^{bc}	87.3	37.9	16.2	13.5^{e}	23.9
(1:2)	7 days	55.2^{d}	90.7	42.2	15.9	13.6^{e}	23.0
	14 days	52.3^{e}	87.4	39.8	15.6	12.1^{e}	23.0
P-value	Ratio	<.001	<.001	<.001	<.001	<.001	<.001
	Day	<.001	0.650	0.062	0.009	0.186	0.266
	Interaction	0.009	1.578	0.314	0.406	0.042	0.441
	UTR vs others	<.001	<.001	<.001	<.001	<.001	<.001
SEM		0.990	0.311	1.559	0.069	0.550	0.880

$^{a-f}$ Values on the same column with different superscripts differed (P <0.05)

UTR = Urea treated rice straw for 14 days, MSGB = Monosodium glutamate by-product, RS = Rice straw, SEM = standard error of the means

Table 2. *In sacco* dry matter digestibility of UTR and MSGB treated rice straw at 0, 7 and 14 days in beef cattle

Items	UTR	RS:MSGB (1:2)			UTR vs Others	0 vs 7, 14	7 vs 14	SEM
		0 day	7 days	14 days				
In sacco DM digestibility, (%)								
0 hr	14.4^{c}	45.4^{b}	47.8^{a}	45.3^{b}	<0.001	0.173	0.016	2.894
24 hr	29.7^{b}	52.3^{a}	54.5^{a}	52.3^{a}	<0.001	0.289	0.094	2.159
48 hr	43.0^{b}	59.8^{a}	60.7^{a}	61.4^{a}	<0.001	0.327	0.800	1.686
72 hr	47.3^{c}	60.9^{b}	64.6^{a}	63.6^{ab}	<0.001	0.036	0.551	1.555

^{a,b,c} Values on the same column with different superscripts differed (P <0.05)

UTR = Urea treated rice straw for 14 days, MSGB = Monosodium glutamate by-product, RS = Rice straw, SEM = standard error of the means

REFERENCES

AOAC (Association of Official Analytical Chemists). 1995. *Official Methods of Analysis, 16*th *ed. Animal Feeds* (Association of Official Analytical Chemists, Virginia, USA).

Kananurak P, Kanto U, Kanthapanit C. 1987. Effect of fermented mother liquid (FML) supplementation of growing and finishing swine rations. *Kasetsart Journal (Natural Science)* 20, 163-170.

Keaokliang O, Siwichai S, Kongmun P, Prasanpanich S. 2012. Effects of monosodium glutamate (DSCL) by-product mix molasses replaced concentrates feed in Holstein Friesian crossbred heifers. *The Proceeding of 50th Kasetsart University Annual Conference* 1, 92-99.

Padunglerk A, Prasanpanich S, Kongmun P. 2016. Use of monosodium glutamate by-product in cow diet on performance of lactating dairy cows. *Animal Science Journal* doi: 10.1111/asj.12572.

SAS, 1996. *SAS User's Guide: Statistic*, Version 5 Edition, (SAS. Inst Cary, NC., USA).

Van Soest PJ. 2006. Rice straw, the role of silica and treatments to improve quality. *Animal Science and Technology* 130, 137-171.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non starch Polysaccharides in Relation to Animal Nutrition. *Journal of Animal Science* 74, 3583-3597.

Wuisman Y, Hiraoka H, Yahaya MS, Takeda M, Kim W, Takahashi T, Karita S, Horiguchi K, Takahashi T, Goto M. 2006. Effects of phenylalanine fermentation byproduct and sugarcane molasses on fermentation quality and rumen degradation of whole crop barley (Hordeum vulgare L.) silage in situ. *Grassland Science* 52, 72-79.

O-34-5

Efficacy of Probiotics (BACTOSAC-P) in Nursery-Fattening Pigs Diets

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Introduction

According to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO, 2001), probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts. In particular, strains belonging to Bifidobacterium and Lactobacillus, which are the predominant and subdominant groups of the gastrointestinal microbiota, respectively (Guarner and Malagelada, 2003), are the most widely used probiotic bacteria. Mode of action and benefits for using probiotics have been documented as a new class of feed additives useful for animal performance and health. Probiotics can act not only as growth promoters and feed savers, but also as nutritional bioregulator useful to animal production. These effects have been found in pigs as well as in poultry, calves and rabbit (Kozasa, 1978 Crawford, 1979 Fuller, 1989 Nguyen, 1991 Khajarern and Khajarern, 1994 Khajarern et al. 1996 Khajarern and Ratanasethakul, 1998). Tournut (1990) reported that administrated Toyocerin (*Bacillus Toyoi* or Paciflor in sow feeds from 2 to 3 weeks before farrowing to weaning, were able to decrease significantly mortality of suckling piglets. These effects have been also reported by Khajarern and Khajarern (1994). Supplementation of Toyocerin in both gestating and lactating sow and creep feeds significantly improved the reproductive performance in sows and the prevention of diarrhea and mortality in suckling piglets. The results indicated that Toyocerin could satisfactory replace antibiotics in creep feed. Another mechanism of action effect of probiotics is serve to reinforce the non-specific immunity system of animals if continuous usage. Using yeast have been documented in several important ways for probiotics, immunological properties of the inner layer of yeast cell wall (Stewart and Russell, 1998), mycotoxin adsorbing properties (Gulderblom et al., 1988 Nelson et. al., 1993 Bullerman et al., 1996), cholesterol lowering properties (Nicolosi et al., 1999) and improvement of functional properties such as solubility, fat binding capacity, emulsion capacity and foaming capacity of yeast extracts (Mrowka et al., 1999). The use of yeast in pig diets results in improvement of appetite, palatability, digestibility of feedstuffs and feed efficiency in pigs from weaning to slaughter house (Stephen, 1993 Mahan and Parratt, 1996 Mahan et al., 1999). The objective of present study was to evaluate probiotics (Bactosac-P, KMP Biotech Co, Ltd. Thailand) the mixture of *Lactobacillus acidophilus* and *plantarum*, *Pediococcus pentosaceus*, *Streptococcus faecium*, *Bacillus subtilis* and *lichenifermis* and yeast (*Sacchalomyces cerevisiae*) in nursery and fattening pigs diets.

MATERIALS AND METHODS

The experiment was conducted in a well manage commercial farm (Reuang Siri Farm,) in MahaSarakham Province, Thailand. A total of 360 crossbred nursery piglets [Duroc x (Yorkshire x Landrace)] with average BW 6.70-6.90 kg (21 days of age) were divided into three treatments with 4 replications of 30 piglets each (2 replications for males and 2 replications for females). All nursery piglets were allotted to treatment on the basis of gender, body weight and genetic background. All weaned piglets were house in an evaporation regulated pens and fattening were house in conventional pen throughout the study (146 days period). All nursery piglets were given *ad libitum* access to feed and water. One nine-hole feeders and two nipple waterers were available per pen. Piglets were weighed at each period of changing feeds (nursery = 35, starter = 18, grower = 39 and finisher = 54 days). Feed intake and mortality of diarrhea are recorded every day until termination. Piglets uniformity was accounted as percentage units that fell into two times of standard deviation based on average live weight at each periods (nursery, starter, grower and finisher). The three treatments consisted of the control diet for treatment 1 and the control diet supplemented with probiotics Bactosac-P two levels (0.50 and 1.00 kg/t) for treatment 2 and 3 levels and deduction antibiotic 20% from the control for nursery, starter, grower and finisher diets. The composition of experimental diets was shown in the **Table 1** and **Table 2**. The diets were fed in mash form and water was allowed *ad libitum*. Body weights were weighed at initial and each period of changing diets, feed intake and mortality were also recorded.

STATISTICAL ANALYSIS

Data from this experimental were subjected to analysis of variance using the General Linear Model (GLM) procedure of SAS system (SAS, 1986) Duncan's Multiple Range Test (Duncan, 1955) was used to determine treatment differences. Dressing percentage was analyzed by using mean replicate final body weight as a covariate. All statements of significance were based on the probability level of 0.05

RESULTS AND DISCUSSION

The results of supplementation Bactosac-P two levels (0.05 and 0.10%) for the whole period of evaluation 146 days of Bactosac-P supplementation in diets (nursery period thoughtout finisher period) showed remarkable increasing ($P<0.05$) Body weight gain (BWG) and ADG with improvement 2.65 and 4.21%, better feed efficiency ($P<0.05$) with improvement 4.62 and 6.38%, decreasing FCG (**Table 3**) and lead to increasing net profits return per head with economic benefits return 189.66 and 240.90 Baht per head over the control unsupplementation Bactosac-P group (**Table 4**) .

CONCLUSION

The results from this study clearly demonstrated that supplementation of Bactosac-P with two levels (0.05 and 0.10%) in four periods of fattening pig diets (nursery, starter, grower and finisher) for 146 days elicits flavorable biological responses in both performance (growth and feed efficiency) and cheaper in feed cost per head with higher economic benefits return over the control unsupplementation Bactosac-P group. Addition Bactosac-P at the level of 0.10% in the four periods of fattening diets showed the best and maximum response on growth performance, feed efficiency and showed the highest economic benefits return.

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KEYWORD : Nursery - Fattening Pigs, Probiotics, Performance, Carcass quality, Economic benefits return

Table 1. Composition of experimental basal diets for Nursery (6-20 kg BW)

Ingredients	**Content (%)**		
	T1	**T2**	**T3**
Broken rice (7.5% CP)	48.91	48.91	48.86
Soybean meal (44% CP)	5.00	5.00	5.00
Full fat soybean	8.00	8.00	8.00
Fish meal (58% CP)	3.00	3.00	3.00
Protiplus F	17.25	17.25	17.25
Prelac	3.00	3.00	3.00
Biopro 480	7.50	7.50	7.50
Rice bran oil (unrefined)	3.79	3.79	3.79
DL-Methionine	0.22	0.22	0.22
L-lysine	0.30	0.30	0.30
L-Threonine	0.13	0.13	0.13
Monocalcium phosphate	1.29	1.29	1.29
Calcium carbonate	0.67	0.67	0.67
Salt	0.20	0.20	0.20
Premixes[a]	0.50	0.50	0.50
Colistin 20%	0.030	0.024	0.024
Ammoxycilin 50%	0.060	0.048	0.048
Tiamulin 10%	0.150	0.120	0.120
Probiotics *	-	±	±
Cost, Baht/kg **	22.07	22.26	22.60
Composition by calculated:			
CP, %	21.43	21.43	21.43
ME, kcal/kg	3,245	3,245	3,245

[a] Premixes provide per kilogram of diet: A 2,500 IU, D_3 250 IU, E 20 IU, B_{12} 0.2 mg, Pantothenic acid 12 mg, Riboflavin 4 mg, Thiamine 2 mg, Choline choride 1 g, K_3 0.5 mg, Biotin 0.3 mg, pyridoxine 2 mg, Folic acid 0.3 mg, Etoxyquia 12.5 mg, Cu 250 mg, Fe 100 mg, Zn 100 mg, Mn 4 mg, I 0.4 mg, Se 0.3 mg, Co 0.14 mg, Ammoxycilin 300 mg, Colistin 120 mg and Tiamulin 100 mg

* Probiotics: Bactosac-P = 690 Baht/kg

** Cost, Baht/kg: Nursery; T1=22.07, T2=22.26 and T3=22.60

Table 2. Composition of experimental basal diets for starter (20-30 kg BW), grower (30-60 kg BW) and finisher (60-market weight)

Ingredients	**Content (%)**		
	Starter	**Grower**	**Finisher**
Broken rice (7.5% CP)	50.99-51.04	28.12-28.17	27.17-27.27
Corn (8% CP)	13.75	20.00	25.00
Corn silage	-	10.00	10.00
Full fat soybean	7.50	5.00	5.00
Rice bran (12.5% CP)	5.00	5.00	5.00
Soybean meal (44% CP)	8.35	17.50	16.50
Fish meal (58% CP)	3.00	3.00	2.00
Meat meal (50% CP)	3.00	-	-
Chicken meal	-	1.00	1.00
Bone meal	-	2.25	2.00
Rice bran oil (unrefined)	5.35	5.85	4.10
DL-Methionine	0.21	0.19	0.29
L-lysine	0.31	0.34	0.15
L-Threonine	0.13	0.09	0.06
Monocalcium phosphate	0.89	-	-
Calcium carbonate	0.45	-	-
Choline chloride	-	0.07	0.07
Lime stone	-	0.46	0.71
Salt	0.28	0.33	0.35
Premixes[a]	0.50	0.50	0.50
Doxcy 20%	-	0.10	-
Colistin 20%	0.03	-	-
Ammoxycilin 50%	0.06	-	-
Tiamulin 10%	0.15	0.15	-
Probiotics *	±	±	±
Cost, Baht/kg **	17.93	14.09	13.07
Composition by calculated:			
CP, %	19.02	17.01	14.45
ME, kcal/kg	3,290	3,200	3,080

[a] Premixes provide per kilogram of diet. The formula as same as table 1

* Probiotics: Bactosac-P = 690 Baht/kg

** Cost, Baht/kg: Starter; T1=17.93, T2=18.12 and T3=18.46: Grower; T1=14.09, T2=14.35 and T3=14.68 : Finisher; T1=13.07, T2=13.41 and T3=13.75

Table 3. Effects of addition Bactosac-P to the diets on the performance in Overall period (146 days period)

Parameter	Control	Bactosac-P		Pooled
	No added	0.05%	0.10%	SEM
	T1	T2	T3	
Initial no. of pigs	120	120	120	
Final no. of pigs	117	118	119	
Survival rate	97.50	98.32	99.17	1.755
Initial weight, kg	6.93	6.76	6.81	1.301
Final weight, kg	100.60	102.85	104.38	2.826
Av. Body weight gain, kg	93.67[b]	96.09[ab]	97.57[a]	2.084
Improvement, %		+2.58	+4.16	
Av. Daily gain, g	641[b]	658[ab]	668[a]	14.412
Improvement, %		+2.65	+4.21	
Av. Daily feed intake, g	1,498	1,466	1,461	33.220
Improvement, %		-2.14	-2.47	
Feed : Gain (FCR)	2.336[a]	2.228[b]	2.187[b]	0.064
Improvement, %		+4.62	+6.38	

[a,b] Means within row with no common superscript differ significant (*P<0.05*)

* Av. Net profit/head calculated from [(Average overall body weight gain x Sale price of pigs (75 Baht/kg)) - (Feed cost/kg/BWG x Weight gain)]

Table 4. Effects of addition Bactosac-P to the diets on the profit and economic production in Overall period (146 days period)

Parameter	Control	Bactosac-P		Pooled
	No added	0.05%	0.10%	SEM
	T1	T2	T3	
Feed cost/kg BWG, Baht	34.39	33.56	33.56	1.135
Av. Net profit/head, Baht*	3,828.21	4,017.87	4,069.11	148.278
Economic benefits return/head, baht		+189.66	+240.90	

REFERENCES

Bullerman, L. B. 1996. Occurrence of Fusarium and fumonisins in grains and in foods. Fumonisins in Foods, edited by L. S. Jackson, J. W. de Vries and L. B. Bullerman (New York: Plenum), P. 27–38.

Crawford, J. S. 1979. Probiotic in animal nutrition. Proc. 1979 Arkasnas Nutr. Conf. P. 44-55.

Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics 11: 1–42.

FAO/WHO; 2001. Health and nutritional properties of probiotics in food including powder milk with live Lactic acid bacteria. www. fao. org.

Fuller, R. 1989. Probiotics in man and animals. Journal of Applied Bacteriology 66: 365–378.

Guarner, F. and J. R. Malagelada. 2003. Gut flora in health and disease. Lancet; 361: 512-519.

Khajarern, S. and J. Khajarern. 1994. Effects of probiotics (Toyocerin) in sow and creep feeds on resistance of diarrhea in piglets. Proceeding of 13th IPVS Congress, Bangkok, Thailand, 26-30 June 1994. P. 294.

Khajarern, J., S. Khajarern. And C. Ratanasethakul. 1996. The efficacy of probiotics (Toyocerin) in broiler breeder feed for inducing immunostimulant and bioregulator effect. Proceeding and Abstract of the 20th World's Poultry Congress, New Delhi, India. 2-5 September 1996. P. 26.

Khajarern, J. and C. Ratanasethakul. 1998. Probiotics in breeder feed. World poultry-Elsevier Volume 14 No. 7. 1998. P. 18-19.

Kozasa, M. 1978. Feed additives of new type. Viable bacterial spores preparation Toyocerin Powder, shiryo-to-shiryo-kogno. J. Feed and Feed Industry 18(12): 45-48.

Mahan, D. C., T. R. Cline and B. Richart. 1999. Effects of dietary levels of selenium-enriched yeast and sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum glutathione peroxidase activity, carcass characteristics, and loin quality. J Anim Sci. Aug; 77(8): 2172-9.

Mahan D. C. and N. A. Parrett. 1996. Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine. J Anim Sci. Dec; 74(12): 2967-74.

Mrowka E., J. Rozmierska, A. Komorowska and K. M. Stecka. 1999. Functional properties of selected yeast products. Zywnosc 3: 7-16.

Nguyen, T. H. 1991. Probiotics a nutritional bioregulator. PIGS-Misset Jan/Feb 1991. P. 27.

National Swine Improvement Federation. 1988. Guidelines for uniform swine improvement programs. Washington, D. C.

Nelson P. E., A. E. Desjardins and R. D. Plattner. 1993. Fumonisins, mycotoxins produced by Fusarium species: Biology, chemistry and significance. Annu. Rev. Phytopathol 31: 233-252.

Nicolosi R., S. J. Bell, B. R. Bistrian, I. Greenberg, R. A. Forse and G. L. Blackburn. 1999. Plasma lipid changes after supplementation with B-glucan fiber from yeast Am. J. Clin. Nutr. 70: 208-212.

SAS. 1986. User's Guide: Statistics (Version 5 Ed.) SAS Inst. Inc., Cary, NC.

Stephen, J. 1993. The use of yeast in nonruminant. Feed Mix 1, 4: 28-33.

Stewart G. G. and I. Russell. 1998. In: An Introduction to Brewing Science &Technology. Series III. Brewer's yeast. The Institute of Brewing, 33 Clarges Street, London W1Y 88EE, England.

O-34-6 Investment in Research and Development Programs on Goat in Central Luzon, Philippines

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Introduction

Central Luzon is an important goat producing region in the Philippines. In 2009, it ranked no. 5 in goat inventory with more than 321,000 head, representing 8% of the country's 4.42 million inventory. During the same year, backyard production represented 99 percent while commercial production was 1% (BAS, 2010). However, the backyard raisers have limited resources, information and skills to make goat farming a more viable enterprise. In view thereof, the government implemented R and D programs in Central Luzon to answer problems that hinder goat farming. The R and D programs of seven institutions in the region, namely (in alphabetical order) Aurora State College of Technology (ASCOT), Bulacan Agricultural State College (BASC), Central Luzon State University (CLSU), Department of Agriculture-Regional Field Office Region 3 (DA-RFU3), Pampanga Agricultural College (PAC), Ramon Magsaysay Technological University (RMTU), and Tarlac College of Agriculture (TCA) were examined. The process of technology transfer and their effects to improve the goat industry in the region were evaluated, in particular, the adoption of the different technologies as an offshoot of technology transfer. The project was conducted to serve as a benchmark assessment of R&D investment, and to set stage for planning and promotion of new technologies in the future.

Objective

This project evaluated the R and D investment on goat and assessed its contribution to the raisers' households in Central Luzon.

Methodology

The primary respondents were 172 goat raisers from Nueva Ecija, Zambales, Tarlac and Aurora and R and D implementers from seven institutions in the region. They consisted of 12 program, project or study leaders of research activities and 8 extension providers who were in-charge of promotion and transfer of technologies. They were determined from the records of the different institutions and from the Central Luzon Agriculture and Resources Research and Development Consortium (CLARRDEC). CLARRDEC is a conglomeration of 26 agencies and institutions in Central Luzon conducting and promoting research and development in agriculture, forestry and natural resources which have been grouped to work together along specific objectives, share expertise and resources and complement each other to push further the horizon of R&D in the region (clarrdec.net).

Secondary data of R and D activities and investment were collected from technical reports and proceedings. Focus group discussions (FGD) were also employed with the R and D workers. All data were analyzed descriptively and presented using means, range, and percentages.

In addition, primary data were collected from goat raisers through personal interview. Adoption of the different technologies on goat as indicator of the success of the technology transfer was analyzed following the steps developed by ACIAR, to wit: a) description of the technologies adopted b) description of the goat raisers c) determination of the indicators of adoption, i.e., level of adoption d) examination of adoption pathways and e) identification of factors influencing the level of adoption.

The number and proportion of component technologies adopted that ranges from 0 to 12 (0 to 100%) were used to indicate the level of adoption. The 12 component technologies were: complete confinement, MPTS supplementation, concentrate supplementation, UMMB supplementation, UTRS supplementation, use of chemical drenches, use of chemical feed block, use of feeds with anthelmintic properties, controlled breeding, upgrading, selection, and artificial insemination (AI).

The model of technology adoption was estimated using the Statistical Packages for Social Science (SPSS):

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + b_{11}X_{11+} b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14} + b_{15}X_{15+} b_{16}X_{16} + b_{17}X_{17} + \varepsilon$$

where:

Y=Level of technology adoption, in % of 12 technologies adopted
X_1=Age of respondents (years)
X_2=Education of respondents (years in school)
X_3=Experience of respondents in goat raising (years)
X_4=Civil status of respondents (1 for married and 0 otherwise)
X_5=Sex of respondents (1 for male and 0 for female)
X_6=Household size
X_7=Household income (pesos)
X_8=Location (1 for Nueva Ecija, 2 for Zambales, 3 for Tarlac, and 4 for Aurora)
X_9=Initial goat inventory
X_{10}=Current goat inventory
X_{11}=Initial capital investment
X_{12}=Type of respondent (1 for trained and 0 for non-trained)
X_{13}=Production type (1 for slaughter and 0 otherwise)
X_{14}=Source of pasture (1 for communal and 0 otherwise)
X_{15}=Number of mass media used for information seeking
X_{16}=Membership in organization (1 if member and 0 otherwise)
X_{17}=Presence of government programs on goat in the locality (1 if there is (are) and 0 otherwise)
ε =Error term

Results and Discussion

Goat R and D Activities and Process of Technology Transfer

Research and development activities were conducted to generate, verify and promote technologies on goat production. These are in line with the identified R and D responsibilities of each institution and the CLARRDEC. As of 2009, there were 69 R & D projects/studies implemented in the region 47 were completed and 22 were on-going. Over half of all projects (60.86%) were implemented by CLSU, 15% by DA-RFU, 10% by PAC, 7% by TCA, and less than 5% by RMTU, BASC and ASCOT. CLSU is the leading institution in goat R&D in the region with the Small Ruminant Center (SRC) taking the lead as a nucleus center.

Research projects were 67% classified by field as genetic improvement (19%), feeds and nutrition (27%), health and diseases (12%), socio-economics (6%), and biotechnology (3%). Development was 33% (Figure 1) with 16 projects/studies. As used in this study, development pertains to either extension projects or applied researches aimed to promote goat technologies and enterprise development to make goat farming a sustainable source of household employment and income.

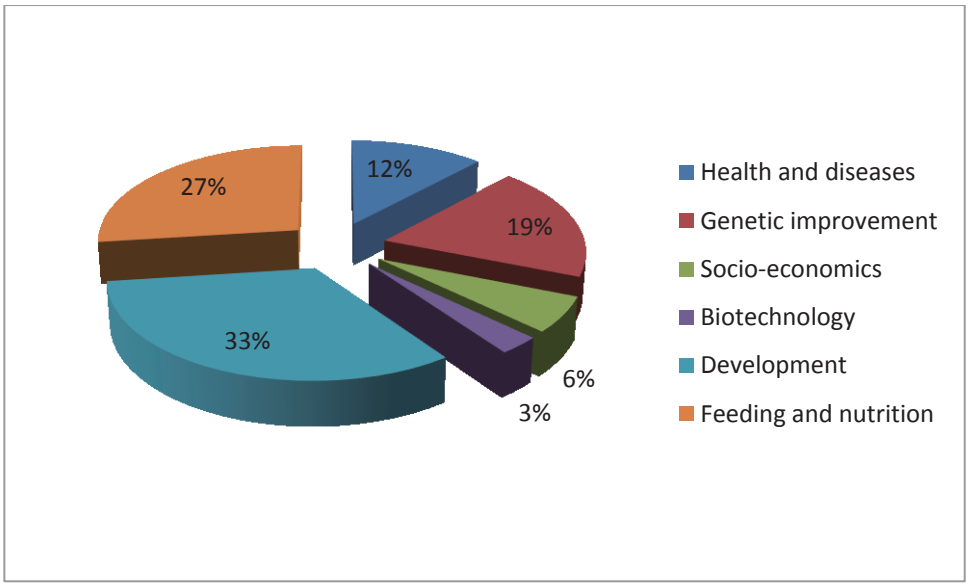


Figure 1. Proportion of R&D activities by field conducted by the different institutions in Central Luzon, 1999 to 2009

Some of the development projects implemented were: the Rural Enterprise Development (RED), the Barangay Integrated Development Approach for Nutrition Improvement (BIDANI), Kambingan sa Barangay, Angat Buhay sa Pamayanan, and Farmers Livestock School on Integrated Goat Management (FLS-IGM). All of these aimed to promote goat raising and transform it to a sustainable enterprise through technological interventions, capacity building and enterprise development.

Amount of Investment on Goat R and D

The total budget for goat R and D and support services in the region from 1992 to 2009 was P44.49 million (Figure 2). Research captured 51% with Php 22.66 million, development had Php 12.03 mil or 27% share, production with Php6.91 mil or 16% share, facility improvement with Php 2.22 mil or 5 %, and human resource development with Php 0.67 mil or 1% share. Apparently, funds for goat R and D is slanted towards research, but this is justified by more research projects than development projects implemented. Research projects on genetic improvement and feeding nutrition shared about 34% each, health and diseases by 23% and socio-economics by 7%. Researches on biotechnology were relatively a new topic with research funds of only 3%.

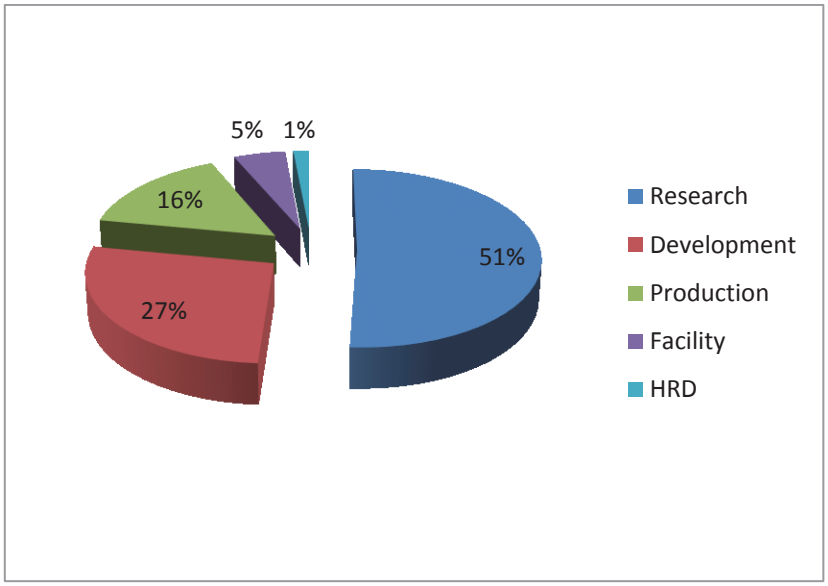


Figure 2. Share to total R&D fund of various disciplines of the different institutions in Central Luzon, 1999 to 2009

The Philippine Council for Agriculture, Aquaculture and Natural Resources Research and Development (PCAARRD) has been in the front line in supporting the goat industry in the region. It has contributed 23% or Php 10.14 mil over the years. PCARRD had been one of the major partners of CLSU in most of its R & D activities to develop the small ruminant industry. PCAARRD also provided funds to TCA to implement FLS-IGM to hasten technology adoption. Of the total funds from PCAARRD, 91% was provided to CLSU and 9% to TCA. Meanwhile, funds for research was Php 3.61 mil, for development activities Php 3.12 mil, and for production Php 1.36 mil. The shares of research and development to the total funds from PCARRD were not far different 36 percent for research and 31 percent for development, indicative of PCARRD's balanced support for research and development to further improve the goat sector in the region. PCAARRD also provided funds to improve the facilities of the SRC and to develop human resources with Php 1.52 mil and Php 0.53 mil, respectively.

Similarly, the Department of Agriculture (DA) through its attached agencies provided Php 14.88 mil to implement goat R and D projects. Bulk of these funds (Php 6.23 mil or 33%) was for the implementation of the genetic improvement program. About a quarter (Php 3.35 mil) was for development activities while the rest (Php 5.3 mil or 36%) was for other research fields, production and facility improvement. CLSU and DA-RFU3 were the major recipients with Php 10.61 mil (71%) and Php 3.25 mil (22%), respectively. TCA also received Php 1.0 mil from DA-BAR to enhance adoption of goat technologies through conduct of FLS-IGM. ASCOT also received Php 0.02 mil to implement its Small Ruminant Integrated Research and Development.

Additional funds were provided by other national and international funding agencies (26%). The seven institutions shared own agency funds CLSU shared 9%, PAC 6% and DA-RFU3 3% while other agencies shared less than 1%. The data on investment further reflects the participation of many agencies in funding goat R and D projects to accelerate the development and promotion of the goat industry in the region.

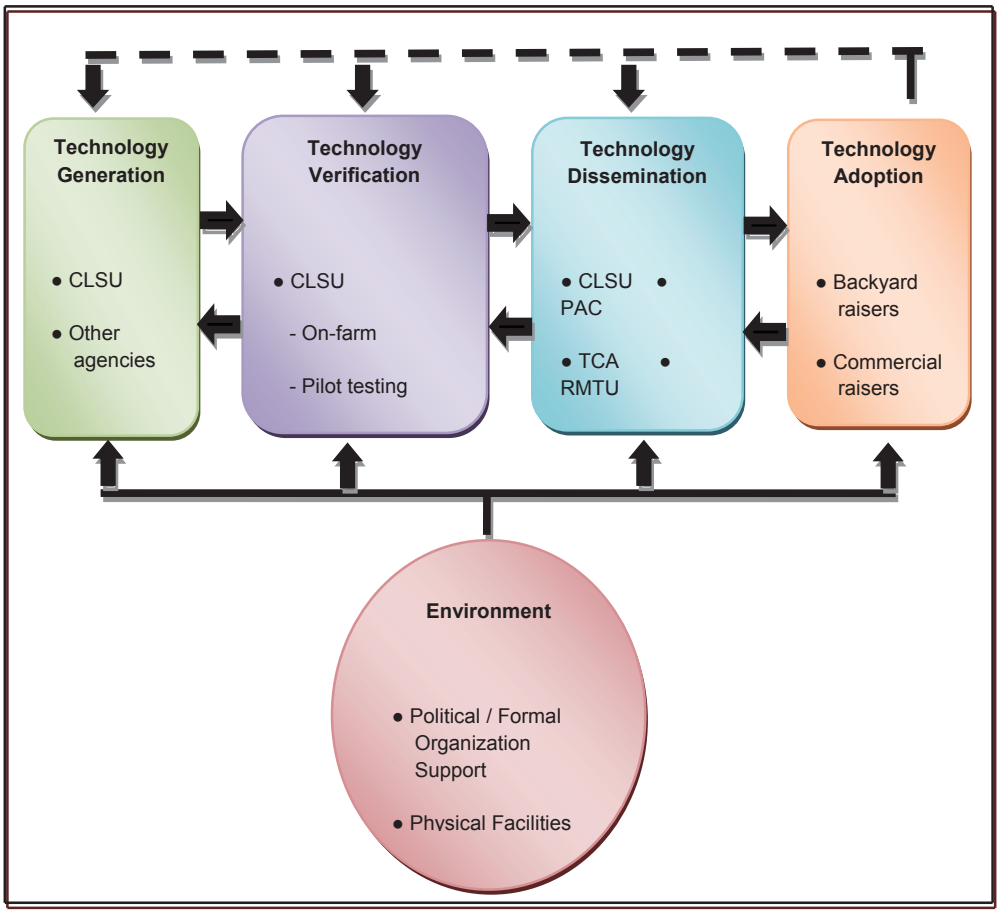
R and D Outputs on Goat

The R and D investment and programs on goat in the region were able to generate, verify and disseminate technologies to both backyard and commercial goat raisers in the region. CLSU had done most of the generation and verification works while other institutions did the dissemination part through various technology transfer mechanisms. The following technologies were generated to improve goat farm productivity.

1.Upgrading technology
2.Three-way cross breeding
3.Multiple ovulation embryo transfer (MOET)
4.Artificial insemination
5.Rapid rotational grazing
6.Pure confinement
7.Housing and flooring design
8.Silage production
9.Legume strata production
10.Grass-legume combination feeding
11.Soluble glass mineral transfer bolus
12.Urea treated rice straw
13.Medicated urea-molasses mineral block
14.Goat-rice, goat mango, goat sheep-fish integration farming
15.Food-feed system

Technology Transfer Process to Promote Goat Technologies

The process of how goat technology transfer (adopted from Rogers, 1983 as cited in Carbonel, 2004) had the following important dimensions: 1) formal in structure with four stages: technology generation, technology verification, technology dissemination, and technology adoption 2) complementation of the different institutions and the local government units (LGUs) depending on their expertise and resources 3) use of different modalities in technology transfer 4) presence of enabling mechanisms which hasten technology transfer, namely political/ formal organization's support, physical facilities, economic, support services, and human/manpower and 5) the importance of feedback mechanism to technology generators, extension providers and end users.



Adopted from Rogers, 1983 as cited in Carbonel, 2004)

Figure 3. Technology transfer process on goat R and D by various institutions in Central Luzon, 1999 to 2009

Training and seminars for knowledge and skills enhancement were the most important activities with 109 activities done by the different institutions from 1989 to 2008. These consisted of one day to season long activities. From 2004 to 2006, there were 862 participants composed of farmers (645), students (102), technicians (11), military men (45), and other clienteles (59). Two examples of trainings conducted are the Farmer Livestock School-Integrated Goat Management (FLS-IGM) and Farmer Field School (FFS) as reported by a few (15%) key informants from TCA and DA-RFU3, respectively. FLS-IGM is a six month-long modality designed by AMPAlo of PCARRD.

Other important modalities of transfer were farm visits, field days, buck loan and dispersal, demonstration farms, formation of goat raisers associations, use of communication media such as radio, print and television, and use of IEC materials. These are considered as the fastest mechanisms to reach out a large base of target clients.

Adoption of and Outputs from Goat Technologies

The adoption pathway of five technological options such as partial confinement, supplementation, health management, genetic improvement and waste management took a much shorter period when they were heard, tried and adopted (Figure 4). The number of raisers who used MPTS and tree leaves with anthelmintic properties are relatively high. In as much that these feed resources are readily available in the locality their use should be further promoted. Information dissemination could play a very important role in this regard. Other technological options such as use of UMMB and other mineral blocks, complete confinement, concentrate supplementation, and controlled breeding are adopted by few raisers whether trained or not. They add cost and are unavailable in the locality, hence the low adoption. Apparently, adoption is also a function of the attributes of the technologies.

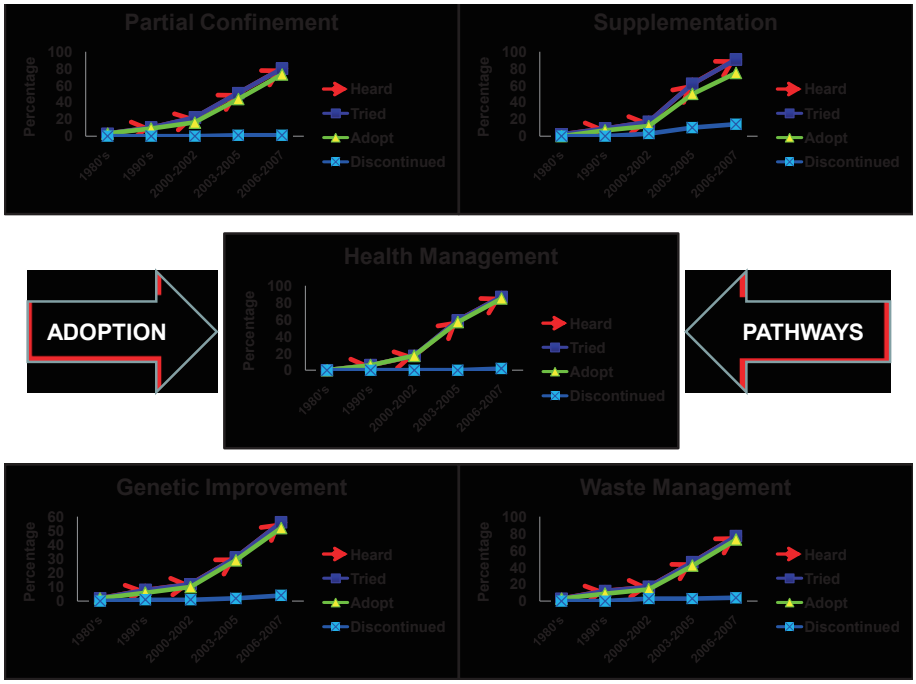


Figure 4. Percent of goat raisers who have heard, tried, adopted and/or discontinued the use of technologies on goat in Central Luzon, 1998 to 2009

There were 6 of 17 variables found to be significant to explain the level of technology adoption household size, household income and initial investment (socio-demographic and economic variables), type of respondent (institutional variable), initial inventory and source of pasture (technological variables). Except for the latter, all the other significant variables have positive coefficients (Table 1). When household size, household income, initial investment, and initial inventory are increased individually, the level of technology adoption is also increased by the amount of their respective coefficients.

Table 1. Results of multiple regression analysis showing the variables that explain the level of technology adoption on goat in Central Luzon, 2009

Parameter	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	5.218	1.339		3.898	.000
Age of grower	.005	.011	.037	.478	.633
Sex of grower	-.426	.328	-.094	-1.299	.196
Civil status of grower	-.637	.472	-.102	-1.350	.179
Education (years)	-.070	.047	-.111	-1.486	.139
Farm location	-.174	.143	-.099	-1.218	.225
Household size***	.208	.067	.216	3.127	.002
Household income*	3.13E-006	.000	.121	1.658	.099
Membership in organization	.158	.112	.124	1.409	.161
Type of respondent***	1.128	.315	.288	3.576	.000
Purpose of production	-.814	.506	-.110	-1.609	.110
Source of pasture**	-1.051	.462	-.183	-2.274	.024
Initial capital investment*	1.43E-005	.000	.140	1.862	.064
Initial inventory*	.059	.033	.122	1.805	.073
Current inventory	.014	.017	.057	.799	.425
No. of communication media used in goat	.382	.261	.105	1.459	.147
Experience in goat raising	.007	.016	.031	.413	.680
Presence of goat program in barangay	-.081	.357	-.020	-.228	.820
R squared	0.369				
F value***	5.260				.000

*** Significant at 99 percent confidence level
** Significant at 95 percent level
* Significant at 90 percent level

The positive coefficient for type of respondent indicates that the level of adoption was also higher among those who had been trained on goat by the different institutions in Region 3. Apparently, training is a vital mechanism for technology transfer to increase adoption. Raisers needed knowledge and skills on goat raising that they could apply to increase farm performance, and these could be derived from trainings. Source of pasture is significant with negative coefficient indicating that adoption of technologies by raisers is limited by not having their own source of pasture.

The application of more management practices and technologies which the raisers learned from trainings and other extension modalities could be attributed to the improvement of the farms' performance. Training in particular has increased the raisers' knowledge and skills that helped them improved their farming.

Conclusion and Recommendation

The primary objective of all R and D institutions and other stakeholders on goat is to further develop the sector and make goat farming as a sustainable enterprise among smallhold farmers in the region. In the light of the above findings, the following are recommended: invigorate R and D activities on goat in the region through

complementary efforts of different R&D institutions, enhance technology adoption through more trainings and other extension modalities, and capitalize on existing resources and proximity of the region to Metro Manila to make the entire Central Luzon a goat hub in production and technology.

KEYWORD : goat, research, development, investment, Central Luzon

REFERENCES

BUREAU OF AGRICULTURAL STATISTICS. 2010. Goat Population Statistics

Carbonel, N. R. 2004. Analysis of communication variables associated with utilization of upgraded goat production technology in Nueva Ecija, Philippines. Unpublished Ph. D. Thesis. University of the Philippines at Los Banos. Philippines

Gordon, J. and J. Davis. 2007. ACIAR impact assessment: Guidelines for practitioners

Orden, E. A., A. B. Serra, S. D. Serra, K. Nakamura, L. C. Cruz, and T. Fujihara. 2000. Direct effects of copper and selenium supplementation and its subsequent effects on other plasma minerals, body weight and hematocrit count of grazing Philippine goats. Asian-Aus. J. Anim. Sci. 2000. 13(3):323-328

Orden, E. A., E.M. Cruz, T. Ichinohe, and T. Fujihara. 2002. Effects of *Leucaena leucocephala and Glicridia sepium* supplementation on outflow rate, microbial protein yield and growth of sheep fed with ammoniated rice straw. Philippine J. Vet. Anim.Sci. 2002, 28 (1):63-71

Orden, M. E. M., W. E. Jamandre, E. O. Brown, E. A. Orden, E. M. Cruz, A. M. P. Alo, and E. C. Villar. 2005. Traders' preference for goat characteristics in selected markets of Pangasinan, Philippines. Animal Science Journal (2005) 76, 179-185

O-34-7

Backyard Raisers' Attitudes Toward Artificial insemination in Goat in the Philippines

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Introduction

Goat has been part of the Philippine landscape since time immemorial, with about 1M Filipinos depending on it for livelihood. At the moment, however, goat population stands at 3.6M head (PSA, 2016), decreasing by about 1% annually since 2010. This is attributed to two major factors - high slaughter rate and low productivity. Low productivity generally stems from the farmers' limited knowledge on proper production and lack of access to quality breeder animals. This leads to long production cycles of more than nine months and low conception rate of only 75% for the dam and high pre-weaning mortality rate (25%) and low slaughter weight (15kg) for the kids. The challenge therefore is to teach farmers the various aspects of goat production and give them access to artificial insemination (AI) to improve farm productivity.

Objectives

The study determined the backyard goat raisers' attitudes towards artificial insemination (AI) in goats, *Capra hircus* L., as basis for further development of the AI delivery system in the Philippines.

Methodology

A survey of 234 backyard goat raisers was conducted in 2014-2015 in six goat producing regions in the country, namely Ilocos Region (Region 1), Cagayan Valley (Region 2), Central Luzon (Region 3), Eastern Visayas (Region 8), Northern Mindanao (Region 10), and SOCSARGEN (Region 12). Using a structured questionnaire, the farmers' acceptability and willingness to have their goats inseminated and their willingness to pay for AI service were determined.

Responses were analyzed descriptively and the factors associated with their behavior determined by binary logistic regression estimated using SAS. Two regression models were estimated, one, for raisers' willingness to have their goats inseminated and another for goat raisers' willingness to pay for AI as the dependent variables. Values 1 and 0 denote willingness and unwillingness, respectively.

The logit model is based on the cumulative logistic probability function as specified by Pyndick and Rubinfeld, 1991 (as cited in Bhandari, 1999). Moreover, the equation and representations were derived from Greene (2005) and Hill et al (2008) as used similarly by Mariano et al (2014) in their study to determine factors influencing farmers in adopting modern rice technologies is the Philippines.

$$P_{1/i} = F(\alpha + \beta X_i) = \frac{1}{1 + e^{-(\alpha + \beta Xi)}} = \frac{e^{(\alpha + \beta Xi)}}{1 + e^{(\alpha + \beta Xi)}} \qquad (1)$$

Where:

F = cumulative logistic probability function

e = base of natural logarithm

$P_{1/i}$ = probability that the individual makes a certain choice

$P_{1/i}(1 + e^{\alpha + \beta Xi}) = e^{(\alpha + \beta Xi)}$

$P_{1/i} = (1 - P_{1/i}) * e^{(\alpha + \beta Xi)}$

$P_{1/i} / (1 - P_{1/i}) = e^{(\alpha + \beta Xi)}$

β = vector of unknown parameters

X_i = vector of independent variables

The linear logit model is:

$\text{Log } e\, P_{1/i} / (P_{2/i}) = P_{1/i} / (1 - P_{1/i}) = \alpha + \beta X_i = Z_i \qquad (2)$

The probability that the individual farmer is willing to have his goat inseminated (or pay for AI service) is represented by $P_{1/i}$ and the probability that the raiser is not willing is represented by $1 - P_{1/i} = P_{2/i} = 1 / (1 + e^{\alpha + \beta Xi})$.

The survey was done by trained project staff and assisted by the personnel of the Department of Agriculture-Regional Field Offices (DA-RFOs), Local Government Units (LGUs), Provincial Agricultural/Veterinary Offices (PAOs/PVOs), and four participating State Colleges and Universities (SCUs).

Results and Discussion

Characteristics of the goat farmers. The 234 respondents were all classified as backyard raisers, as they have an average herd of 12 goats, 6 of which are does. In the Philippines, backyard operation is characterized by the presence of Majority of them (75%) have been raising goats only since 2001. The rise in the number of goat raisers from the 1950s to 2000s is attributed to the initiatives of government institutions like DOST-PCAARRD on goat R&D since the start of the millennium.

Average household size is quite big at 5 heads. In the Philippines under backyard level, household size is a critical factor in animal raising, as members provide the needed farm labor.

Age of the goat farmers interviewed ranged from 18 to 76 years, affirming that goat raising can be participated in by both young and old.

Most (40%) are crop farmers with livestock farming as supplementary source of income. Average annual income across regions is PhP 132,410 (US$2,817) with goat farming contributing a little more than 5%. This income from goats is mostly from the sale of live animals.

Sixty percent (60%) of the respondents are members of goat related organizations, which are their sources of information, knowledge and new technologies on goat farming.

Thirty-five percent (35%) have had training on goat production via the Farmer Livestock School on Goat Enterprise Management, where goat AI is one of several modules taught. Only 5.1% were actual beneficiaries of the training on AI delivery protocols. The rest had no skill training on AI.

Current goat breeds raised. As shown in Figures 2a & 2b, majority of the does and bucks raised were upgraded native goats (Native x Anglo Nubian or Native x Boer). Only 13% of the does are purebred (Anglo Nubian or Boer) and crossbred (Anglo Nubian x Boer). Data shows that 36% of the bucks in the farms surveyed were purebred and crossbreds. Interestingly, some of the backyard farmers spent money to really purchase purebred bucks to upgrade their herd although many of the farms (109 farms or 47%) do not have their own bucks for breeding purposes.

Breeding practices employed in the farms. In the surveyed farms, 53% have their own breeder bucks and 47% either borrow from their neighbors or pay for the services of bucks-for-hire 78% reported using natural mating in their farms.

AI was practiced by 22%, in combination with natural mating. It is practiced by more raisers from Regions 2 and 8, where the goat AI delivery system was piloted and where more farmers have no access to quality bucks.

Because of the limited access of smallholders to quality bucks, the Department of Science and Technology (DOST) thru PCAARRD introduced R&D initiatives to refine the AI delivery protocol and pilot the delivery system in Regions 1, 2, 3, 8, 10 and 12 starting 2013. It was later made part of the DA's *Unified National Artificial Insemination Program* (UNAIP). It was also included in the Farmer Livestock School on Goat Enterprise Management designed by PCAARRD and implemented by local government units in the same six pilot regions.

Raisers' attitudes toward goat AI. Although most have heard about goat artificial insemination, only 30% of the respondents have had the chance to avail of AI. Of those who availed, success rate was low at 6-36%. The generally low percentage of AI success across areas surveyed can be attributed to the lack of training of most farmers on heat detection, which resulted to problems on timing of AI, failed monitoring of recurrence of heat among inseminated does, and missed follow-up insemination. As AI in goats is relatively a new breeding method in the country, not all the respondents have been covered by the training provided by the DOST- PCAARRD and the DA projects hence not all the respondents who availed of AI had the basic knowledge on the reproductive physiology of goats and skills on the proper protocols before, during and after insemination.

Despite these initial results, about 80% of the goat raisers expressed willingness to have their goats inseminated or continue to avail of AI and 74% said they are willing to pay Php100-Php150 ($2.15-$3.23) for a successful AI service (Table 1). Four of the six regions have more than 80% of the raisers willing to have their goats inseminated and two regions have more than 80% of raisers willing to pay for AI service. Outright reasons mentioned for this positive regard for AI were the quality of offspring seen in farms who have availed of AI and the presence of AI technicians in the locality.

Factors influencing willingness to practice AI. Using the characteristics in Table 2, the estimated logistic model for

raisers' willingness to have their goat inseminated was plotted as follows:

As analyzed in Table 3, among the different variables, regional grouping was found to be a significant predictor of having AI. Specifically, Regions 2 and 3 showed positive coefficient and significant p-value for farmers' willingness to avail of AI. The awareness created by the R&D efforts on AI in goat done by the government thru LGU technicians and SCU program implementers in this region contributed to the positive regard of farmers for AI.

Among the farmer characteristics, level of education, degree of awareness (having heard of AI), and attendance to the FLS-GEM showed positive coefficients and significant p-values (Table 3). The result regarding education was expected, such that those with higher education (more than the primary level) have higher predisposition of understand the physiology of goats and the need for AI than those who had less than 6 years of schooling. Moreover, those who underwent the FLS-GEM for 28 weeks definitely had a good understanding not only of breeding and AI but all the aspects of goat production hence, the variable is a good predictor of whether farmers will accept or reject AI. For those who have heard of AI, the most significant change they heard was the increase in size of kids from AI, positively affecting therefore their decision to avail of AI.

The chi-square test statistics was significant at 5% level, indicating the significance of the model. Among the different variables, 'FLS-GEM' and 'having heard of AI' had the highest marginal effects of 12% and 10%, respectively. All other variables have 6% or lower marginal effects. The mean values of the different variables were substituted to the estimated logit model to determine the probability of raisers willingness to avail of AI. The result showed a relatively high probability of 93%. This value is supported by the result of the interview wherein 80% said they were willing and only 20% unwilling to avail of AI.

Factors influencing willingness to pay for AI service. The estimated logistic model for raisers' willingness to pay for AI services is:

For willingness to pay, aside from Regions 2 and 3, Region 10 was found to have positive coefficient and significant p-value. This means that raisers in these regions have better chances of agreeing to pay for AI service than those in other regions. Among the farmers' characteristics, sex along with education were also significant, but the former has a negative coefficient. This implies that female raiser-respondents were less likely to pay for AI service than their male counterparts. As in the first logit model, the variables 'heard AI' and 'FLS-GEM' were also predictors that explain willingness to pay for AI service. The benefit from AI that the raisers heard from FLS-GEM entice them to agree to pay for AI service. The variables 'heard AI', 'FLS-GEM', 'civil status', and 'Region 10' had the highest marginal effect, 16%, 14%, 12%, and 11%, respectively. The possibility of goat raisers paying for AI service was computed at 89%.

Conclusion and Recommendations

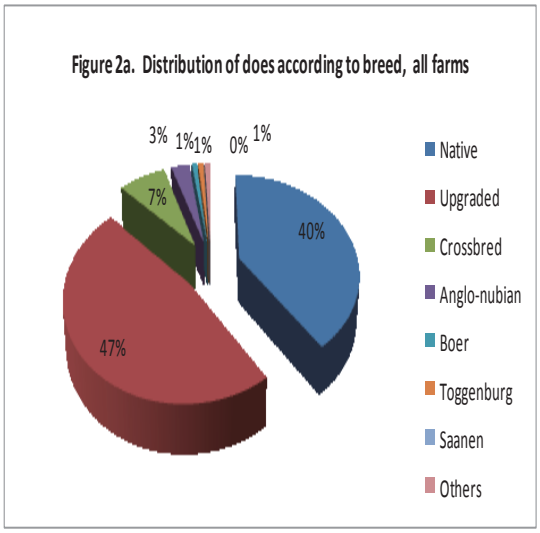
The challenge to teach farmers the various aspects of goat production and give them access to artificial insemination to improve farm productivity is hinged not only on the readiness of government to rollout the technologies but also on the acceptability by farmers of the AI technology for goat, a relatively new technology in the country.

As shown by the results of the study, willingness to avail of AI service and pay for a successful insemination is affected by the readiness of the regions. Where AI services (technicians and logistics) are available like in Regions 2 (Cagayan Valley) and 3 (Central Luzon), farmers have shown an equivalent readiness to accept the technology and even pay for AI. Moreover, degree of awareness, be it participation in a season-long training like the FLS-GEM, short orientation trainings or even just news about AI, can significantly encourage farmers to try and even pay for AI services especially when access to breeders is a problem.

Considering these findings, it may sit well with government, especially the R&D Institutions promoting AI, to address first the following before widespread roll-out of the AI delivery system in the countryside:

Ensure that the regions (to where AI will be promoted) have existing AI service providers and that the goat AI delivery system has been made part of UNAIP. It is necessary that more LGU technicians and village-based AI technicians are trained and supported with the necessary equipment and facilities for semen storage and processing.Thorough training on the physiology of the animal as well as on the protocols prior, during and after AI should be implemented before accrediting an inseminator to conduct AI, especially for a fee.Promotion of AI in various media can be done to create widespread awareness of AI as an alternative breeding method especially in areas where access to quality breeding bucks is limited. This will create needed awareness for beneficiaries. Training of farmers availing AI should likewise be thorough, as the timing of AI, monitoring of recurrence of heat among inseminated does, and seeking for follow-up insemination are responsibilities of farmers.

KEYWORD : Goat, Artificial insemination, Backyard, Attitudes, Philippines



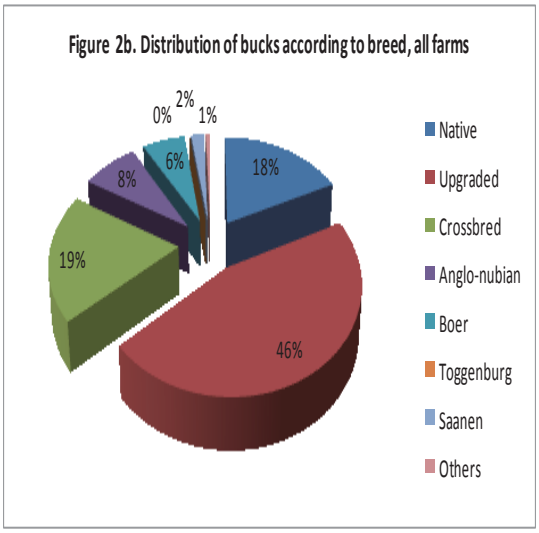


Table 1. Goat raisers' regard for the AI services in the Philippines (N=234).

	Willingness to avail of AI		Willingness to pay for AI	
	Willing	Not willing	Willing	Not willing
Region 1, no. reporting	30 (69.8%)	13 (30.2%)	29 (67.4%)	14 (32.6%)
Region 2, no. reporting	27 (81.8%)	6 (18.2%)	26 (78.8%)	7 (21.2%)
Region 3, no. reporting	53 (86.9%)	8 (13.1%)	51 (83.6%)	10 (16.4%)
Region 8, no. reporting	16 (94.1%)	1 (5.9%)	10 (58.8%)	7 (41.2%)
Region 10, no. reporting	26 (86.7%)	4 (13.3%)	25 (83.3%)	5 (16.7%)
Region 12, no. reporting	34 (68.0%)	16 (32.0%)	31 (62.0%)	19 (38.0%)
All areas, no. reporting	186 (79.5%)	48 (20.5%)	172 (73.5%)	62 (26.5%)

Table 2. Characteristics of goat raisers surveyed vis-à-vis their regard for AI.

Characteristic	Willingness to avail of AI		Willingness to pay for AI	
	Willing	Not willing	Willing	Not Willing
Age, years, mean	49.8	50.10	49.78	50.13
Education, years, mean	4	3	3	3
Experience, years, mean	8.17	7.17	7.5	9.19
Married, no. reporting*	151 (78.2)	42 (21.8)	145 (75.1)	48 (24.9)
Male, no. reporting*	122 (81.9)	27 (18.1)	116 (77.9)	33 (22.1)
Household income, Php	144,259	179,156	137,023	195,678
Ave. goat inventory per farm	15	14	15	14
No. of does per farm	6	6	8	6
Native	2	3	4	3
Other breeds	4	3	4	3
Attended FLS-GEM	21 (95.5)	1 (4.5)	20 (90.9)	2 (9.9)
Attended other training	135 (78.0)	38 (22.0)	128 (78.0)	45 (22.0)
Membership in organization	108 (79.4)	28 (21.6)	103 (75.7)	33 (24.3)

*Figures parentheses are %

Table 3. Estimated coefficients and marginal effects of the logit model

Variable Group		Willingness to Have Goats Inseminated			Willingness to Pay for AI Service		
		Coefficient	P level	ME	Coefficient	P level	ME
	Constant	-1.0561	0.0624		-2.7504	0.0524	
Regional resource	R1				0.4054	0.4975	0.03679
	R2	0.9172	0.0954	0.05513	1.1922	0.0548	0.10818
	R3	1.0947	0.0290	0.06580	0.8862	0.1003	0.08041
	R10	0.9333	0.1325	0.05610	1.2677	0.0510	0.11503
Farmers' characteristics	Age				0.0084	0.6087	0.00076
	Sex	-0.3316	0.3828	-0.01993	-0.8410	0.0328	-0.07631
	Education	0.1420	0.0530	0.00854	0.0856	0.0523	0.00777
	Experience	0.0300	0.1865	0.00180	-0.0177	0.3173	-0.00161
	Civil status				1.2782	0.6737	0.11598
	HH size				0.0378	0.6342	0.00343
Farm resource	Native goat	-0.1079	0.1513	-0.00649	0.0498	0.4476	0.00452
	Other breeds				-0.0111	0.4070	-0.00101
Institutional	Heard of AI	1.7318	0.0001	0.10410	1.7726	0.0004	0.16084
	Participated in FLS-GEM	1.9924	0.0672	0.11976	1.6188	0.0557	0.14689
	Org. Membership				0.3972	0.3608	0.03604
	Training				-0.0599	0.9088	-0.00544

REFERENCES

BHANDARI, H. N. 1999. The economics of groundwater irrigation in ricebased system terai of Nepal. Unpublished PhD thesis. UPLB.

GREEN, W. H. 1993. Econometric analysis. New York USA. McMillan Publishing Company

MARIANO, M. J., R. VILLANO, E. FLEMING. 2012. Factors influencing farmers' adoption of modern rice technologies and good management practices in the Philippines. Elsevier. Agricultural Systems 110 (2012) 41-53

PHILIPPINE STATISTICS AUTHORITY. 2016. In: http://countrystat.psa.gov.ph/selection.asp (accessed June 20, 2016).

PINDYCK, R. S. AND D. L. RUBINfeld. 1991. Econometric models and economic forecast. 3rd Edition. New York. McGraw Hill.

O-34-9

Milk Composition of Upgraded Philippine Native Goats

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Introduction

Dairy goat farms in the Philippines today are concentrated in herd build-up and increasing milk production per animal. Constant infusion of bucks from elite herds abroad combined with efforts to improve management practices have been the center of interest of progressive DG owners in the Philippines. Differences in genetics and nutrition for goats are probably the main factors affecting the extreme variation in milk yields and components. Reliability and uniformity of animals in terms of milk yield and components is yet to be realized within the goat herd.

Materials and Methods

A total of sixty dairy goats including Upgraded Philippine native goats (Philippine Native x Anglo-Nubian) in the first and second parity from the Small Ruminant Center with average body weight of 40 ± 5 kilogram were used in this study to characterize milk component concentration. Goats were fed ration made up of 57%grass and 25%tree legumes based on 3% of the BW with 250g rice bran.

Monthly milk collection was conducted to measure milk yield and components from different breeds of lactating goats at the Small Ruminant Center. Breeding and pedigree information of animals are encoded in the herd database such as date of parturition, breed, parentage, identification and kid information. Morning samples were collected from both side of the udder and then immediately cooled down and kept refrigerated. Milk samples were pooled with milk samples from PM milking and then heated to temperature between 33-38 ℃ before analysis. Pooled AM/PM milk samples were analyzed using a LactoScan™ SLP that uses ultrasonic technology to measure fat, protein, lactose, solids, solid-non-fat, added water, temperature, conductivity and freezing point.

Results and Discussion

Fat

Milk fat has the most complex fatty acid composition of the edible fats. Results on milk fat are presented in Figure 1. Unlike protein and carbohydrates, fat composition in milk varies widely in the composition due to genetic, lactational, and nutritional factors (Fox, 1995). Beside from the process milk has undergone, the percentage of milk fat in milk is used to grade or classify milk such as medium cream, whole milk, reduced fat milk, low fat and others.

Results showed that the average milk fat concentration (5.8%) across breed was higher compared to USDA (3.5%) milk fat values for goat. Among the breeds upgraded goats gave the highest value of 7.09% followed by Boer, Saanen, Anglo-Nubian, La Mancha and Alpine with 6.78%, 5.60%, 5.11%, 4.97% and 4.34%, respectively. Upgraded goats yielded twice (102%) the milk fat concentration over that reported by USDA for goats. The higher milk fat translates to more nutritional energy per serving of milk.

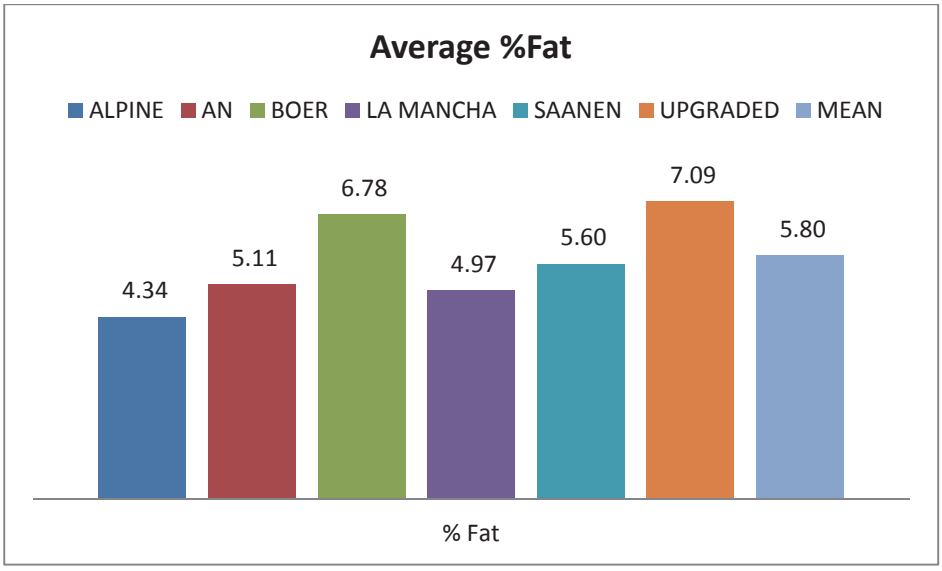

Figure 1. Milk fat concentration (%) from goats maintained at the Small Ruminant Center

Lactose

Lactose is a disaccharide sugar derived from galactose and glucose that is found in milk. Lactose makes up around 2-8% of milk, by weight (Carper, 2014). This carbohydrate needs the enzyme lactase to be broken down and absorbed by the body. The inability to digest milk also known as lactose intolerance comes from the insufficient production of lactase.

Mean lactose concentration was 4.76% across breeds (Figure 2) and were nearly the same amongst the breed except milk lactose values 5.02% from Upgraded goats. Alpine 4.34% was nearly the same values reported by Mioc (2008) with 4.54% lactose. Meanwhile, Saanen goats at the Small Ruminant Center were higher in milk lactose concentration at 5.60% compared to 4.46% in Croatia. The mean lactose level is still lower than those found on cattle, sheep and buffalo (Table 1) which maybe a small advantage of goat's milk for people with lactose intolerance.

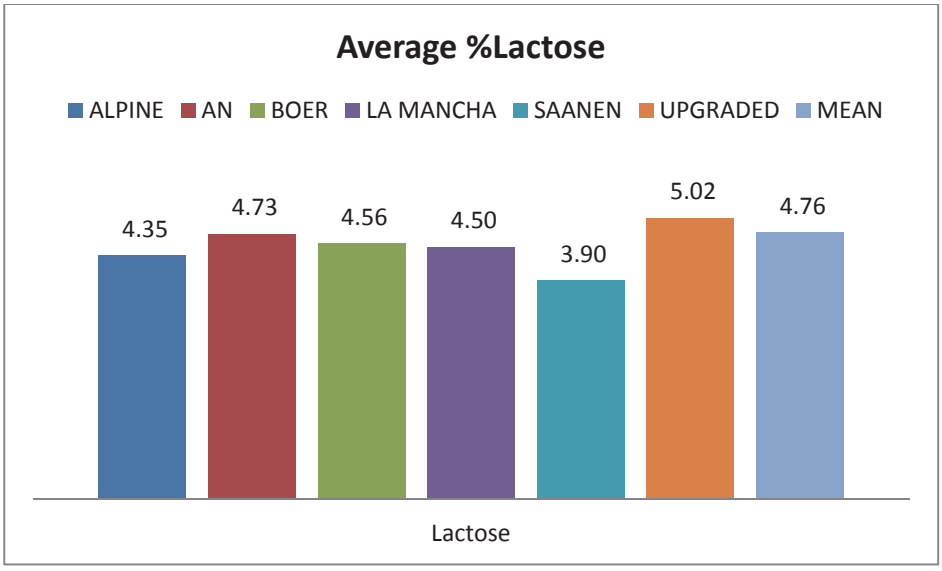

Figure 2. Milk lactose concentration (%) from goats maintained at the Small Ruminant Center

Table 1. Differential milk composition analysis, per 100 grams, by species

Constituents	Unit	Cow	Goat	Sheep	Water buffalo
Water	g	87.8	88.9	83	81.1
Protein	g	3.2	3.1	5.4	4.5
Fat	g	3.9	3.5	6	8
Carbohydrate (i.e the sugar form of lactose)	g	4.8	4.4	5.1	4.9
Calcium	mg	120	100	170	195
Energy	kcal	66	60	95	110
	kJ	275	253	396	463

*USDA National Nutrient Database for Standard Reference
**McCane *et. al*, International Laboratory Services

Protein

Milk proteins contain all 9 essential amino acids required by humans. Total milk protein content and amino acid composition varies with goat breed and individual animal genetics. Goat milk protein forms a softer curd (protein clumps that are formed by the action of stomach acid on the protein), which makes the protein more easily and rapidly digestible.

Milk protein concentrations among breeds were nearly the same with an average of 3.19% (Figure 3) with the lowest mean value of 2.62% from Saanen goats. The same values were similar to goat milk protein from USDA National Nutrient Database for Standard Reference (3.1g/100g) which ranks goat's milk lowest compared to buffalo, sheep and cow's milk.

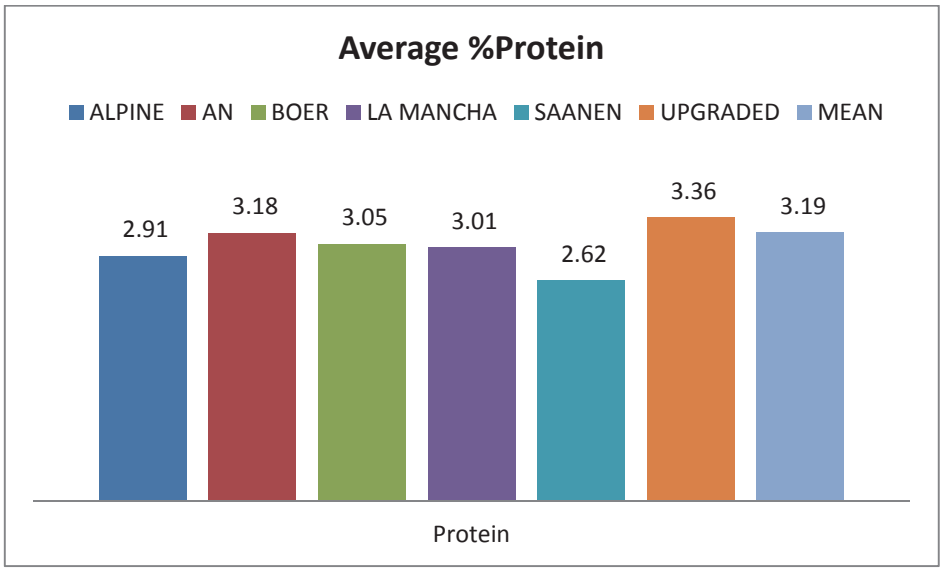

Figure 3. Milk protein concentration (%) from goats maintained at the Small Ruminant Center

Conclusion and Implications

Milk fat and milk protein concentrations of Upgraded Philippine goat was higher compared to other breeds. Results indicate that milk produced by upgraded Philippine native goats could be a potential source of butter fat though milk yield is relatively low than purebreds.

KEYWORD : milk composition, upgraded goat, milk fat, milk protein

O-35-2

Monitoring system of body temperature in calves by using infrared thermography

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Objective

The economic losses of young calves which susceptible to infectious pathogens causing diarrhea and pneumonia, because of their immature immune systems, are serious. Early detection of infected animals is important to implement effective treatment and control disease within herds. Body temperature is one of suitable parameters to exhibit health condition of animal. Infrared thermography is a non-invasive sensing technique, and is superior to measure quickly object's temperatures. In this study, we attempted to establish an automated system to monitor health condition of animal by using the infrared thermography.

Methodology

Our system can individually and automatically record their facial temperature, focusing on ophthalmic max temperature by using an infrared camera (InfRec TS9100, Nippon Avio, Co., Ltd.), and ambient temperature by using a thermometer when young calves are drinking milk fed by robots (Fig. 1).

Results

In operation of our system using multiple animals in a Japanese Black cattle breeding farm, we confirm that this system can comprehend health condition of animal by monitor of ophthalmic max temperature correlating with rectal temperature during drinking milk (Fig. 2). In addition, this system has an optional function which correct ophthalmic max temperature according to daily fluctuation of ambient temperature by monitoring ambient temperature of animal continuously.

Conclusions

Our system enables early detection and effective treatment of abnormal animal, and hence it would contribute to reducing animal suffering and improving the economic losses in farm. This system is patent pending in Japan.

KEYWORD：infrared thermography, monitoring system, health condition, body temperature, detection of abnormal animal

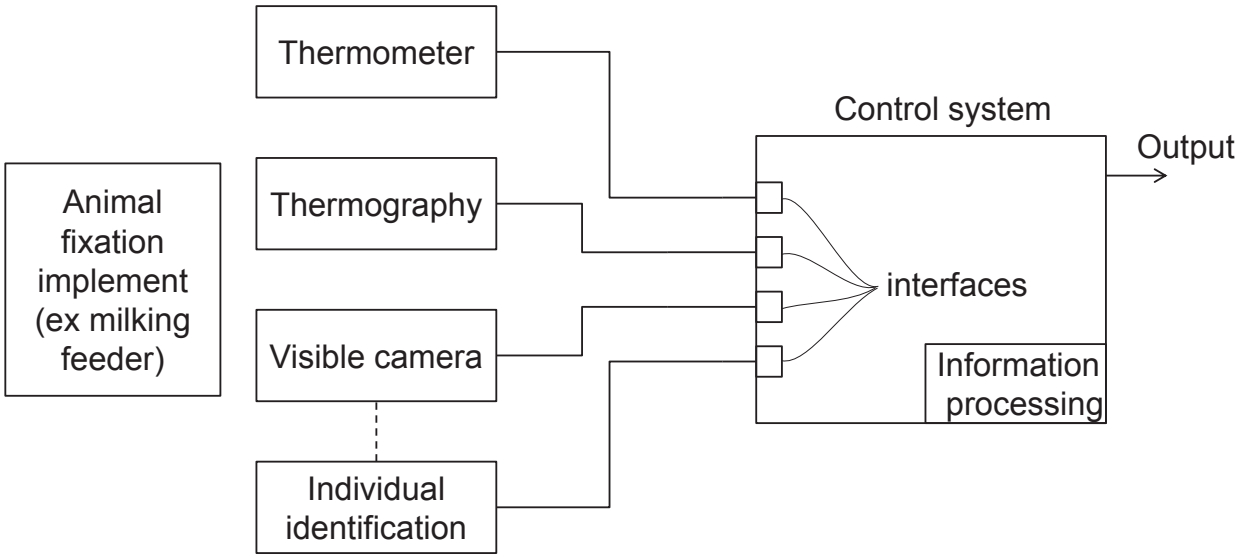

Fig. 1. Component of our system

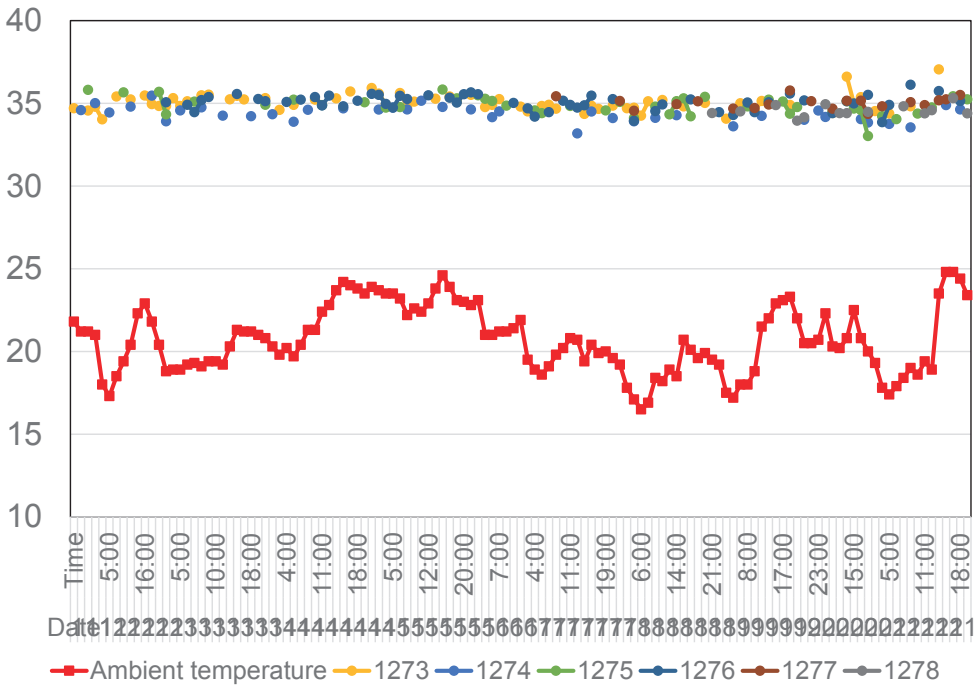

Fig. 2. Individual ophthalmic temperatures and ambient temperature of calves in a farm monitored by our system.

O-35-4 Physiological parameters of young cattle transported from Hawai ei to US west coast during summer.

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Introduction

Cattle was introduced onto the Hawaiian island dates back to 1793 with the arrival of large merchant ships. The first cattle were gifts by the merchant captain Vancouver to the Hawaiian king. Over the years the numbers increase. Today the industry is a vital part of diversified agriculture in Hawaii's economy valued at over $64m at farm gate (1).

Hawaii's history of large plantations of sugarcane and pineapple limited the production of feeds for livestock. When the last feedlot operation on Oahu closed in early 1990s, weaned calves were shipped to the west coast for growing out and finish (2). Hawaii has three of the top twenty-five largest cow-calf operations in the USA (3). Shipment from Hawaiian Islands were in livestock containers versus livestock ship. Over the years, the type of containers used were modified to increase capacity and improve on animal welfare. There are also shipment by 747 cargo plane but the number of cattle via this route is limited.

Transportation stress can result in 3-15% shrinkage (4). Different activities of human handling has been identified to result in different shrinkages in cattle (5). Grandin (6) provide a review that identified various contributors of stress and handling. One factor identified was the novelty of the environment/surroundings on cattle. However, there can be an adaptation of the animals to handling, hence tame animals (7) and hand-raised animals are less likely to be violent or object to handling (8). The American Association of Bovine Practitioners (AABP) provide guidelines for transportation of cattle and calves (9). The animals must be supplied with feed and water if the transportation exceeds 28h but an exemption is made for air and ship transportation where feed and water are available. The Australian has a more extensive guideline for land transportation and some of the requirements can be found of page 57 of the guide (10).

Objectives

There is no report(s) on transportation of wean calves from the Hawaiian Islands to the ports of the US west coast. Hence, the attempt of this study is to evaluate the following:
The changes in core body temperature in calves from the ranch to the designation where they will grow up prior to entering the feedlot, andThe changes in blood parameters to ascertain the physiological status of the animal prior to transportation and upon arrival at the designated site.
The overall goal of the study was to demonstrate that the handling methods employed by Hawaii ranchers result in minimum stress on the animals over a long haul (over 5days of transportation). This is a precautionary approach to alleviate any concerns the public may have on handling and transportation of animals and also to identify if there is any factor in the shipping process that needs to be refined/change to enhance animal welfare.

Materials and Methods

Beef calves weaned from one of the large ranch in Hawaii was used in the study. This ranch had been shipping animals to the US mainland for 15+ years with little to losses. Two studies were conducted one in winter and one in summer. In each shipment, there were 24 animals. There are 12 animals which are of Angus breed (black or dark hair coat) and 12 animals which are of Charolaise breed (white hair coat). Only heifers were used in the study as they were fitted with a temperature data logger in the vagina (11, 12).

Following weaning, animals were processed by receiving vaccinations, ear tagging, etc. The calves had all the pastures they could consume in the ranch. They were supplemented with feed (a grain-forage pellet) that they will received during transportation. Within 2 weeks, they animal readied for shipping. A day prior to shipping, they are brought to the corral. Blood for physiological parameters were drawn from the jugular vein following restraining

with a halter and squeeze chute. The animals were fitted with a blank CIDR with a TidBit data logger V2, Figure 1 (by OnSet Computer Corp., Bourne, MA http://www.onsetcomp.com/products/data-loggers/utbi-001). The weight was recorded.

Blood samples were processed in the farm and the plasma were frozen immediately with dry ice. These samples were then packed in dry ice and insulated container and shipped to Antech Diagnostic, Portland, OR. for analyses. A total of 39 blood parameters were evaluated, Table 1.

The "cowtainers" is a double deck 12.2m trailer with wide opens for airflow. Each deck is further divided into 2 compartments. There are 3 animals in each compartment. In addition, each compartment is monitored by a HOBO data logger for temperature and humidity.

Animals were loaded the following morning after the sampling day and trucked to the dock where the "cowtainers" were staged until shipment by barge from Kawaihae to Honolulu. The journey normally begins aroun 1700 - 1830h. The barge would arrive at the Honolulu harbor and the "cowtainers" were truck to another staging area until loading for the transport to the US mainland West Coast. The loading onto the Maston ocean liner is usually at night and the ship takes off at about midnight heading east of the Hawaiian Islands. The journey from Hawaii to the west coast port is 5-7 days, depending the port is Oakland or Seattle and weather conditions. The winter shipment was to Seattle, Washington and the summer shipment was to Oakland, California. The "cowtainers" were then offload and trucked to the site where the animals will be house for growing out prior to the feedlot.

Upon arrival at the designation, the animals were processed again the vagina temperature logger were retried, animal weights were taken and blood samples drawn for physiological parameters evaluation.

Core body temperatures were analyses by hair coat color over time at different locations or transportation. Blood plasma parameters were individual values compared to normal standards for cattle of the age group.

Results and Discussion

Figure 1 showed the blank CIDR with the TidBit V2 data logger for core body temperature acquisition. The temperatures of the animals increased when they are moved and handled (Figure 2). The very activity of fear, novel environment and movement elicited an increase in core body temperature. The body temperatures were also elevated in the staging areas of the dock. Nevertheless, these temperatures return to normal. The body temperatures were within the normal range throughout the ocean transportation period. Thus, the data indicated the potential hazard site for transportation. Special attention to animals in the staging areas would ensure proper animal welfare issues are addressed when needed.

Table 1 showed the blood parameters analyzed. Except for the 2 animals that were ill, the rest of the 42 animals showed normal profiles. These two animals also happened to the lightest in weight. Hence, the data suggest that it is important to put animals within similar weight range in each compartment.

The influence of hair coat color and size of animal on core body temperature will be discussed in the presentation.

Shrinkage for the journey over a 5-7 days period was merely 6.9% and this is within the range seen with land transportation within 36h (13, 14). The low shrinkage experienced in this study is probably due to the presence of feed and water at all times. These animals regained their weight and put on additional gains one week after arrival at the designation.

There was no detection of any respiratory problems in the animals shipped. Bovine respiratory disease (BRD) has been reported to be common infection in animal transportation.

Summary

In summary, the data suggest that preparation of the animals (pre shipping vaccination), feed adaptation, presence of feed and water, and regular monitoring during transportation are helpful for animal welfare and animal well-

being. The system of shipping employed by the Hawaiian ranchers post little stress on the weaned calves over the long journey.

Acknowledgement

This research was funded by USDA-HATCH funds to the College of Tropical Agriculture and Human Resources. Institutional Animal Care and Use protocol # 12-1391. Special thanks to the stockers and the staff of the participating ranch for their assistance.

KEYWORD：cattle, transportation, body temperature, physiology, blood parameters

Figure 1. Showing a blank CIDR with a TidBit V2 temperature data logger which was inserted into the heifers .

Figure 2. Core body temperature of calves during handling and ocean transportation.

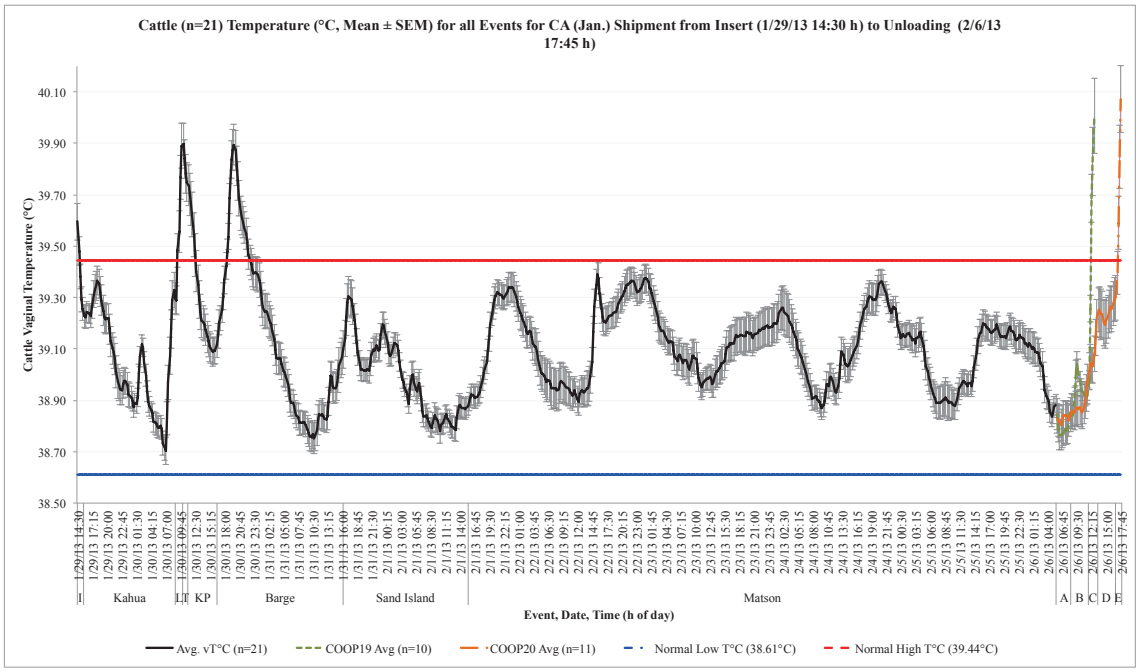

Table 1. Sample of blood plasma parameters analyses by Antech Inc. OR.

Super Chem			CBC
Total Protein	Total Bilirubin	Sodium	WBC
Albumin	BUN	Chloride	RBC
Globulin	Creatinine	Potassium	HGB
SGOT	Phosphorus	Amylase	MCV
SGPT	Glucose	Triglyceride	MCHC
Alk Phosphatase	Calcium	CPK	Platelet Count

REFERENCES

1. National Agriculture Statistic Service (NASS). 2014. Statistical of Hawaii Agriculture 2014. https://www.nass.usda.gov/Statistics_by_State/Hawaii/Publications/Miscellaneous/Top%2020%20commodities%202014.pdf; accessed June 16, 2016.
2. Schreiber, C. 2013. Hawaiian cattlemen ship calves across ocean with few problems. Livestock Weekly. http://www.livestockweekly.com/papers/00/07/13/whlshipping.asp; accessed July 4th, 2016.
3. The National Cattlemen and Beef Association. 2012. Directions celebrating 25 years. http://www.beefusa.org/CMDocs/BeefUSA/Media/National%20Cattlemen/Directions%20Lists.pdf; accessed July 4th, 2016.
4. Hunt, L. 2013. Economics and Marketing: How shrinkage affects returns for feeder and slaughter cattle. Alberta Feedlot Management Guide. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/beef11854; accessed July 7th, 2016.
5. Richardson, C. 2005. Reducing cattle shrinkage. Ontario Ministry of Agriculture, Food and Rural Affairs. Factsheet. http://www.omafra.gov.on.ca/english/livestock/animalcare/facts/05-063.htm; accessed July 7th, 2016.
6. Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:249-257. http://www.grandin.com/references/handle.stress.html; accessed July 7th, 2016.
7. Boandale, K.E., J.E. Wohlt and R.V. Carisa. 1989. Effect of handling of a local anesthetic and electrical dehorning on plasma cortisol in Holstein calves. J. Dairy Sci. 72:2913-2197. http://www.ncbi.nlm.nih.gov/pubmed/2794176; accessed July 7, 2016
8. Hastings, B. E., D. E. Abbott, and L. M. George. 1992. Stress factors influencing plasma cortisol levels and drenal weights in Chinese water deer (*Hydropotes inermis*) Res. in Vet. Sci. 53: 375-380. http://www.ncbi.nlm.nih.gov/pubmed/1465513; accessed July 5th, 2016.
9. American Assoc. of Bovine Practitioners. 2014. Transportation recommendation for cattle. http://www.aabp.org/resources/aabp_guidelines/transportationguidelines-03-11-2014.pdf; accessed July 5th, 2016.
10. Australian Animal Welfare Standards and Guidelines. 2013. Land transportation of livestock. http://www.animalwelfarestandards.net.au/files/2011/02/Land-transport-of-livestock-Standards-and-Guidelines-Version-1.-1-21-September-2012.pdf; accessed July 5th, 2016.
11. Hillman, P.E., K.G. Gebremedhin, S.T. Willard, C.N. Lee and A.D. Kennedy. 2009. Continuous measurements of vaginal temperature of female cattle using a data logger encased in a plastic anchor. Applied Engineering in Agriculture, ASABE. 25(2):291-296. https://elibrary.asabe.org/azdez.asp?AID=26332&T=2; accessed July 5th, 2016.
12. Godfrey, R.W., A. J. Weis, P. E. Hillman, K. G. Gebremedhin, C. N. Lee, and R. J. Collier. 2012. Evaluation of body temperature and sweating rate of Senepol cows in the tropics. J.Anim.Sci. joint meeting. http://m.jtmtg.org/2012/PresDetail.aspx?view=sci&selectby=daytime&dt=7/16/2012&ap=PM&sespage=1&sessionID=5362&prespage=1&presID=52626&prestype=abs;
13. Smith, G.C., T.Grandin, T. H. Friend, D. Lay, Jr. and J. C. Swanson. 2004. Effect of Transport on Meat Quality and Animal Welfare of Cattle, Pigs, Sheep, Horses, Deer, and Poultry. In Livestock behaviour, design of facilities and humane slaughter. http://www.grandin.com/behaviour/effect.of.transport.html; accessed July 5, 2016.
14. Barnes, K., S. Smith and D. Lalman. 2013. Managing shrink and weighing conditions in beef cattle. OK Coop. Extension Factsheet ANSC-3257. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-1986/ANSI-3257web.pdf; accessed July 6th, 2016.

O-35-5

FIELD TRIAL ON THE EFFICACY AND TOXICITY OF SELECTED FASCIOLICIDES IN BUFFALOES (Bubalus bubalis).

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[2]Philippine Carabao Center

Introduction/Objectives

In the Philippines and many other Asian countries, swamp and riverine buffaloes are significant sources of draft power, meat, milk and by-products. As of 2010 the carabaos population in the Philippine island is about three million (BAS, Department of Agriculture, 2013). Living in tropical countries, these animals are continuously challenged by both internal and external parasites (Manuel, 1983). Fasciolosis, caused by *Fasciola hepatica* or *F. gigantica,* is considered the most important helminth infection of ruminants in tropical countries contributing to socio economic problems (Spithill *et al*., 1999 Amor *et al.,* 2011). It is still the leading cause of morbidity and mortality in ruminants in the Philippines (Copeman *et al.,* 1997). Adult parasites are found in the bile ducts, and the immature flukes in the liver parenchyma, of infected final hosts. Clinical disease is usually characterized by weight loss, anaemia and hypoproteinaemia.

Other than controlling the Fasciola population in the pasture, the most common method of worm control used by famers is the use of chemical anthelmintics. However, resistance to various anthelmintics have been recently reported and these leads to treatment failure. Parasites have been familiar to some the most commonly available anthelmintics, however, new drug combinations have been out in the market and offers alternative for parasite control.

Hence this study compared the efficacy of the commonly used anthelmintics (triclabendazole bolus) and anthelmintic combinations (ivermectin + clorsulon, and closantel + levamisole combinations) in controlling infection *Fasciola spp*. in water buffalo. The efficacy of one-dose and two-dose treatments were compared among treatments and examined the effect of these doses in the SGPT level in the animals.

Materials and Methods

Identification of Experimental Animals. Buffaloes owned by farmers of PCC assisted cooperatives from Muñoz, Nueva Ecija were used in the study to determine the efficacy of fasciolicides. A total of 32 buffaloes naturally-infected with *Fasciola* spp. regardless of sex, non-pregnant (in case of caracows), ages 8-mo or above were used in the study for the treatment groups. Meanwhile, caracows on their first trimester of pregnancy ranging from one to four months were used for the control.

There were two sets of treatment: one dose-treatment (Set 1) and two dose-treatment in 1-month interval (Set 2). The individual pre-treatment faecal egg count using standard sedimentation technique was taken two weeks before administration of anthelmintics. At day 0, the 32 animals were ranked according to their FEC such that heavy, moderate, and low burdens of fasciolosis with equal representations of each group to the sets of treatment. The animals were treated with anthelmintics: 1) triclabendazole bolus (TBZ), 2) ivermectin + clorsulon (IVER+CLOR) and 3) closantel + levamisole (CLOS+LEV), in their respective designated groups according to the manufacturer's recommended dose.

Faecal Collection and Analysis. About five grams of stool were collected directly from the rectum of each animal. Stool samples were collected directly from the rectum with a gloved hand and were placed in properly labelled zipped plastic bags. Collected samples were placed in a cooler to be transported to the laboratory for analysis.

The post-treatment collection of faecal samples and analysis of faecal egg counts were done on the 14th day after treatment. Efficacy data were based on the post-treatment FEC performed 14 days after treatment. The faecal egg count (FEC) was computed using this formula (Coles *et al*., 1992)

FEC (epg)= (no. of egg x amount of faecal suspension/ amount examined x amount of feces used) x 100

Where: epg= egg per gram

While the efficacy of the anthelmintic compound was determined based on reduction of egg excretion at 14 days post-treatment using the formula below (Coles *et al*., 1992).
Efficacy = (pre-treatment FEC - post-treatment FEC)/ pre-treatment FEC x100
Liver Enzyme Assay. The effects of the treatment anthelmintics on the liver function were determined by SGPT level determination. Initial SGPT levels were determined on day 0, prior to drug administration, and succeeding tests were done on 1st week post-treatment. A 10ml blood sample was collected from the jugular vein of the animal using a red-topped vacutainer. Samples were placed in an ice cooler before they were submitted to a diagnostic lab.
*Statistical Analysis***.** Analysis of Variance (ANOVA) was used for the overall comparison of the different treatments using 95% confidence interval and *P* value set at P<0.05

Results and Discussion

The efficacy of available and combined anthelmintics were evaluated for their efficacy against *Fasciola* spp in buffaloes (Table 1). The efficacy of one-dose treatment of the three drugs used: TBZ, IVER+CLOR, and CLOS+LEV, is shown in Table 1. No significant differences were found in the efficacy of the three drugs used in the study. The statistically comparable efficacy (*P*<0.05) of treatments were 74.47% (TBZ bolus), 72.03% (IVER+CLOR), and 79.17% (CLOS+LEV). The efficacy of the three drugs is considered insufficiently effective based on the criteria by the World Association for the Advancement of Veterinary Parasitology (WAAVP) which sets a cut-off of >80% for an anthelmintic drug to be considered moderately effective. Similar low TBZ efficacy was found by Sanyal and Gupta (1995) with regards to using the dose recommendation of TBZ by manufacturers.

The efficacies of TBZ, IVER+CLOR, and CLOS+LEV in water buffaloes after two-dose treatments with interval of one month (Set 2) were higher than set 1 (Table 1). TBZ had an efficacy of 84.53%, 81.2% of IVER+CLOR while slightly higher efficacy was recorded for CLOS+LEV (87.50%). Similarly, statistical analysis shows no significant differences *(P*<0.05*)* among all treatments. Based on WAAVP standards on anthelmintic efficacy, administration of anthelmintics two times at one month interval is considered moderately effective for TBZ and IVER+LEV while higher efficacy for CLOS+LEV.

Administration of two doses in one month interval increased the average efficacy of the anthelmintics as compared to one-dose treatment, but not significantly recognizable. The concept of two-dose treatment with a month interval is to target the emerging adult Fasciola that has been protected from the first treatment due to its secluded location at the liver.

The drugs used in the study were reported to have efficacy to different developmental stages of *Fasciola* spp. Triclabendazole is effective against early, immature, and mature stages (Shorkier et al., 2013). Ivermectin and clorsulon combination is only effective against adult stages of the nematode and trematodes, respectively (Kumar and Vatsya, 2013). Closantel and levamisole, on the other hand, claims to be effective against adult and late immature stages. Though this study did not establish the specific stage at which the drugs used were effective, calculation of efficacy based on the reduction of egg count, nevertheless, has been considered reliable in drug efficacy field trials. Combination of anthelmintics such as Ivermectin and Clorsulon, Closantel and Levamisole showed no additive effect in contrast to the findings of Shorkier *et al.* (2013). The low efficacy of the anthelmintic can be accounted to resistance of the parasite (Prichard, 1994 Moll et al., 2000, Brockwell *et al*.,2014) due to frequent administration of the drugs (Ancheta, *et al*., 2004)

Liver Function Test

The Serum glutamic-pyruvic transaminase (SGPT) levels in buffaloes before and after treatment with TBZ bolus, IVER+CLOR, and CLOS+LEV in single dose- treatment (Set 1) and double dose treatment (Set 2) were determined to establish toxic effect of the anthelmintics Table 2).

The SGPT values of all animals are higher compared to the published normal values (6.9-35 u/L) in cows (MedHealth.net,2014).This shows that animals with worms suffer from higher SGPT values. Result of the study

showed that use of anthelmintics,1 or 2 doses, did not significantly increase the SGPT values of the animals.

References show that the increase in the SGPT values are related to toxicity of the drug to the liver, primarily because an increase in the enzymes means the liver overworked for the release of these enzymes in the blood hence the detected high values (Coles *et al*, 1992).

Conclusion

The data obtained from this study shows low efficacy of the compared anthelmintics: Triclabendazole, Ivermectin + Clorsulon, and Closantel + Levamisole, against *Fasciola* spp in buffaloes and increasing the dose treatment does not significantly increase the efficacy. There is no significant change in the SGPT secretion of buffaloes treated with the anthelmintic and their combination, though high SGPT level is apparent in helminth infected animal.

KEYWORD : Closantel + Levamisole, Fasciolla spp, Ivermectin + clorsulon, Triclabendazole bolus, Fasciolicides

Table 1. Efficacy of TBZ, IVER+CLOR, and CLOS+LEV against *Fasciola* spp infection using FECRT in one-dose treatment (set 1) and two-dose treatment (set 2)

Drug	Dose	Mean Efficacy of Anthelmintics	
		Set 1	Set 2
TBZ	1 bolus/75 kg	74.47[a]	84.53[a]
IVER + CLOR	0.02 mL/ kg	72.03[a]	81.25[a]
CLOS + LEV	0.2 mL/ kg	79.17[a]	87.50[a]
control	No treatment	-49.17[b]	10.83[b]

Table 2. SGPT values of treated animals in one-dose (SET 1) and 2-dose (set 2) treatment

Anthelmintic	Mean SGPT Level (U/L)			
	SET 1		SET 2	
	Pre Tx	Post Tx	Pre Tx	Post Tx
TBZ	46.25[a]	47.43[a]	54.75[a]	54.48[a]
IVER+CLOR	45.40[b]	44.23[b]	41.38[b]	45.68[b]
CLOS+LEV	53.56[c]	47.28[c]	38.58[c]	37.50[c]
CON	45.23[d]	55.78[d]	54.58[d]	63.75[d]

Means with the same letter within rows are not significantly different at $P<0.05$

REFERENCES

AMOR, N., A. HALAJIAN, P. MERELLA, S. FARJALLAH, K. SAID and B. B. SLIMANE. 2011. Genetic characterization of *Fasciola spp*. from Tonekabon City (Northern Iran) based on the ribosomal internal transcribed spacer regions. *Pakistan J. Zool*. 43(6): 1061-1067.

ANCHETA, P.B., R.A. DUMILON, V.M. VENTURINA, W.A. CERBITO, R.J.DOBSON, L.F. LEJAMBRE, E.C.VILLAR, G.D. GRAY. 2004. Efficacy of benzimidazole anthelmintics in goats and sheep in the Philippines using a larval development assay. Veterinary Parasitology vol 120, 1-2:107-121

BUREAU OF AGRICULTURAL STATISTICS. 2013. Cattle and carabao industry performance report. Retrieved on September 23, 2013 from World Wide Web:http:// www.bas.gov.ph.

BROCKWELL, Y.M., T.P. ELLIOT, G.R. ANDERSON, R. STANTON, T.W. SPITHILL, AND N.C.SANGSTER. 2014. Confirmation of Fasciola hepatica resultant to Triclabendazole in naturally infected Australian beed and dairy cattle. International Journal for Parasitology: Drugs and Drug Resistance 4:48-54

COLES C., F.H.M. BORGSTEEDE, S. GEERTS, T.R. KLEI, M.A. TAYLOR, and P.J. WALLER. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 1992;44:35–44.

KUMAR, R.R. and S. VATSYA. 2013. Efficacy of Ivermectin-Clorsulon combination against mix anthelmintic infection in goats- a case report. Veterinary Research International. 1,1: 31-32

MANUEL, G. 2000. Efficacy of triclabendazole against roundworms of goats (*Capra hircus*) at the Small Ruminant Center, Central Luzon State University. Unpublished undergraduate thesis. College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

MOLL, L., C. P. H. GAASENBEEK, P. VELLEMA, and F. H. M. BORGSTEEDE.2000. Resistance of *Fasciola hepatica* against Triclabendazole in cattle and sheep in the Netherlands. *Veterinary Parasitology* 91 (2000): Pp. 153–158.

PRICHARD. R. 1994. Anthelmintic resistance. Vet Parasitology. 54(103):259-68

SANYAL, P.K. and S.C. GUPTA. Efficacy and pharmacokinetics of Triclabendazole in buffalo with induced fasciolosis. Veterinary Parasitology 63:75082

SHORLIER, K.M., S.M. ABOELHADID, and M.A. WALEED. 2013. Efficacy of five anthelmintics against a natural Fasciola species infection in cattle. Beni-Suef University Journal of Basic and Applied Sciences 2:41-45.

O-35-6

Preculcture freezing and incubation on bacteriological isolation from subclinical mastitis milk samples in dairy cows

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ABSTRACT: The purpose of this study was to investigate the efficiency of preculture freezing technique on bacterial isolation of subclinical mastitis milk from dairy cows. Twenty-four individual cow milk samples with CMT score +3 from five small holder dairy farms in Khon Kaen dairy cooperative farm were collected for bacterial isolation. Somatic cell count (SCC) of the milk samples was measured by the DeLaval Cell Counter (DCC) apparatus. The bacterial isolation performing with fresh milk samples served as the conventional culture method. For the preculture freezing method, milk samples were kept frozen at -20ºC for 12 h prior to bacterial isolation. The average SCC value of the milk samples was 2.17 x 10^6 cells/mL, with the range of 1.21-3.38 x10^6 cells/ml. The overall percentage of bacterial positive samples found by both methods combined was 70.83 percent (17/24). However, the percentage of positive samples found by the conventional method was 45.83 percent (11/24) whereas that of the preculture freezing method was 25.0 percent (6/24). Interestingly, four positive samples were detected only with the preculture freezing method. *Streptococcus* and *Staphylococcus* species were found in milk samples by both methods. In conclusion, the use of preculture freezing technique in combination with conventional culture method improved the sensitivity of bacterial isolation from subclinical mastitis milk.

INTRODUCTION

Subclinical mastitis causes great economic losses to dairy farmers, because it has been linked to increased replacement cost and considerable veterinary expenses (Koop et al., 2010). It was reported that thirty one percent of the cows (within a herd) were afflicted with subclinical mastitis (Pitkälä et al. 2004) causing an increase in the somatic cell count (SCC) and milk production loss (Forsbäck et al., 2009). Several studies have found that milk production loss was due largely to subclinical mastitis (Hortet and Seegers, 1998 Koldeweij et al., 1999). Complementary, Ott (1999) reported that total production loss due to subclinical mastitis in the USA was $108.00 per cow for herds with average BTSCC of 200,000-399,999 cells/ml and $295.24 per cow for herds with average BTSCC 400,000 cells/ml and above resulting in a total losses of approximately $1 billion to the USA dairy industry. Morin et al. (1993) indicated that mastitis-associated economic losses were rang from US$161.79 to $344.16 per lactating cow/year.

Common mastitis pathogens can be divided into contagious and environmental pathogen. Subclinical mastitis is caused mostly by contagious pathogens. Coagulase-negative *staphylococci (CNS)* and *Staphylococcus aureus* are frequently isolated from cases of subclinical mastitis, whereas *Streptococcus spp*., *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Mycoplasma spp*., and other pathogens were found at lower frequencies (Contreras et al., 2007). According to Botrel et al. (2010) it was reported that coagulase-positive staphylococci (30.2%), coagulase-negative staphylococci (13.7%), and*Streptococcus dysgalactiae*(9.3%) were predominantly implicated in subclinical mastitis. All mastitis pathogens cause an inflammation to the mammary gland, and increase number of SCC in milk, which subsequently increase the bulk milk somatic cell count (BMSCC). Thus, BMSCC is a good mastitis indicator of dairy herds (Jayarao and Wolfgang, 2003 Jayarao et al., 2004).

Technique for detection and identification of pathogens in milk has become a valuable tool for the determination of udder health in modern farm dairy (Petzer et al., 2012). *Because mastitis*can*occur*in both *clinical* and subclinical form with difference pathogens. The simple diagnostic laboratories in the bacteriological culture of the milk sample is the most common and the best way for isolation of mastitis pathogens (Hicks et al., 1994). However, culture of cow milk samples with a subclinical mastitis were often resulted in no pathogen (Sol et al, 2002). To increase the sensitivity of the culture methods, various techniques have been included to standard culture technique, such as freezing sample milk (Hubackova and Rysanek, 2007 Alrabadi, 2015 Pehlivanoglu et al., 2015), pre-milking and post-milking milk samples (Godden et al., 2002), storage under difference temperature

and sample volume (Zambriski et al., 2012) as well as centrifugation of milk samples before culturing (Gh et al., 2005).

The purpose of this study was to investigate the efficiency of preculture freezing technique on bacterial isolation of subclinical mastitis milk from dairy cows.

Materials and Methods

California Mastitis Test (CMT) was used to detect subclinical mastitis in five small holder farms in Khon Kaen dairy co-operative as described by Philpot and Nickerson (1999). Approximately 2-5 ml of quarter milk samples with a CMT score of +3 were aseptically collected for bacteriological procedures. SCC was analyzed by the DeLaval Cell Counter® (DeLaval International AB, Tumba, Sweden).

Bacterial identification was performed with the two techniques. The first technique was standard culture technique (IDF, 1981), which was the cultured of fresh milk sample. Inoculum of 10 µl of milk sample was spread on a blood agar plate and MacConkey agar plate, incubated for 18 hour, at 37 degrees C, and specific bacterial colonies were examined. Species identifications were done by conventional biochemical tests according to National Mastitis Council (1987) and Quinn et al. (1994). For the secondary technique, the whole milk sample was frozen for 18 hour at -20 degrees C and thawed at room temperature before being cultured as described for standard culture technique.

Results and Discussion

The results of this study showed that average SCC value of the milk samples was 2.17 x 10^6 cells/ml, with the range of 1.21-3.38 x10^6 cells/ml. This level of SCC was show to be related to caused by bacteria (Kirk, 2000). It was found that the herds with bulk tank SCC above 3 x10^6 cells/ml have varying degrees of subclinical mastitispresent (Lyer et al., 2014).

In the Table 1, the experiment demonstrated that the overall percentage of bacterial positive samples found by both methods combined was 70.83 percent (17/24). However, the percentage of positive samples found by the conventional method was 45.83 percent (11/24) whereas that of the preculture freezing technique was 25.0 percent (6/24). In addition, number of bacterial positive milk samples in preculture freezing technique was lower than conventional method. The observation was probably due to freezing and thawing can cause stress to some species of bacteria and diminish culture yield (Zambriski et al., 2012). Bexiga et al. (2011) who stated that freezing milk samples at -20 ºC for 24 hours was not helpful in diagnosing mastitis since they indicated that the number of Gram-positive cocci decreased in the milk samples after the pre-freezing step. Hubackova and Rysanek (2007) reported only a slight increase in the *Staphylococcus aureus* count in the milk after freezing the samples for 3, 7 and 21 days. This decrease may be explained by the possible negative effect of freezing on the growth and survival of the bacterial pathogens. Thus, our results support those studies suggested that freezing and thawing milk samples disrupt the bacterial clusters (Godden et al., 2002).

Furthermore, the result of this study found no Gram-negative bacteria by both method. Nevertheless, the percentages of *Streptococcus dysgalactiae* and *Streptococcus agalactiae* in preculture freezing technique was lower than standard culture technique, this finding was in agreement of Sol et al. (2002) who suggested that percentages of *Streptococcus dysgalactiae* and *Streptococcus agalactiae* were highest using the standard culture technique, while isolation rate of *Streptococcus uberis* was not different among the two methods used (standard culture technique and freezing the whole milk sample). In addition, under low temperatures the reaction rates for enzymes in the organism become much slower and reduce the fluidity of the cytoplasmic membrane (Mossel et al., 1995). Thus, frozen can *inhibit bacterial growth.* However, four positive samples were detected only with the preculture freezing method. *Streptococcus* and *Staphylococcus* species were found in milk samples by both methods. Likewise, Jarassaeng et al. (2012) reported that *CNS* and *Streptococcus spp*. are major isolated microorganisms in subclinical mastitis of smallholder dairy farms in Khon Kaen province in the northeastern of Thailand.

Conclusions

From this study, it can be concluded that milk samples should be preculture freezing technique prior to analysis for isolation of bacterial pathogen in subclinical mastitis, which *inhibit bacterial growth.* Therefore, the use of preculture freezing technique in combination with conventional culture method improved the sensitivity of bacterial isolation from subclinical mastitis milk.

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KEYWORD : bacteriological isolation, subclinical mastitis, dairy cows

Table 1 Comparison of the culture results of the milk samples (n = 24) inoculated by both methods combined

Items	Preculture freezing technique (%)	Standard culture technique (%)
Mastitis pathogens		
Staphylococcus epidermidis	4.16	12.5
Staphylococcus uberis	4.16	4.16
Streptococcus agalactiae	8.33	12.5
Streptococcus dysgalactiae	0.0	4.16
Streptococcus spp.	8.33	12.5

REFERENCES

Alrabadi, N.I. 2015. The Effect of Freezing on Different Bacterial Counts in Raw Milk. International Journal of Biology. 7(4): 9-12.

Bexiga, R., M.T. Koskinen, J. Holopainen, C. Carneiro, H. Pereira, K.A.

Ellis, and C.L. Vilela. 2011. Diagnosis of intramammary infection in samples yielding negative results or minor pathogens in conventional bacterial culturing. J. Dairy Res. 78: 49-55.

Botrel, M.A., M. Haenni, E. Morignat, P. Sulpice, J.Y. Madec, and D. Calavas. 2010. Foodborne Pathogens and Disease. 7(5): 479-487.

Contreras, A., D. Sierra, A. Sánchez, J.C. Corrales, J.C. Marco, M.J. Paape, and C. Gonzalo. 2007. Mastitis in small ruminants. Small Rumin. Res. 68:145–153.

Forsbäck, L., H. Lindmark-Månsson, A. Andrén, M. Åkerstedt, and K. Svennersten-Sjaunja, 2009. Udder quarter milk composition at different levels of somatic cell count in cow composite milk. Animal. 3: 710–717.

Gh, Y.B., M., Rad, S.M., Hosseini, and A. Jafari. 2005. Effect of centrifugation of milk samples on bacteriologic culture. Iranian Journal of Veterinary Research. 6(1): 56-61.

Godden, S.M, J.T. Jansen, K.E. Leslie, N.L. Smart, and D.F. Kelton. 2002. The effect of sampling time and sample handling on the detection of Staphylococcus aureus in milk from quarters with subclinical mastitis. Can. Vet. J. 43: 38-42.

Hicks, C.R., R.J. Eberhart, and W.M. Sischo. 1994. Comparison of microbiologic culture, an enzyme-linked immunosorbent assay, and determination of somatic cell count for diagnosing *Staphylococcus aureus* mastitis in dairy cows. J.Am.Vet.Assoc. 204: 255-260.

Hortet, P., and H. Seegers. 1998. Calculated milk production losses associated with elevated somatic cell counts in dairy cows: A review and a critical discussion. Vet. Res. 29: 497–510.

Hubackova, M., and D. Rysanek. 2007. Effects of freezing milk samples on the recovery of alimentary pathogens and indicator microorganisms. Acta Vet. 76: 301-307.

Hubackova, M., and D. Rysanek. 2007. Effects of freezing milk samples on the recovery of alimentary pathogens and indicator microorganisms. Acta Vet. Brno. 76: 301-307.

IDF. 1981. Factors Affecting the Keeping Quality of Heat Treated Milk (Bulletin Doc. 130). IDF, Brussels, Belgium.

Jarassaeng, C., S. Aiumlamai, C. Wachirapakorn, M. Techakumphu, J.P.T.M. Noordhuizen, A.C.Beynen, and S. Suadsong. 2012. Risk Factor of Subclinical Mastitis in small Holder Dairy Cow in Khon Kaen Province.

Thai. J. Vet. Med. 42(2): 143-151.
Jayarao, B.M. and D.R. Wolfgang. 2003. Bulk-tank milk analysis: A useful tool for improving milk quality and herd udder health. Vet. Clin. North Am. Food Anim. Pract. 19(1): 75-92.
Jayarao, B.M., S.R. Pillai, A.A. Sawant, D.R. Wolfgang, and N.V. Hegde. 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87(10): 3561-3573.
Kirk, J.H. 2000. subclinical mastitis and somatic cell counts. Veterinary Medicine Teaching and Research center. University of California.
Koldeweij, E., U. Emanuelson, and L. Janson. 1999. Relation of milk production loss to milk somatic cell count. Acta Vet. Scand. 40: 47–56.
Koop, G., T. van Werven, H. J. Schuilling, and M. Nielen. 2010. The effect of subclinical mastitis on milk yield in dairy goats. J. Dairy Sci. 93: 5809–5817.
Lyer, P.A., M. Albaik, and I. Baghallab. 2014. Mastitis in Camels in African and Middle East Countries. J. Bacteriol Parasitol. 5(3): 1-11.
Morin, D.E, G.C. Petersen, H.L. Whitmore, L.L. Hungerford, and R.A Hinton. 1993 Economic analysis of a mastitis monitoring and control program in four dairy herds. Journal of the American Veterinary Medical Association. 202: 540–548.
Mossel, D.A.A., J.E.L. Corry, C.B. Struijk, and R.M. Baird. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley and Sons. 699 p.
National Mastitis Council. 1987. Laboratory handbook on bovine mastitis. Madison, WI, National Mastitis Council, Inc. pp. 1-32.
Ott, S.L. 1999. Costs of herd-level production losses associated with subclinical mastitis in U.S. dairy cows. National Mastitis Council Annual Meeting Proceedings. 152.
Pehlivanoglu, F., H. Yardimci, and H. Turutoglu. 2015. Freezing and thawing milk samples before culture to improve diagnosis of bovine staphylococcal mastitis. Veterinarski Arhiv. 85(1): 59-65.
Petzer, I.M., J., Karzis, T.J. Van der Schans, J.C. Watermeyer, N. Mitchell-Innes, S. Eloff, and G.T. Fosgate. 2012. 'Comparing effects of freezing at -196 ℃ and -20 ℃ on the viability of mastitis pathogens. J. Vet. Res. 79(1): 1-6.
Philpot, W.N, and S.C. Nickerson. 1999. Mastitis Counter Attack, A strategy to Cobat Mastitis. Babson Bros. Co., Naperville, IL.
Pitkälä, A., M. Haveri, S. Pyörälä, V. Myllys, and T. Honkanen-Buzalski. 2004. Bovine mastitis in Finland 2001 – prevalence, distribution of bacteria and antimicrobial resistance, Journal of Dairy Science. 87: 2433-2441.
Quinn, P.J., M.E. Carter, B.K. Markey, and G.R. Carter. 1994. Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited.
Sol, X., O.C. Sampimon, E. Hartman, and H.W. Barkema. 2002. Effect of pre-culture freezing and incubation on bacteriological isolation from subclinical mastitis samples. Vet. Microbiol. 85: 241-249.
Zambriski, J.A., R.C. Maves, D.V. Nydam, V. Ayvar, D. Cepeda, R. Castillo, A. Díaz-Pino, M. Saito, and R.H. Gilman. 2012. Effect of Storage Temperature and Sample Volume on Brucella melitensis Isolation from Goat Milk. International Journal of Tropical Disease and Health. 2(3): 207-213.

O-35-8 FIELD VALIDATION OF FAMACHA SYSTEM IN THE ESTIMATION OF DEGREE OF ANAEMIA IN GOATSWITH HAEMONCHOSIS

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OBJECTIVES

The study aimed to validate the local field adoption of the FAMACHA System in the control of haemonchosis in goats. It correlated the FAMACHA scores with the PCV, and *Haemonchus* sp.-predominant FEC of ≤ 6 month of goats.

METHODOLOGY

Blood and faecal samples were collected biweekly for 1.5 months from 15 crossbred goats aged ≤ 6 months regardless of sex naturally infected with haemonchosis. Standard McMaster egg counting technique was done to determine the degree of infection while standard protocols to determine PCV were also determined. Larval culture (Hansen and Perry, 1994) was performed to determine the larva under study.

Animals were restrained before collecting faeces directly from the rectum. After the collection of faeces, blood was collected simultaneously from the restrained goat through the jugular vein using sterile 3ml/cc syringe. Blood was directly placed in heparinized mircohematocrit tubes sealed with laboratory clay and placed in iceboxes. Samples were sent to the laboratory for centrifugation.

FAMACHA scoring of the sample animal was performed simultaneous with blood abd feces collection. The colour of the ocular conjunctiva was evaluated following the recommendations of the FAMACHA©-method (Van Wyk and Bath, 2002). The guide shows five colour classes: 1 (red) and 2 (red-pink) being considered as non-anaemic 3 (pink) borderline 4 (pink-white) anaemic and 5 (white) severely anaemic.

For FAMACHA scoring, the lower eyelid of the animal was gently pulled down with the finger exposing the ventral conjunctiva. The upper eyelid was pushed down to cover the eyeball and the membrane nictitans. The colour of the lower conjunctiva was then evaluated by comparing it directly with the FAMACHA chart as shown below. Only goats that were given scores of 4-5 were recommended for drenching (Bath *et al*., 2001). Goats that had scores of 3.0 (borderline) were reported to the farm owner for them to decide whether deworming was necessary. None of the animals were dewormed by the owner during the study period.

Correlations were based on the correlation coefficient, which was calculated by using the Pearson square method. Reliability was based on the expected agreement, which was calculated using the method described by Viera and Garrett *et al.*, (2005).

RESULTS AND DISCUSSION

The parameters used to evaluate the presence of anemia are presented in Table 1. The relationship between the parameters used to evaluate anemia (FAMACHA score, PCV, and FEC) associated with worm load are presented in Table 2.

The correlation between FEC and PCV was also significant at $P< 0.05$. The correlation between FEC and PCV was also significant at *P.*

FAMACHA scores were negatively correlated (r=-0.703) with PCV). FAMACHA scores were higher in samples where PCV values were low. Low PCV may indicate the presence of anaemia and higher FAMACHA score was suggestive of anaemia (Bath et al., 2001). The relationship established in these results implies that FAMACHA score could be a good tool to estimate PCV. However, it must be noted that the PCV values reported in the study were within the normal range indicating that anaemia was not present in the examined animals. As mentioned previously, most of the animals' FAMACHA scores fell in the borderline classification, for which the owner opted not to deform the animals upon his own assessment that the animals look healthy. PCV represents the percentage in the blood that are red blood cells, which would indicate the level of anaemia. However, PCV values should not be a "stand-alone" diagnostic tool, but should be supported with other response criteria. In most cases, PCV was used as a screening method for anemia detection but subsequent tests such as complete blood count (CBC) should be performed for further confirmation.

FAMACHA score was positively correlated (r=0.788) with FEC (Table 2). The relationship established in the present study implied that an increase in FEC had corresponding increase in FAMACHA score.

Theoretically, an increase in FEC, particularly in *Haemonchus*-predominant nematode population would predispose the animal to suffer from anaemia. While this was true in the present study, the low FEC observed in the samples appear to be low to substantially produce anaemia. Faecal egg count may be a less accurate predictor of adult worm burdens. The current findings led the researcher to assume that most of the worms present in the animals were still immature and hence, were not capable of laying eggs. If heavy infection occurred over one to two weeks with*H. contortus*, animals may lose substantial amount of blood with few eggs in the faeces as the pre-patent period is about three weeks (Miller, 2000).

The negative correlation (r=-0.570) between FEC and PCV implies that the FEC decreases as the PCV increases. This was expected to occur considering that FEC was known to be predominated by *Haemonchus* sp. However, as what has been emphasized earlier, interpretation of FEC and PCV should be taken with caution. In the current study, whilst the relationship between FEC and PCV were consistent with the general observations both values were within the normal range. The scenario may be different if the study was done during the rainy months when there was scarce pasture for grazing and higher rate of worm reinfection and larval establishment.

The agreements of each FAMACHA score are summarized in Table 3. There was substantial agreement between FAMACHA scores and FEC (0.788) as well as in PCV (0.703). Moderate agreement at 0.570 was established between PCV and FEC.

CONCLUSION

The mean values for PCV, FEC and FAMACHA score of 15 ≤ -6 month old crossbred goats were 29.02 %, 256 epg, 2.98 respectively. There was significant negative correlation between FAMACHA score and PCV (-0.703), and FEC and PCV (-0.570) while positive correlation was seen between FAMACHA score and FEC (0.788).

Results of the current study suggest that FAMACHA Card may be used to indicate anaemia due to *Haemonchus* spp.-predominant worm infection in goats under smallholder farming system. The success rate of estimating anaemia by the FAMACHA rating system based on the percentage of correct score category and the corresponding PCV values was 100% in categories 2 vs. 4 and 2 vs. 3. All of the presented results of the study also show connection to the management system described in the farm in relation to the problem of haemonchosis in the herd such as the feeding management and deworming program.

The present study validated the potential use of FAMACHA system as a small hold farms in the country.

KEYWORD : FAMACHA, Packed Cell Volume, Faecal Egg Count, Haemonchus contortus

Table 1. Mean faecal egg count, PCV and FAMACHA scores of goats after three collections

Animal I.D.	FAMACHA Score*	Packed Cell Volume (%)	Mean Faecal Egg Count (epg)
172	3.00	29.00	2000
173	2.67	28.00	2000
174	3.00	29.67	2670
175	3.00	26.67	3000
171	3.33	30.00	2670
170	2.67	32.33	2670
169	3.00	30.33	3000
168	3.67	27.00	3000
176	2.67	29.33	2670
178	3.67	24.00	3670
179	3.00	31.33	2670
180	3.00	28.33	2670
181	3.33	26.67	2670
182	2.33	31.00	1330
183	2.33	31.67	1670
MEAN	2.98	29.02	2560

*FAMACHA score: 1 (optimal), 2(acceptable), 3 (borderline), 4 (dangerous), 5 (fatal)

Table 2. Correlation between FAMACHA score, PCV and FEC

	Correlation coefficient (r)	*P* value
FAMACHA score vs. PCV	-0.703	0.0040
FAMACHA score vs. FEC	0.788	0.0005
FEC vs. PCV	-0.570	0.0260

Table 3. Agreement of the different score categories of the FAMACHA system

FAMACHA score versus indices of Haemonchosis	Agreement*
FAMACHA score vs. PCV	Substantial
FAMACHA score vs. FEC	Substantial
PCV vs. FEC	Moderate

*Correlation was based on the kappa statistics agreement: <0 (less than chance agreement), 0.01-0.20 (slight), 0.21-0.40 (fair), 0.41-0.60 (moderate), 0.61-0.80 (substantial), 0.81-0.99 (almost perfect)

REFERENCES

BATH, G. F., and J. W. HANSEN. 2001. Sustainable approaches for managing haemonchosis in sheep and goats, final report of FAO technical cooperation project no. TCP/SAF8821 (A).

MILLER, J. 2000. Internal and external parasites of goats. Louisiana State University. Retrieved on January 8, 2015 from the World Wide Web: http://www.luresext. edu/goats/training/parasites.html.

NOBLE, E.R. and G.A. NOBLE. 1982. Parasitology Textbook. The biology of animal parasites. Leas and Febiger, Philadelphia. 522pp

PRICHARD, R. 1994. Anthelmintic resistance. *Vet Parasitol*, 54 (1-3), 259-268.

SANGSTER, N. C. 1999. Anthelmintic resistance: past, present and future. *International Journal of Parasitology*, 29: 115-124.

SCHNYDER, M. 2005. Multiple anthelmintic resistance in *Haemonchus contortus* isolated from South African boer goats in Switzerland. *Vet Parasitol*. 128 (3/4):285-290, 2005.

SOULSBY, E.J.L. 1982. Helminths, arthropods, and protozoa of domesticated animals. Ed.7. Lea and Febiger, Philadelphia, PA.

TERRILL, T. H., and R. M. KAPLAN. 2001. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. *Veterinary Parasitology* 97 (4): 261-268.

VAN WYK, J.A. 2001. Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort *J. Vet. Res.* 68, 55–67.

VAN WYK, J.A., and G.F. BATH. 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Vet. Res*. 33, 509–529.

VATTA, A. F., R.M. KAPLAN, J.M. BURKE, T.H. TERRILL, J.E. MILLER, W.R. GETZ, and S. MOBINI. 2004. Validation of the FAMACHA eye color chart for detecting clinical anaemia in sheep and goats on farms in the southern United States. *Vet Parasitol*, 123 (1-2), 105-120.

VIERA, A.J., and J.M. GARRETT. 2005. Understanding inter-observer agreement. The kappa statistic. May 2005, *Fam Med* 2005; 37(5):360-3.

WALSH, T. K., A.A. DONNAN, F. JACKSON, P. SKUCE, and A.J. WOLSTENHOLME. 2007. Detection and measurement of benzimidazole resistance alleles in Haemonchus contortus using real-time PCR with locked nucleic acid taqman probes. *Vet Parasitol*, 144 (3-4), 304-312.

WOLSTENHOLME, A.J., I. FAIRWEATHER, R. PRICHARD, G.Y. SAMSON-HIMMELSTJERNA, and N.C. SANGSTER. 2004. Drug resistance in veterinary helminthes. *Trends in parasitology*. 20:469-476

ZAJAC, A.M. and T.A. GIPSON. 2000. Multiple anthelmintic resistance in a goat herd. *Vet Parasitol*. 2000. Jan., 87 (2/3):163-172.

O-35-9

Stable Management and Colic Prevalence with Different Condition in Indonesian Horse Farming

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Objective

Horses are one of important livestock in Indonesia, and there is no significant attention by the government for the development. The existence of the horses have a strategic value for utilization as working animals, one of them as transport horses. On the other hand, horses development was encouraging as its utilization as a means of sport facilities such as horse racing and equestrian that has positive effects for the development as an industrial commodity which is able to increase the economic value of the horses.

This study is expected to obtain basic information for the development of scientific study of the horses, and to understand the general conditions of Indoensian equine farming systems. This study aimed to looking for at the extent to which pattern and types of maintenance and stable management and the influence of the frequency rate of colic in horses.

Methodology

The research was conducted from May to October 2015 in three different stable management types transportation (andong) horses in Yogyakarta region, racehorses in Salatiga, Central Java region, and Cavalry Horse, in Indonesian Cavalry Detachment Army Parongpong, Bandung, West Java region, and in the Parasitology Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada. As many as 553 horses are divided into three groups 176 andong horses in Yogyakarta,144 racehorses in Salatiga and 233 cavalry horses in Parongpong. Data retrieved by conducting direct interviews by a questionaire with the owners or farm managers. The study also took data on helminth infections in horses that can be seen through in the feces samples of horses in the laboratory. Data taken include the identity of the breeders or owners and farm managers, purpose and circumstances, horse selection, reproductive performance and stable management. The study also took environmental data to analyse the influence of the environment to the horse performances.

Result and Conclusion

Table 1. Stable management parameter

Transportation	Racing	Cavalry
Feeding Frequency (%)		
2 times	47.8	-
	-	-
3 times	47.8	-
	-	-
4 times	4.4	12.5
	-	

More than 4 times
-
87.5
100

Feedstuffs (%)

Pellet + Grass
-
16.67
100

Mix 1[a]
100
-
-

Mix 2[b]
-
70.83
-

Mix 3[c]
-
12.5
-

environmental conditions

Temperature (^{0}C)
30 ± 2
22.27 ± 2.27
19.45 ± 4.12

Humidity (%)
60.06 ± 5.06
69 ± 9
78.05 ± 11.74

Barn floor (%)

Soil
89.13
79.17
-

Cement floor
87.0
20.83
100

Paving block

2.17
-
-

Bedding (%)

None
34.73
-
58.82

Sand
10.87
-
-

Sawdust
54.35
100
41.18

Bedding replacement (%)

Never
34.73
-
58.82

1 month
43.48
100
11.76

2 month
4.35
-
-

3 month
17.39
-
-

4 month
2.17
-
-

Deworming programs (%)

Never
21.02
-

-

3 month
7.39
26.18
-

4 month
15.91
29.83
-

5 month
30.11
23.18
100

6 month
25.57
20.82
-

Vaccination (%)

1 time
-
15,45 %
100 %

2 times
-
23,39 %
-

3 times
-
13,30 %
-

4 times
-
30,04 %
-

Never
100 %
17,81 %
-

[a] rice brand + wheat brand + peanut straw
[b] local pellets + oats + importede complete feeds
[c] rice brand + wheat brand + corns + oats

Stable management closely related to the productivity performances of the horses that will be achieved. In addition to productivity erformances is also closely related to the health condition of the horses. equine health

problems such as worm infections and colic are quite dangerous and fatal. Worm infection in horses can not be seen directly and negatively on horses. According to Syamsi (2011), colic are symptoms that every horse owners must pay attention because it can caused death in horses and in some cases, colic can lead to death within hours.
The frequency and types of horse feeding management can also cause colic. Widyananta (2000) mention that easy fermentable feed ingredients (grains) and moldy feeds can resulted colic. feeding excess can caused dilatation of the stomach, this is because the digestive system of a horse is small and always enough to facilitate gastric dilatation (Stafford, 1993).

Table 2. Prevalence infection rate of Helminthiasis

Transportation (%)
Racing (%)
Cavalry (%)
Infected
35
16.42
94.74
Clear
65
83.58
5.26
Infected
35
16.42
93.42

Problems often arise on an equine farm that worm infected the digestive tract. How the spread of worms in horses is through the mouth as they eat forage contaminated by third stage larvae (the infective larvae). Sumartono (2010) explains that one of the entrances to the host stadium definitive worm infection through the mouth along with feeds, drinking water or because licked. Barn and barn floor conditions are also one of the causes of worm infections. Irregular bedding replacement enclosures, barn and stable environment sanitary which not done properly and the barn floor moisture will facilitate the development of the parasite worms. Temperature range required by the nematodes to hatch is 18 to 38^{0}C and high humidity is very helpful to ease the worm eggs to hatch within 3 to 4 days (Levine ,1994).
Noble (1989) found that the worm will not be able to hatche in temperatures above 40^{0}C. Nielsen (2007) adds that Strongylus infective larvae phase can survive in extreme climatic conditions, but they will still have the optimum environmental conditions to grow. In the summer, when conditions are very humid then the ability to survive the larvae will declined, but still can infect horses. In the winter, where there are a couple of days with freezing temperatures and periods of snow-covered land, larvae can survive for several days, although some studies conducted in the UK showed a lower survival rate of larvae until next spring (Nielsen, 2007).
The important factor to prevent the worm infection is deworming program in horses. Papini (2015) explains that the same group dewormer used, the high-frequency treatment, and under doses used of dewormer is the cause of resistant of worms to an anthelminticum. According to Relf (2014), grazing activities of the horses are potential condition of helminth parasite infection throughout their lives, and type of small Strongylus considered as the most dangerous. Access to the grazing paddocks is a favorable condition for the occurrence of the infection by infective stage larvae that live freely in the pasture (Papini, 2015).

Table 3. Colic Prevalence Parameter
Transportation (%)
Racing (%)
Cavalry (%)
Colic occurence
30.68

10.42
57.94

Symptom

Rolling
29.63
6.67
5.19

Frequent wake-lay down
83.33
86.67
13.33

Nervous
11.11
13.33
-

Front legs hit
35.19
20
8.89

Anorexia
-
-
72.59

Treatment

Traditional
48.15
-
-

Medication
14.81
100
100

Combination

33.33
-
-

Result

Survive
81.48
80
96.15

Death
18.52
20
3.85

Signs of equine colic is moving constantly, pain, sweating, anxiety, anorexia, abdominal section turned constantly, lay down and rolling (Rossdale, 1987). Horse is suffering from colic due to the specificity of the anatomy of the digestive tract that the stomach is small and always fully charged which causes easy dilatation, the horse has a digestive tract that is long in the abdominal cavity is narrow, the horse is difficult vomiting because it has epiglottis great, horses, including mammalian species can not stand to pain (Sisson, 1958).
Worm infections can caused colic in horses. Belschner (1969) adds the worm infection can cause colic in horses is often called a worm colic. Colic is also caused by larval migration strongylus vulgaris to the anterior mesenteric artery resulting in thickening of the artery walls and blocked blood flow to the intestines aorta, the artery blockage can lead to rupture of the intestine which caused colic and death (Anonim, 1961).
Colic can also be caused by a blockage of the intestine, impaction colic is caused by an obstruction in the form of food ingredients in the colon. Impaction colic can occur for various reason, one of which is for horses it often takes a powder that is usually used for the base in large quantities (Hayes, 1990). Total mount of concentrate should not be more than 0.5 percent of body weight for concentrates will be easily absorbed in the small intestine and if the amount of excess will lead to colic (Syefrizal, 2008).
The results showed that helminth infection rate from highest to lowest is the Cavalry horses with 94.74% of faecal samples infected, Transportation horses with 35% of faecal samples infected and race horses with 16.42% of faecal samples infected. The highest occurence rate of colic is in the Horse Cavalry Detachment Army with a percentage of 57.94%, transportation horse farms by 30.68% and racehorse stables with10.42%. In conclusion, there is a positive correlation between helminth infection rate and the occurence of colic and different types of stable management.

KEYWORD : Horse, Management, Colic, Helminthiasis

O-35-10 The Learning Achievement on the OIE Standards in Animal Welfare at Slaughter and Transportation Training Program of Farmers

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ABSTRACT

The objectives of this research were 1) to development of the OIE Standards in Animal Welfare at Slaughter and Transportation Training Program, 2) to compare the pretest scores with posttest score after learning from the training program, 3) to study the farmers' satisfaction on the training program. The population in this study was 28 farmers who participated in the training program. The data were collected by questionnaire and test. Analyzed data by mean, percentage, standard deviation, and t - test. The results were 1) the farmers' posttest scores after studying through of the training program were statistically significant higher than the farmers pretest scores at .05 level 2) the participants had satisfaction in the Training on OIE standards in animal welfare at slaughter and transportation at much level, and 3) majority of the participants had the problems and suggestions to have training programs more in the concentrates and silage feeding management, then pasture and forage, artificial insemination, tanning of goat, meat processing, farming accounting and farm management. Furthermore the participants had suggestions in the training courses should have been added care and treatment of livestock and breeding improvement system of goat.

INTRODUCTION

The World Organization for Animal Health (OIE) improved standards in animal welfare at slaughter and transportation program has been developed as a capacity building program to assist country members implement the welfare standards on land transport and slaughter. The training program has been applied for transport and slaughter of animals. The program is contained as a training of trainers program to train the specialists who are then able to train other in-country veterinarians and technician at slaughterhouses to apply best practices in slaughter. The training approach is designed to enhance the Knowledge, Attitude, and Practice of trainees. The contents of this training program contained the importance of understanding animal behavior and how they see their environment. The trainees learn to appreciate facility design and staff animal interaction from the animal's point of view, along with possible adjustments that can be made to facility designs and handling procedures to help create a low stress environment that contributes to animal wellbeing. The training program is run on three training sessions as following 1) the basic concepts in animal welfare and animal welfare assessment, discrimination between stress and distress, and why animal welfare in pre- slaughter and slaughter is important, 2) the different ways people learn and how to structure training programs to facilitate easy learning, and 3) the trainers run a seminar for each country stakeholders on animal welfare in pre-slaughter and slaughter.

MATERIAL AND METHODS

Research site: The study was conducted in Banpongyae Village, Raimai Pattana Sub-district, Cha-Am District, Phetchaburi Province located in the lower central region of Thailand. The area is one of the major agricultural areas in Thailand. Hosted by The World Organization for Animal Health and Silpakorn University maintains a policy of helping improve the standards in animal welfare at slaughter and transportation program has been developed as a capacity building program. The area was chosen as the study site for its nearness to the Silpakorn University where the researchers work.

Respondents: The population in this study was 28 farmers who participated in the training program. Complete enumeration was applied for the sampling of respondents.

Research Instrument: The instruments of this study were 1) the pretest and posttest composed of 20 true-false questions about the animal welfare standards, animal behavior, transportations, and slaughter, 2) the questionnaire intended to find out the respondents' opinion towards satisfaction in the Training on OIE standards in animal welfare at slaughter and transportation. Perception opinion towards satisfaction of was measured through the following Likert scale:

Level	Scale	Range of the mean scores
Very much	5	4.51 – 5.00
Much	4	3.51 – 4.50
Fair	3	2.51 – 3.50
Poor	2	1.51 – 2.50
Very poor	1	1.01 – 1.50

The questionnaire also contained the semi-structured questions to elicit responses from the respondents about needs and the problem in their livestock works.

Data Analysis: Data were analyzed and presented through percentage, mean and standard deviation, t-test was employed to test hypothesis at .05 level.

Table 1 The analysis compare between pretest and posttest scores

Test	$\bar{x}$	S.D.	*df*	t
Pretest scores	9.03	3.08	35	14.37*
Posttest scores	16.39	0.64		

*p < .05

RESULTS AND DISCUSSION

The analysis of pretest and posttest score in Table 1 shows that respondents gained knowledge after the training (t-value = 14.37). This indicates that the farmers had a good learning achievement towards the OIE Standards in Animal Welfare at Slaughter and Transportation Training Program. As the research finding, the respondents' learning achievement on training had improved after training. Therefore, this research is beneficial to both of The World Organization for Animal Health, and farmers, since it as the goal of development and technology transfer.

Table 2 The respondents' opinion towards satisfaction in the training program

	Evaluation lists	$\bar{x}$	S.D.	Level of satisfaction
1.	Contents is suitable and valuable	3.77	0.75	Much
2.	The knowledge can apply to livestock works	3.68	0.95	Much
3.	Suitability of the approach on training program	4.05	0.84	Much
4.	The timing of training program was appropriate	4.09	0.75	Much
5.	Suitability of the trainers	4.36	0.73	Much
6.	Suitability of the place	3.95	0.90	Much
7.	Benefits on the training program	4.14	0.77	Much
8.	In general on the training program	4.27	0.77	Much
	Grand $\bar{x}$	4.08	0.80	Much

Table 2 shows that the respondents had satisfaction at much level on opinion towards the training program in all of items (Grand mean = 4.08). The results indicate that the participants highly satisfaction with all the statements presented to them regarding the training program especially on the trainers. The results have shown like that because the farmers need to improve their productivity to be the quality standard. The after training test proved their respondents gained knowledge and were able to apply the knowledge from this training to their work.

Nevertheless, the almost respondents still had the problems and need to have training programs more in the concentrates and silage feeding management, then pasture and forage, artificial insemination, tanning of goat,

meat processing, farming accounting and farm management. Furthermore the participants had suggestions in the training courses should have been added care and treatment of livestock and breeding improvement system of goat.

CONCLUSION

The studied found out the farmers gained a good learning achievement towards training program on OIE Standards in Animal Welfare at Slaughter and Transportation Training Program. The respondents had expressed high level of opinion satisfaction on the training program. The respondents need to have training programs more for the next time especially the contents about concentrates and silage feeding management.

KEYWORD : Animal welfare, World Organization for Animal Health, Slaughter and Transportation, Training program, Learning Achievement

REFFERENCE

Animal Welfare Standards. 2015. Internal Animal Welfare Standards Project. Available Source: http://www.animalwelfarestandards.org/. October 13, 2015.

Poommarin, P., et al. 2015. Satisfaction of Farmers' on the OIE Standards in Animal Welfare at Slaughter and Transportation Training Program. Proceedings of The 12th National Conference Kasetsart University KPS Campus, Thailand, 89 - 94.

Poommarin, P., et al. 2016. Knowledge Management of Dairy Cattle Farm Management for Farmers in Krabyai Sub-District, Ban pong District, Rachaburi Province. The 5th National Animal Science Conference of Thailand, Thailand, 850 - 857.

World Organization for Animal Health. 2014. Concepts in Animal Welfare, 3rd edition. Available Source: http://www.worldvet.org/docs/universities_and_academic_institutes _caw.pdf. October 14, 2015.

O-36-1

The Effect of Lighting Regimens on Broiler Growth:3. Status of Corticosteroid Hormone and Feeding Behaviour

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Introduction

Light, one of the most important environmental factors in determining animal behaviour, influences animal's life cycles and controls their behaviour to a great extent. The natural behaviour of chicken such as pecking among other, foraging, drinking, walking, running, perching, standing, sitting, resting, preening, wing stretching, flying, dust bathing (Appleby *et* al.,2004). Light is made up of various wavelengths to stimulate the eye's retina broiler, then affect the behavior of broiler (especially on feeding behavior), thus affecting growth. Blue lighting (BL) which characterized by short wavelength seems to stimulate broiler growth at the end of the production cycle (27-49 day) without significant effects on total feed consumption, food conversion ratio and mortality rate (Mohamed, *et* al.,2014). In this study, corticosterone levels were measured, as both factors are stress indicators. The glucocorticoid cortisol (or its equivalent corticosterone) is a steroid hormone produced by the adrenal glands. When exposed to stress, (but also to for example physical exercise, arousal or illness), the hypothalamus releases corticotropine-releasing-hormone (CRH) to the pituitary gland, which in turn excretes adrenocorticotropic hormone (ACTH), signaling to the adrenal glands to release corticosterone. (Ericsson, 2014).

Objective

The objective of this study was to investigate effect of intermittent (B12) and continuous blue lighting (B24) in order to determine their effects on behaviour and status of corticosterone hormones in broiler.

Materials and Methods

The research was conducted at poultry farm in the village of Wonokerto, Turi, Sleman, Yogyakarta. Hormonal analyses were conducted at the Physiology Laboratory, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia. A total of 2700 Lohmann DOC were grouped randomly into 3 treatment groups : the control group (N), the group treated with intermittent blue lighting (B12), and the group treated with continuous blue lighting. Each treatment dividen into 3 replicates 300 broilers each.

Feeding Behaviour. Feeding time started to be counted to all of the birds which given black marked in their back moved to feed. Duration was measured by recording the duration of feeding (minutes). Feeding frequency chicken is calculated in units of time. Observations carried out during 24 hours using CCTV camera.

Hormone corticosterone. Blood samples were taken at ages 1, 7, 14, 21 and 28 days, methods of analysis of the blood to determine the status of corticosterone levels using Enyzme Linked Immunosorbent Assay (ELISA).

Data Analysis. The data were statistical analyses using an analysis of variance technique with a completely randomized design followed by Duncan New Multiple Range Test.

Results And Discussion

Feeding behaviour. The results for feeding duration were shown in Table 1. The chicken feeding duration reared under B24 had a highest than the others, however the feeding frequency had lowest among others (Tabel 2). The peak of feeding activity in chicken reared under B24 were in the night. The feeding duration of all of the treatment increased in every weeks, however did not occurred to the feeding frequency.

Corticosterone Hormones. Analysis of level corticosteron (Table 3) revealed significant differences between treatment B24 and N. The corticosterone of the birds reared under B24 had a lowest level ($P < 0.05$).

$^{a, b, c,}$ different superscript in the same row and column showed significant differences ($P < 0.05$)

Discussion

The previous researches concerned the monochromatic light of red, green, blue and white without yellow. Because

of four types of cone in the retina of eye, poultry probably see color differently from trichromatic humans (Lewis and Morris, 2000). Behaviour is a useful indicator of animal well-being. A composite average feed ingestion behaviour of broilers in a treatment may mask useful dynamic information (Puma et al., 2001). Welfare is often difficult to measure, but behaviour can be one of the strongest indicators of animal welfare available to scientists (Duncan, 2005). Behaviour can aid in the interpretation of an animal feelings, both positive (such as playful behaviours, comfort behaviours and exploratory behaviours) and negative (including frustration, fear or pain). The color of light influences biological processes through hormonal activity produced by the pineal gland. Different light colors result in differences in feeding behaviour that ultimately affect the productivity of broilers (Mohamed, *et* al.,2014). Behaviour measured as the feeding duration by giving blue lighting produces feeding duration higher than normal (Table 1) (fig. 1) is different the feeding frequency with B24 is lower than normal treatment (Table 2) (fig. 2). Blue lighting control broilers for moderation in activity (aggressive) thus affecting the feeding duration became longer and a lower feeding frequency. Continuous blue lighting stimulates androgen plasma broiler grower phase thus improving feeding consumption. Rozenboim et al.,2004, said that blue lighting can stimulate androgen plasma becomes more spurring the formation of proteins more quickly. Marked by the blue lighting of short wavelength stimulates the growth of broiler at the end of the production cycle (27-49 days) with no significant effect on the total feed consumption, food conversion ratio and mortality rate, but it has a major role in reducing stress, reducing the fear response stress and has a calming effect on the broiler (Mohamed, *et al*.,2014). Poultry behaviour is strongly influenced by the intensity of the light, a brighter light will encourage an increase in activity, while a lower intensity effective in controlling aggressive actions that could lead to cannibalism (Sulistyoningsih, 2009). Corticosterone hormone levels in the given treatment B12 and B24 lower compared to the treatment N. Age 14 and 28 days of use of B24 can significantly reduce levels of (Table 3) blue lighting had shorter wavelenght than a normal, so broiler were more relaxed. Sunarti, 2004, said that the dim light will release the androgen and corticosterone, androgens participate in the process of bone formation, further stated that during the dark period turned out to be a low level of corticosterone. Low levels of corticosterone caused the chicken to be quieter so that the feeding duration increased.

Conclusion

The of use continuous blue lighting has a calming effect on the broiler (feeding duration and feeding frequency). Continuous blue lighting may be a good tool for improving welfare management of broiler

KEYWORD : blue light, feeding behaviour, corticosterone hormone

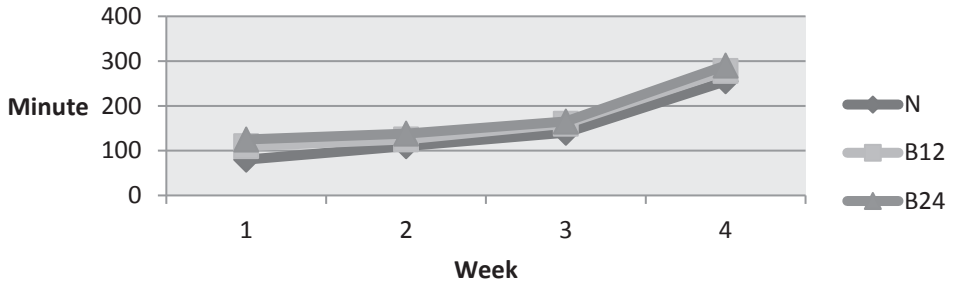

Figure 1. The mean feeding duration of broiler in blue lighting (minutes/day)

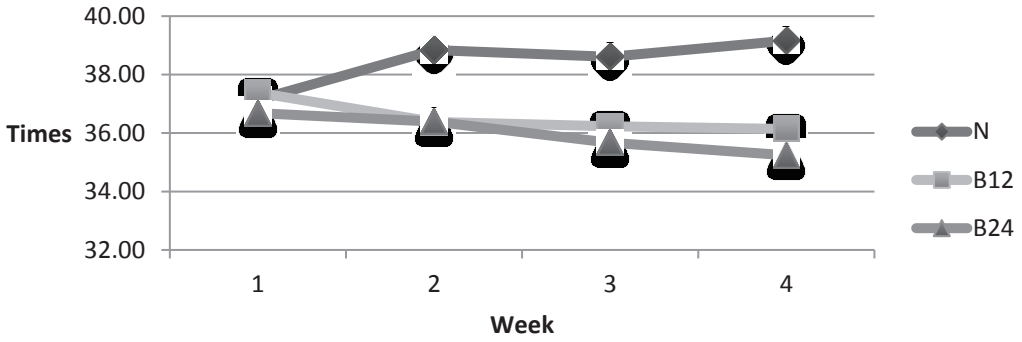

Figure 2. The mean feeding frequency of broiler in blue lighting (times/day)

Table 1: The mean feeding duration of broiler on blue lighting (minutes/day)

treatment	Week 1	Week 2	Week 3	Week 4
N	80	110	140	255
B12	110	125	160	278
B24	125	138	165	290

Description: N = normal, B12 = intermittent, B24 = continuous

Table 2. The mean feeding frequency of broiler on blue lighting (times/day)

treatment	Week 1	Week 2	Week 3	Week 4
N	37,14	38,84	38,61	39,16
B12	37,38	36,38	36,23	36,14
B24	36,68	36,39	35,67	35,24

Description: N = normal, B12 = intermittent, B24 = continuous

Table 3. Average levels of corticosterone (ng/ml)

treatment	A1	A7	A14	A21	A28
N	7,2899±2,13	8,0370±2,23	7,8514±1,12^{b}	8,7625±2,65	6,6482±0,42^{b}
B12	6,3000±0,37	9,93324±4,04	6,0193±0,74^{a}	7,1144±0,19	6,5203±0,77^{a}
B24	6,3374±0,74	5,9270±0,78	5,1744±1,06^{a}	6,8375±1,14	4,5708±0,63^{a}

Description: A = Age; 1,7,14,21,28 = day

REFFERENCE

Appleby, M.C, J.A. Mench, and B.O Hughes. 2004. Poultry Behaviour And Welfare. Center of Agriculture Bioscientific (CABI) Publishing. London.

Duncan IJH. (2005). Science-based assessment of animal welfare: farm animals. Rev. Sci. Tech. Off. Int. Epizoot. 24:483-492.

Ericsson, M. 2014. Early experiences in the chicken production and its long-term effects on behaviour. Department of Physics, Chemistry and Biology, Linköping University, SE-581 83 Linköping, Sweden.

Mohamed, R. A., M. M. Eltholth, and N. R. El-Saidy. 2014. Rearing Broiler Chickens Under Monochromatic Blue Light Improve Performance and Reduce Fear and Stress During Pre-Slaughter Handling And Transportation. Biotechnology in Animal Husbandry 30 (3), p 457-471. Institute for Animal Husbandry, Belgrade-Zemun.

Puma MC, Xin H, Gates RS, Burnham DJ (2001). An instrumentation system for studying feeding and drinking of individual poultry. Appl. Eng. in Agric. 17(3): 365 — 374.

Rozenboim, I., I. Biran, Y. Chaiseha, S. Yahav. A. Rosenstrauch, D. Sklan dan O. Halevy, 2004. The Effect of Green and Blue Monochramatic Light Combination on Broiler Growth and Development. Journal of Poultry Science. 83: 842-845.

Sulistyoningsih, M. 2009. Effect Lighting (Lighting) On Performans And Protein Consumption On Chicken. Proceedings of the National Seminar ISBN 978-602-95207-0-5.

Sunarti D. 2004. Lighting For Prevention Stress In Perspective Tropical Animal Poultry Industry Welefare.Pidato Inauguration in cattle Poultry Science at the Faculty of Animal Science Diponegoro University. Semarang.

O-36-2

Effects of Genotypes, Rearing System and Sex on Meat Yield Characteristics of Chicken at 48 Weeks of Age

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Introduction

Meat is held in high esteem in most communities. It has prestige value. The importance of meat in the diet is as a concentrated source of protein which is not only of high biological value but its amino acid composition complements that of cereal and other vegetable proteins. It is also a good source of other vitamins and minerals. Although there has been an increase in the amount of meat available in developing countries but the quantities are still small. The daily per capita availability of protein from meat increased by 24% but this was an increase from only 4.9 g to 6.1 g in contrast these figures increased in developed countries by 8%, from 27.4 to 33.9 g per day (FAO,1984). In contrast, as per capita income rises in Third World countries the demand for meat products is rising faster than that for cereals and outpaces supplies. The problems of meat production are complex and include multiple biological, economic and social factors. The practices of the small-holder system of livestock production need to be gradually developed so as to fit local conditions and meet increasing demands (Bender, 1992). Therefore, meat production could be increased through chicken rearing. Chicken meat is the cheapest meat throughout the world. Regardless of the religion and age almost all the people are fond of chicken meat. People of any age can take chicken meat without hesitation because low fat and cholesterol content compared to other meats. The quality of the meat is mainly influenced by genotype of animals and its environment, especially either nutrients or stress undergone during growing period or before slaughter.

The high live weight, high weight gain, feed conversion, carcass traits and adaptation potential depend on numerous factors, including the genotype and the gender (Hristakieva et al., 2014). Many researchers have reported a substantial effect of the genotype on live weight, feed conversion, carcass composition and carcass weight (Ojedapo et al., 2014 Razuki et al., 2011 Havenstein et al., 2003 Santos et al., 2004 Markato et al., 2006 Nikolova and Pavlovski 2009). A number of experiments have showed that the live body weight was also influenced by the gender, feed intake and utilization, abdominal fat content and the carcass composition (Scheuermann et al., 2003 and Musa et al., 2006).

Good dressing percentage, desired conformation, as much meat on the carcass as possible, optimal distribution of fat tissues, appropriate skin colour and least damage possible occurring during fattening, loading and unloading, the proportions of major basic carcass parts (breast, drumstick and thigh) as well as the presence of certain tissues are considered quality traits depend on a number of factors (Holcman et al., 2003 and Sütö et al., 1998). Among the probable biological factors, the greatest impact is produced by genotype, sex and age (Bokkers and Koene 2003, Hellmeister et al., 2003). Among numerous non-genetic factors that may have a considerable efect on meat quality, a broiler rearing system has been recognized over the past years by a large number of authors as being particularly important (Bokkers and Koene 2003 Hellmeister et al., 2003 and Ristic 2003). Many author evaluated free-range genotypes, among them Fanatico et al. found no significant interaction between genotype and production system (Fanatico et al., 2005). The superiority and genetic improvement of meat-type chickens in terms of growth is well documented (Zelenka et al., 2001 Damme and Ristic, 2003 Gerken et al., 2003 Havestein et al., 2003 and Lonergan et al., 2003). How-ever, there are almost no studies concerning the meat yield characteristics of pure breed in comparison with cross bred at the same age of birds in farming and semi-scavenging system.

Considering the aforementioned, the main objective of this study was to evaluate the effect of breed, rearing system and sex on meat yield characteristics of chicken raised under farming and semi-scavenging condition. In this study four genotypes namely Deshi (Indigenous, non-descriptive), Fayoumi (pure breed), Rhode Island Red (RIR, pure breed) and Sonali (cross bred, Fayoumi × RIR) were used to investigate meat yield characteristics.

Materials and methods

The experiment was carried out at Rajshahi University, Rajshahi, 6205, Bangladesh. A total of 48 chickens (24 males and 24 females) were reared up to 48 weeks of age (12 Deshi, 12 Fayoumi, 12 RIR and 12 Sonali). Half of the chicken (12 males and 12 females) were reared under farm in 4 replications with 4 genotypes (3 males and 3 females of each) and similarly, another 24 chicken were reared under semi-scavenging system. Before the distribution of the chicken, farmers were properly trained up about poultry husbandry management and data collection. The chickens were vaccinated against the most common chicken diseases namely Newcastle and Infectious Bursal (Gumboro) diseases with broad spectrum antibiotic (oxy-tetracycline 20% powder) was given for three days after distribution to minimize the risk of disease outbreak. During rearing in farming system the chicken were fed ad libitum on a balanced diet. In semi-scavenging condition chicken were given 50g of supplementary balance feed 25g in the morning and 25g in the afternoon. They were allowed to scavenge around the homestead and in the neighborhood for whole day. There was a continuous supply of drinking water in the shelter as well as during the scavenging periods of the day.

Data collection

At the end of the experiment, 2 chickens (1 male and 1 female) weighing average of pen weight from each replication of 4 genotypes were selected and slaughtered for processing under farm and semi scavenging conditions. Feed was withdrawn and water was supplied *ad libitum* during 12 hours of fasting prior to slaughtering to facilitate proper bleeding and then sacrificed, weighed, eviscerated, dressed, dissected, and the meat stripped from carcass. The recorded data of each bird were live weight, dressing yield, total meat yield, breast meat, thigh meat and drumstick meat. Meat yield traits were converted into percentage of individual live weight prior to analyzing the data statistically.

Statistical analysis

All recorded parameters on meat yield and the calculated variable were analyzed for a 4 (genotypes) × 2 (farming condition) factorial experiment in a Completely Randomized Design (CRD) with the help of a Computer package program GenStat 16th Edition. Significant differences among the means were isolated by calculating Least Significant Differences (LSD).

Result and Discussion

The edible meat yield characteristics of chicken breeds raised under two management systems are evaluated under this study. Genotype, rearing system and sex did not interact to influence the live weight and dressing yield ($P>0.05$, data not shown) in chicken. The influence of genotype, rearing system and sex on the total meat yield (%) is shown in the **Figure 1**. It was revealed from the findings that the highest total meat yield (44.44%) was obtained from Deshi male chicken reared in farming condition followed by Deshi male chicken reared in semi-scavenging condition (42.80%). On contrary, lowest total meat yield (27.88%) was observed in Sonali male reared in semi-scavenging condition. The results indicated that the Deshi and RIR males reared both in farm and semi scavenging had significantly higher ($P<0.01$) total meat than in their females counterparts. The differences exhibited in terms of the sex influence. While Fayoumi males and females chicken reared both in farm and semi scavenging did not differ ($P>0.05$) because they are almost equal in size genetically. Sonali male chicken reared in farm had higher total meat than that of their female counterpart.

Figure 2 shown the influence of genotype, rearing system and sex on the breast meat yield (%). It was noticed that the highest breast meat yield (14.80%) was obtained from Deshi male chicken reared in farming condition followed by Deshi male chicken reared in semi-scavenging condition (14.16%). On the other hand, lowest breast meat yield (8.75%) was observed in Sonali male reared in semi-scavenging condition. Deshi males reared both in farm and semi scavenging had higher breast meat ($P<0.01$) than that of their females counterparts. Fayoumi female chicken reared in farm had higher ($P<0.01$) breast meat than that of male. But Fayoumi male reared in semi scavenging had higher breast meat than that of female. On the other hand, RIR and Sonali farm reared males had higher breast meat than of their female counterparts. While RIR and Sonali females reared in semi scavenging had higher ($P<0.01$) breast meat than that of their males counterparts.

Further, the influence of genotype, rearing system and sex on the thigh meat yield (%) is represented in the **Figure 3**. It was found that the highest thigh meat yield (14.01%) was obtained from Deshi male chicken reared in farming

condition followed by Sonali male chicken reared in farming condition (13.45%). On the other hand, lowest thigh meat yield (8.69%) was observed in RIR female reared in semi-scavenging condition. Deshi and RIR males chicken reared both in farm and semi scavenging had higher (P<0.01) thigh meat than that of their females counter parts. On the other hand, Fayoumi and Sonali males chicken reared in farm had higher thigh meat than that of their females counter parts. In general, it is believed that larger chicken produced higher proportion of thigh meat than that of small one. While Fayoumi and Sonali chicken reared in semi scavenging did not differ between sexes (P > 0.05).

The influence of genotype, rearing system and sex on the drumstick meat yield (%) is shown in the **Figure 4**. It was observed from the finding that the highest drumstick meat yield (9.21%) was obtained from Deshi male chicken reared in farming condition followed by Sonali male chicken reared in farming condition (9.02%), but the lowest drumstick meat yield (5.37%) was observed in Deshi female reared in semi-scavenging condition. Deshi and RIR males chicken reared both in farm and semi scavenging had higher (P<0.01) drumstick meat than in their females counterparts. For Fayoumi male chicken reared in farm had higher (P<0.01) drumstick meat than that of their female. On the other hand, semi scavenging reared female had higher (P<0.01) drumstick meat than that of their male. Sonali male chicken reared in farm had higher (P<0.01) drumstick meat than that of their female. In both system males had higher drumstick meat production than that of their female ones, this implies that males chicken had larger size than females.

Conclusion

The present study indicates that there is significant effect of genotype, environment and sex on different meat quality characters. It reveals that overall meat quality find to be higher in Deshi male chicken under farming condition followed by male of other genotypes. Therefore, it is noteworthy to conclude that for meat production sex is the key consideration to maximize the output.

Acknowledgements

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KEYWORD：Meat yield, Genotype, Chicken, Rearing system, Impact

O-36-3

Analysis of growth curves in Betong chicken

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1.Introduction

Betong chicken is the indigenous chicken which found in the lower southern region of Thailand. Betong chicken is a meat type and popular in this area, due to meat is softer and the taste is better than other Thai indigenous chicks, but it is not flabby as broiler's meat(Gongruttananun and Chotesangasa, 1996). However, increasing Betong chicken production into a profitable knowledge on growth characteristics and patterns are required. The growth patterns will allow the design of nutritional or feeding regimens for Betong chicken for efficient use of feed and profitability.

Growth models are used to optimizing the management to improve the efficiency of livestock production for animals of various ages (Schinckel and de Lange, 1996). These models have different characteristics, different mathematical limitations. Therefore choice growth model should be concern that appropriate for describes growth pattern. The objective of this study was to compare nonlinear models (Gompertz, logistic and Bertalanffy) in order to describe the growth of Betong chicken.

2. Materials and Methods

2.1. Experimental Animals

The data used for the growth analysis came from experimental flocks at faculty of Agricultural Technology, Rajabhat Songkhla University. A total number of 4026 body weight (BW) records from 59 maleerels and 307 females. BW were measured from hatching to 140 days of age were used. Chicken were fed a diet containing 17% CP and3,000 kcal of ME/kg. They had ad libitum access to feed and water.

2.2 Statistical Analysis

The growth models used in study were Gompertz, Logistic and Bertalanffyas follow:

Gompertz :W=A × exp(-B × e^Ct)

Logistic : W=A/((1-B × 〖exp〗 ^Ct))

Bertalanffy : W=A × 〖(1-B × 〖exp〗 ^Ct)〗 ^3

where W is the corresponding weight at time t, A is the asymptotic mature weight, B is integration constant, C is rate of mature weight and exp is exponential constant.

Growth model parameters for the Gompertz, Logistic and Bertalanffy were estimated using PROC NLIN (Marquart algorithm) (SAS Institute, 1999).The statistic used to comparethe goodness of fit was the coefficient of determination (R^2) and mean square error (MSE) were define as

$$R^2=(1- \Sigma_i^n 〖(y_i-\hat{y}_i)〗^2)/(\Sigma_i^n 〖(y_i-\bar{y})〗^2),$$

$$MSE=(\Sigma_i^n 〖(y_i-\hat{y}_i)〗^2)/n$$

where $\hat{y}_i$ is the predicted value for y_i, and y_iis the observed record for BW.

3.Results and Discussion

Means and standard deviations of BW of Betong chicken for each sex are presented in Table 1. The effects of sex on BW at hatching was not detected, however, BW between sexes had significant differences started at 14 days of age and continue.

The fitted parameters for 3 growth models are presented in Table 2. The model with small MSE and high R^2were preferred among models. The result showed that MSE of logistic model had lower than others models (Gompertz and Von Bertalanffy) in both sex. Additionally, $R^2$of logistic model had lower than others model in female. This result indicated that logistic model seem to be not appropriate. The result agree with Yakupoglu and Atil (2001) which reported Gompertz and Bertalanffy models seemed to be appropriate to describe the association between age and BW in broilers base on R^2. The MSE was highest for the male, due to a low number of records. This result

agree with Ersoy et al. (2006). Considering the growth parameters in the present study were closer to the values previous reported by Norris et al. (2007) and Yang et al. (2006)

Figure 1 shows the results of maleand female for the Gompertz Logistic and Bertalanffy models. The Logistic model overestimates the initial BW and underestimates the final BW. This result agree with Rizzi et al. (2013) who study in Italian local chicken populations. The Gompertz and Bertalanffy can be considered a model that has appropriate of the data ofthe studied populations.

Conclusion

The results of this study indicate that all the three growth models evaluated adequately predicted the growth curve parameters. The Gompertz and Bertalanffy models are appropriate for describing the age - BW relationship in the Betong chicken. However, further research is required using other data sets Betong chicken to validate due to data of male were small.

KEYWORD：Growth models, Gompertz, Logistic, Bertalanffy

Table 1. Means and standard deviation for body weight at different ages in a Betong chicken

Age (days)	Male		Female	
	Mean	SD	Mean	SD
0	35.01	2.77	34.59	3.01
14	109.38	14.98	103.95	14.96
28	278.71	34.41	245.57	27.91
42	530.97	50.28	450.87	53.21
56	816.94	82.41	656.38	67.82
70	1194.92	130.43	920.66	93.66
84	1453.22	175.04	1097.40	113.10
98	1783.05	213.72	1309.41	143.62
112	1986.78	249.67	1467.36	160.15
126	2141.86	284.17	1644.04	187.93
140	2279.83	266.35	1795.77	214.15

Table 2. Fitting degree and parameter estimated value of three growth models

Sex	Growth model	Model parameters			R^2	MSE
		A	B	C		
Male	Gompertz	2643.9	4.517	0.025	0.986	28845.5
	Logistic	2338.3	-23.786	0.045	0.985	30113.4
	Bertalanffy	2905.8	0.906	0.018	0.985	29347.4
Female	Gompertz	2195.1	3.833	0.021	0.987	14637.2
	Logistic	1873.2	-17.642	0.039	0.974	16299.8
	Bertalanffy	2508.7	0.796	0.014	0.988	14453.4

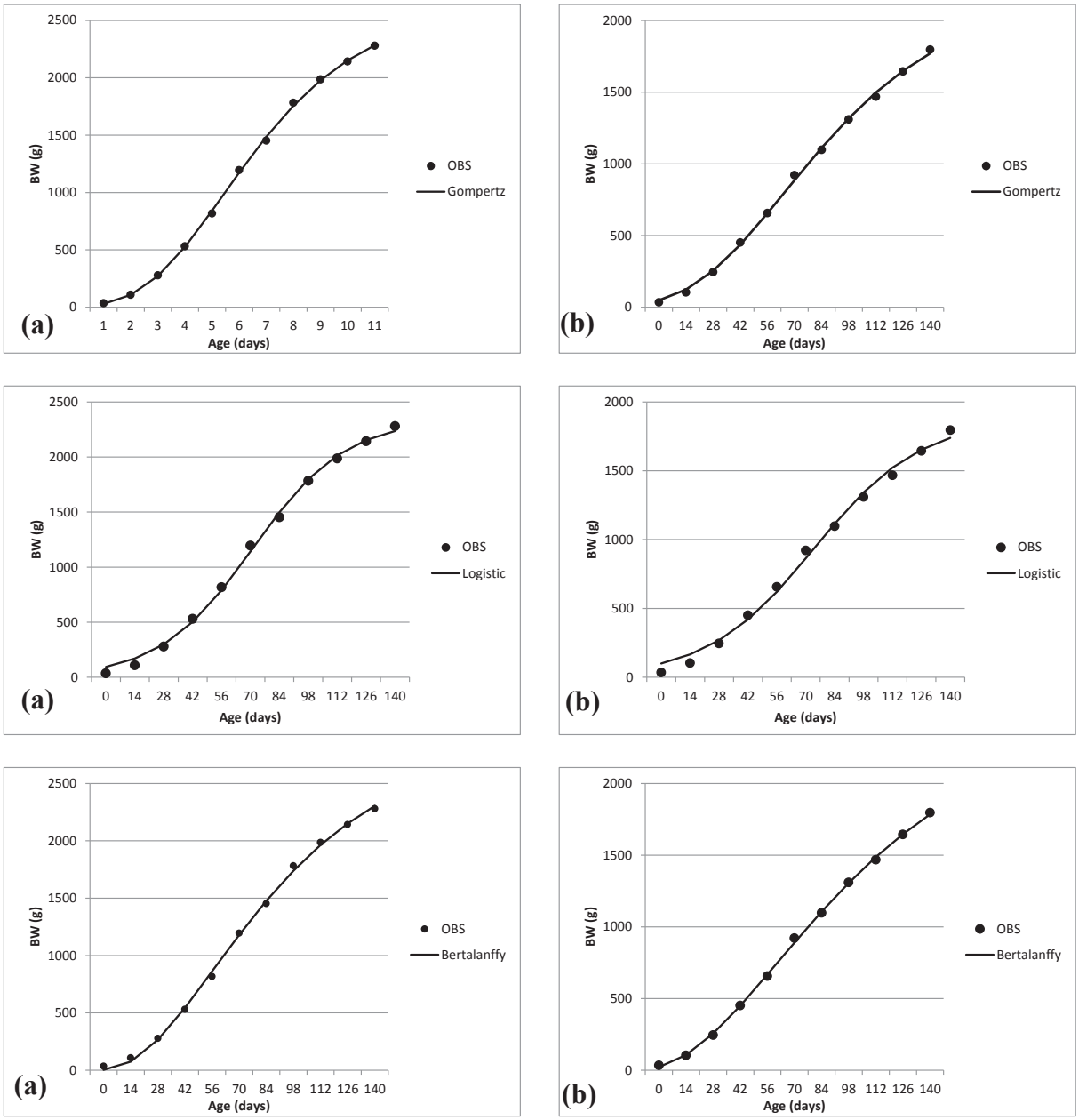

Figure 1. Growth male (a) and female (b) according to Gompertz Logistic and Bertalanffy models in comparison to the observed data.

REFFERENCE

Norris, D., Ngambi, J.W., Benyi, K., Makgahlela, M.L., Shimelis, H.A. and Nesamvuni, E.A. 2007. Analysis of growth curves of indigenous male Venda and Naked Neck chickens. S. Afr. J. Anim. Sci. 37 : 21 -26.

Ersoy, I. E., Mendes, M and Aktan, S. 2006. Growth curve establishment for American Bronze turkeys. Arch. Tierz., Dummerstorf 49 : 293-299.

Gongruttananun, N. and Chotesangasa,R. (1996). A study of growth and carcass yield of Betong chickens compared with those of Native and crossbred Betong x Native chickens. Kasetsart J. (Nat. Sci.), 30:312-321.

Rizzi, C., Contiero, B.and Cassandro, M. 2013. Growth patterns of Italian local chicken populations. Poultry Science 92 :2226–2235.

SAS Institute. 1999. SAS/STAT User's Guide (Version 8). SAS Inst. Inc., Cary, NC.

Schinckel, A. P., and C. F. M. de Lange. 1996. Characterization of growth parameters needed as inputs for pig growth models. J. Anim. Sci. 74:2021-2036.

Yakupoglu, C. and Atil, H. 2001. Comparison of growth curve models on Broilers. II. Comparison of models. Online J. Biol. Sci. 1 : 682-684.

Yang, Y., Mekki, D.M., Lv, S.J., Wang, L.Y., Yu, J.H. and Wang, J.Y. 2006. Analysis of Fitting Growth Models in Jinghai Mixed-Sex Yellow Chicken. International Journal of Poultry Science 5: 517-521.

O-37-1

IDENTIFICATION OF REPRODUCTIVE PERFORMANCE OF ETTAWA CROSSED BREED GOATS ON DIFFERENT BODY CONDITION SCORE USING VAGINAL SMEAR

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OBJECTIVE

Goat played an important role as source of income for the farmers, also a source of animal protein to support the national programme on meat self-sufficient. Some favourable characteristics of goat are prolific, the litter is more than 1 and capability to produce offspring 3 times in 2 years. Un fortunately, due to unoptimal husbandry these goat cannot express its best reproductive performance (Soeharto, 2008).

Reproductive performance of EG goat so far has not been optimal. This can be seen from long calving interval, ranges from 9 to 15 months. After partus, is generally decreasing body condition score (BCS) does, conditions that occur normally but is not expected a drastic reduction (Anonymous, 2006). The low BCS after partum causes a change in the reproduction performance, such as failure ovulation, extend the periode of estrous postpartum and calving interval (Widayati et al., 2011). Thus, BCS influence the changes of hormone gonadotropin circulation, growth as well as the development of the follicle and corpus luteum, and ovarian activity.

Vaginal smear methods were done to obtain an accurate observation of estrous, using epithelial cells taken from the vaginal wall. Epithelial cells was performed as an accurate parameter in determining the reproductive cycle, the cells will be directly related to hormones, especially estrogen in the follicular phase and the luteal phase. Therefore, the study was conducted to determine the reproductive performance of EG goat on body condition score (BCS) through the observation of vaginal cytology.

METHODOLOGY

Description of the study site and materials

Six EG does with body condition score (BCS) of 2.0 to 3.0, not pregnant, lactation, milked once a day, were used in this research. They were feed ad libitum of white pollard and fresh cut of forage (legumes and jackfruit leaves). Vaginal smear samples and reproduction data were obtained from goat farmers in Rejodani area, Sleman, Yogyakarta. Smear analysis were carried out in the Laboratory of Phyisiology and Reproducstion, Faculty of Animal Scince, University of Gadjah Mada. The research was conducted from April until October 2015. The equipment were used including digital thermometer (Magic Star, China), litmus paper (Merck ® universal pH indicator, Germany), object glass, microscopes, tweezers. For vaginal smear staining, methanol, Giemsa, phosphate buffered saline with 1% acetic acid were used.

Research methods

Before conducting the study, each doe was dewormed and submitted to a general physical examination and vaginal inspection. Data began to be taken at 10 days after parturition. . Three areas were evaluated in assigning a BCS: the lumbar region, or area containing the loin muscle the sternum and the rib cage (Spahr, 2005).

Estrous were observed based on the general symptoms of estrous, such as seeking out the male, loss of appetite, crestlessness, and social behaviors such ascrubbing up against herd-mates. Physical signs included redness and swelling around the vulva, and thin mucous discharge from the vulva. Moreover, determination of the does's cycles also be done by observing the vaginal epithelium histology. Each does was taken vaginal smear every morning. The smears were stained 20 minutes of Giemsa solution, and observed using a microscope (Turner and Bagnara, 1988 adapted by Widayati *et al*, 2010).

To strengthen estrous cycle determination, measuring the temperature of the vagina using a digital thermometer (Magic Star) and measuring the pH of the vagina using litmus paper were done. Vaginal temperature measurement was carried out every morning. Measurements of the pH of the vaginal mucus is done with paper pH indicator (Merck ®, Germany) by way of gluing paper pH indicator on the vaginal mucous, and then matched with standard pH 1-14.

Does declared having estrus if showed one sign from the following observations visual signs of vulvar

experiencing 3A, increasing the vaginal temperature and vaginal pH (alkaline or pH becomes > 7), and vaginal smear showed the superficial cells in the form of polygonal or irregular (Figure 2). Reproductive performance was obtained through interviews then analyzed using t-test. Visual signs of estrous were analyzed descriptively. pH and the number of superficial cells was analyzed using Randomized Complete Block Design (RCBD)..

RESULTS

Visual signs of estrous, as presented in Table 1, were not well understood by both groups of goat farmers. Does's behavior at the time of estrous tart also showed behavior changes, such as more aggressively and restless. The determination of the start of estrous was based on the changes in behavior, changes in the visual condition of vulva and changes the histogy of vaginal epithelial cells. Furthermore, according to Hafez (2000) and Widayati *et al*. (2013), at the time of estrous animals showed signs of changing behaviors the vulva was swollen, reddened and wet. Furthermore, blood flows, uterine activity, secretion of glands on cervical and vaginal were increased.

The concentration of epithelial cells and other cells were influenced by estrogen. When estrogen increased the vaginal walls became thicken, and the number of epithelial cells that undergo cornification increased. Otherwise, when there is no estrogen vaginal wall was very thin and intermediate and parabacal cells were found. Morphological changes were seen at the time of estrus, the cells were flat-shaped, pleated, transparent, non-core, and uneven edges. Changes in morphology of each estrous phase were presented in Figure 1. According to Nalley et al (2011), the changes in the estrous cycle were influenced by hormonal factors that showed changes in vaginal epithelial cells. Character vaginal epithelial cells during estrus were shaped superficial keratinized, undergoes cornification, mostly core, has a corner, and sometimes folded (Widayati et.al, 2010).

Based on the results of statistical analysis do not occur significant differences (P <0.05) between the pH of the vaginal fluid of goats with BCS 2 and 3 at the time of estrus and not estrus. The average of pH when estrous was 7.84 ± 0.52 on BCS 2 and 8:39 ± 0:29 on BCS 3.

Statistical analysis showed that there was significant differences (p <0.05) between the PPE goats Peranakan ettawa the BCS 2 and 3 (Table 2). Murdjito et al. (2011) reported that the goat shows postpartum estrus about 45 to 180 days with an average of 95 days. While Chowdhury et al. (2002) cit Tanjung et al. (2015) revealed that the Black Bengal goat postpartum estrusnya ranged from 16 to 136 days with an average of 30 days. Sutama (2009) states that postpartum estrus occurs 3 to 5 months after birth, and factors influencing postpartum estrus include low body weight, low body condition and high prolactin levels are the reasons for the slow postpartum estrus.

CONCLUSION

It could be concluded that visual signs of estrous and the reproductive performance, in terms of postpartum estrous and days open, are better in EG goat with BCS 3 than in BCS 2.

KEYWORD : Reproductive performance, Ettawa crossed breed goat, Body condition score, vaginal smear

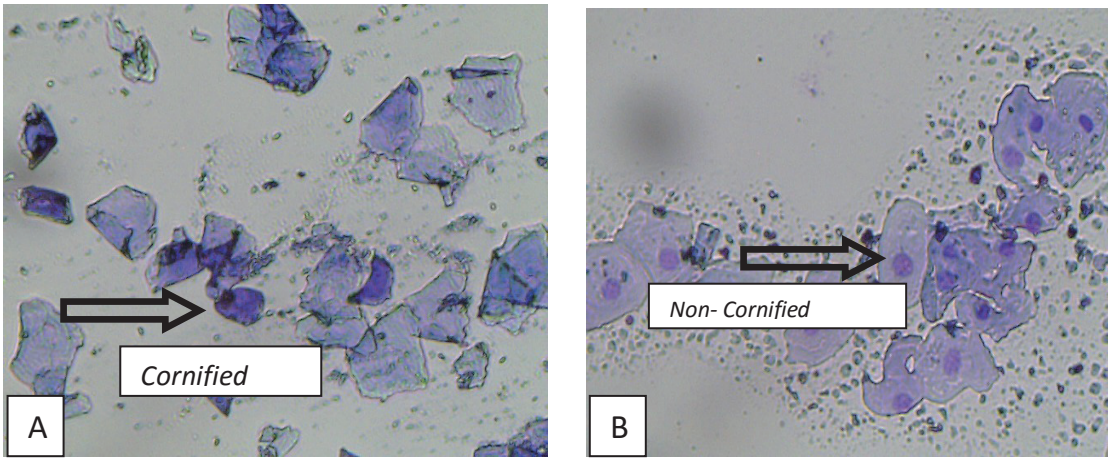

Figure 1. Vaginal histology during estrous of Ettawa Grade goats. The arrow showed some cornified epithelial cells and non cornified cell. a. Does having BCS 2.00, b. Does having BCS > 2.00–3.00.

Table 1. Description of estrous signs of Ettawa Grade goat having BCS 1.00 up to 2.0 and BCS > 2.00 up to 3.00

Criteria of estrous signs	BCS 2 (n=3)		BCS 3 (n=3)	
	Number of goat	Percentage	Number of goat	Percentage
Slightly swollen of vulva	-	0.00	3	100.00
Vaginal discharge	-	0.00	2	66.60
Reddened of vulva	1	33.33	3	100.00
Changes of behaviour	1	33.33	3	100.00

Tabel 2. Average of PPE, DO and S/C of Ettawa grade goats on BCS 2 and 3

BCS	PPE (days)	DO (days)	S/C
BCS 2	67.67±2.516[a]	127.00±6.082[a]	1.96±0.05[a]
BCS 3	57.67±.,516[b]	112.67±2.516[b]	1.56±0.11[b]

[a-b]*different superscript* indicated significance different (p<0,05)

REFFERENCE

Hafez, E.S.E., M.R. Jainudeen and Y. Rosnina. 2000. Hormone, Growth Factor and Reproductin. In: Reproduction in Farm Animal, 7th Edition, E.S.E Hafez ed., Kiawah Island, South Carolina, USA.

Johnston S.D., M.R. Kustritz and P. Olson. 2001. Canine and Feline Theriogenology. WB Saunders comp. Philadelphia.

Najamudin, Rusdin, Sriyanto, Amrozi, Srihadi A.P., dan Tuti L.Y. 2010.Penentuan Siklus Estrus pada Kancil (Tragulus javanicus) Berdasarkan Perubahan Sitologi Vagina. Jurnal Veteriner, vol 11No. 2: 81-86

Murdjito, G, Budisatria, Panjono, N. Ngadiyono, dan Endang B. 2011.Kinerja Kambing Bligon yang dipelihara peternak di Desa Giri Sekar, Panggang,Gunung Kidul. Buletin Peternakan Vol. 35(2): 86-5

Nalley, W. M. M., R. Handarini, M. Rizal, R. I. Arifiantini, T. L. Yusuf, B. Purwantara. 2011. Penentuan Siklus Estrus Berdasarkan Gambaran Sitologi Vagina dan Profil Hormon pada Rusa Timor. J. Vet. 12 (2): 98-106

Spahr LI. 2005. Body Condition Scoring in Meat Goats. Penn State College of Agricultural Science. 23: 11-15.

Sutama, I.K. 2009. Productive and Reproductive Performances of Female Etawah Crossbread Goats In Indonesia. Jurnal Wartazoa Vol. 19 No. 1: 1-6.

Tanjung, A.D., E.T. Setiatin, D. Samsudewa. 2015. Level of Estrogen Homone and Estrus Performance of Different Postpartum Estrus of Jawa Randu Goat. Journal Indonesian Tropical Animal Agricultural. Vol. 40 No. 2: 87-92.

Widayati, D.T., Junaidi, A Aris Junaidi, Suharto, K., Oktaviani, A., Wahyuningsih. 2010. Reproduction Performance of Etawah Cross Bred Goats in Estrus Synchronization by *Controlled Internal Drug Release* Implant and PGF2 ά Continued by Artificial Insemination. World Academy of Science, Engineering and Technology 41: 1077-1079.

Widayati, D.T., M. Suryaputri and Y. Suranindyah. 2013. The Effect of Body Condition Score on Estrous Postpartum of Ettawa Crossed Breed Does in Girikerto Farmer Group, Turi, Sleman. Proceeding the 2nd Animal Production International Seminar Malang, 29th August – 1st September 2013, Malang, pp. 111-116.

O-37-2

Dietary Yeast (Saccharomyces cerevisiae) Probiotic for Growth Performance, Body Weight and Semen Quality Enhancements in Goats

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ABSTRACT

Saccharomyces cerevisiae as probiotic has been widely used to promote rumen fermentation, body weight and production performance in ruminants. This study was to evaluate efficacy of dietary yeast *(S. cerevisiae)* probiotic to enhance growth performance, body weight (BW) and semen quality in Boer goats. Five lean Boer bucks, aging 1-3 years, body condition score 2-2.5 from 5 scores and average weighing 42.60 ± 4.34 kg were conducted for 4 months. They were fed with concentrate and roughage (corn silage and rice straw) for one month. After that they were continuously fed the same feeds, but with supplemented dietary yeast probiotic (*S. cerevisiae* 1 g/kg concentrate) for 2 months. In the fourth month, there was no dietary yeast probiotic in feeding for all bucks. During the experimental period, all bucks were measured total feed intake (for gain:feed calculation) and BW daily. They also were semen collected by electro-ejaculator for semen evaluation once a month. For gain:feed and BW performance, the results showed that the bucks had averages of gain:feed and BW in first, second, third and fourth month as 0.02±0.01, 0.06±0.01, 0.06±0.02 and 0.04±0.01 vs 44.30±4.58, 47.20±5.30, 49.70±6.05 and 51.50 ± 6.13 kg, respectively. They occurred the gain:feed in the second till the fourth month higher than in the first month ($p \leq 0.01$). For semen evaluation, the current study has found that means of sperm concentrations in the first to fourth months were 3474 ± 827.12, 4406 ± 834.85, 4606 ± 905.56,and 4732 ± 618.16 number in 10^6/ml, respectively. Additionally, means (1^{st}-4^{th} month) of percentages of progressive motility (60.00 ± 6.12, 74.00 ± 9.62, 80.00 ± 5.00, 78.00 ± 5.70, respectively), sperm viability (42.62 ± 8.53, 81.45 ± 6.86, 81.10 ± 5.35, 75.20 ± 5.60, respectively) and positive-hypo-osmotic swelling test (41.25 ± 9.07, 77.30 ± 7.25, 75.90 ± 6.86, 71.40 ± 8.32, respectively) in the last three months were greater than those in the first month ($p \leq 0.05$). Conclusion, dietary yeast *(S. cerevisiae)* probiotic has good efficacy to enhance growth performance, BW and semen quality in Boer goats.

INTRODUCTION

Dietary yeast *(Saccharomyces cerevisiae)* probiotic has been frequently used to enhance rumen fermentation, which improves health, productivity and reproductive performance in ruminants. Yeast is utilized to obtain better rumen condition for the growth and activity of cellulolytic anaerobic bacteria (Moallem et al., 2009). In addition, yeast or yeast culture could be an as rich source of vitamins, minerals, amino acids, enzymes and important basic nutrients for rumen bacteria which enhance the better digestibility of ruminants (Polyorach and Wanapat, 2015). Hence, yeast is able to contribute digestibility and maintain stable rumen environment that they are the importance factors for occurring good health, productivity and reproductive performances of animals. Thus, previous studies have reported that dietary yeast culture supplementation could improve the growth rate in calves (Rameshwar et al., 1998) and increase milk production in dairy cows (Piva et al., 1993). The potential of yeast as probiotic on an improvement in average daily gain, feed conversion ratio, milk yield and milk composition were reported in ruminants (Polyorach and Wanapat, 2015). Moreover, the similar reported by Abd El-Ghani (2004) that goats daily fed with yeast culture 3 or 6 g per head under field condition had improvement of digestibility trials and higher milk yield. Furthermore, the studies of dietary yeast supplementation effects on reproductive performance are limited in goats. Particularly, the effects of supplemented dietary yeast on the parameter such as semen quality in male goats have been either seldom or never determined.

However, according to the beneficial effects of dietary yeast on good digestibility contribution and maintenance stable rumen environment result in good health, growth rate, and productivity in goats. Then, authors have the hypothesis that dietary yeast probiotic also is able to enhance fertility on semen quality parameter in the buck. In this study was to evaluate efficacy of dietary yeast *(S. cerevisiae)* probiotic to enhance growth performance, body weight and semen quality in Boer goats.

MATERIAL AND METHODS

Experimental animals

Five lean Boer bucks, aging 1-3 years, body condition scores 2-2.5 from 5 scores and average weighing 42.60 ± 4.34 kg were randomly chosen from the herd for the experiment. They also were kept for four months in experimental stall barn of Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand. This study was conducted during in May to August 2015 (outdoor temperature ranged from 22.50 to 40.50 °C, and the average humidity was 87.50%. The selected bucks were fertile, normal anatomical reproductive organs and free from any reproductive disorders. The bucks were fed daily for one month with concentrates containing 16% crude protein about 2 kg/day, offered roughage (corn silage and rice straw) as 10% of body weight and freely accessed to clean drinking water. After that they were continuously fed the same feeds and water, but the supplemented dietary yeast probiotic (*S. cerevisiae*) 1 g/kg concentrate (total viable yeast count $1.5x10^7$ cfu/1 g probiotic) also would be mixed in the feeds for 2 months. In the fourth month, there had no more dietary yeast probiotic in the same feeding for all bucks. During the experimental period (four months), all bucks were measured total feed intake and body weight daily for gain:feed calculation. They also were semen collected by electro-ejaculator for semen evaluation once a month.

Semen quality evaluation

Each buck's semen was quality evaluated once a month (for four months) with the same person. The used parameters to determine the semen quality in this study were percentages of progressive motility, sperm viability, and positive sperm hypo-osmotic swelling test, and sperm concentration. The progressive motility is individual swimming in a straight direction of sperm. The undiluted semen sample of each buck was dropped on a pre-warmed glass slide (37°C) and placed a cover glass slip. The percentage progressive motility of spermatozoa was assessed with a microscope (40X). For the sperm viability determination, a drop of semen sample and the eosin-nigrosin stain was mixed on a pre-warmed glass slide using applicator stick. This ready mixture sample was thin smeared using another slide (Khan and Ijaz, 2008). After air-drying in room temperature, the smeared sample was observed under a phase contrast microscope at 40X. The live sperm as unstained heads and dead sperm as total or partial stained heads were counted and calculated the percentage of sperm viability (live sperm) from a total 200 spermatozoa in at least 5 different fields of view. The hypo-osmotic swelling test (HOS test) is one of the new procedures which it has been developed for sperm membrane integrity determination. It is used to evaluate of reaction. The all living sperms with intact membrane will be swelling in a hypo-osmotic solution that they are detected of positive sperms HOS test (Jeyendran et al., 1992). The prepared HOS solution was done with dissolved sodium citrate (0.735 g) and fructose (1.351 g) in 100 ml distilled water. Before using, it would be incubated at 37°C for 5 min. Each semen sample (100µl) was mixed with HOS solution (1000µl) and maintained at 37°C for 60 min. After that, the spermatozoa were fixed with formaldehyde (10% formalin 0.1 ml) for observation of positively swollen sperms later. A drop of the mixed semen sample and HOS solution was placed on a glass slide and covered with a cover slip. The positively swollen sperms from a total 200 sperms in at least 5 different fields of observation were counted and converted to the percentage. Sperm concentration was examined by spectrophotometer. Formal-buffer saline (3.42 ml) was mixed with of semen sample (180 µl) in a blank tube. The tube was inserted into the spectrophotometer. Hence, the total sperm numbers also were calculated for sperm concentration in 1 ml.

Statistic analyses

All measured parameters were subjected to be statistically analyzed by ANOVA with repeated measurement and Tukey HSD multiple comparisons to detect significant level at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results showed that the bucks had averages of gain:feed (growth performance) and body weight (BW) in the first, second, third and fourth month as 0.02 ± 0.01, 0.06 ± 0.01, 0.06 ± 0.02 and 0.04 ± 0.01 versus 44.30 ± 4.58, 47.20 ± 5.30, 49.70 ± 6.05 and 51.50 ± 6.13 kg, respectively. The gain:feed ratios occurred in the second till the fourth month higher than in the first month ($p \leq 0.01$). For the BW series, they raised in every month from the first till the fourth month. Especially, the BW in the fourth month was higher than the BW in the first month ($p \leq 0.05$) while they also trended higher in the third than in the first month ($p=0.06$). From these results expressed, the supplemented dietary yeast *(S. cerevisiae)* probiotic was able to contribute and maintain stable

rumen environment, enhance of good digestibility, growth performance and BW, according to the beneficial effects of dietary yeast on daily gain, feed conversion ratio and growth rate in ruminants (Rameshwar et al., 1998 Polyorach and Wanapat, 2015). Moreover, although were the bucks fed without of dietary yeast probiotic in feeds of the fourth month. They also had high gain:feed and BW which were similar with the values of the third month. That mean, the dietary yeast *(S. cerevisiae)* probiotic also could be still effective on growth performance and body weight in bucks after probiotic withdrawal of feeds for 1 month. For semen evaluation of sperm concentrations in the first to, fourth months were not significantly different when they were compared to the value in each month (3,474 ± 827.12, 4,406 ± 834.85, 4,606 ± 905.56,and 4,732 ± 618.16 the number in 10^6/ml, respectively). Additionally, means (1^{st}-4^{th} month) of percentages of progressive motility (60.00 ± 6.12, 74.00 ± 9.62, 80.00 ± 5.00, 78.00 ± 5.70, respectively), sperm viability (42.62 ± 8.53, 81.45 ± 6.86, 81.10 ± 5.35, 75.20 ± 5.60, respectively) and positive-hypo-osmotic swelling test (41.25 ± 9.07, 77.30 ± 7.25, 75.90 ± 6.86, 71.40 ± 8.32, respectively) in the last three months were better than those in the first month ($p \leq 0.05$). Although, the sperm concentration in the first month was not significantly different from other months but the three other semen quality parameters were lowest in this month. After that, these three parameters were significantly increasing in the second month and maintained on high levels in the third month. Furthermore, all semen quality parameters were still good in the fourth month that the bucks were not fed with supplemented dietary yeast probiotic. Hence, the current study has found that means the supplemented dietary yeast *(S. cerevisiae)* probiotic was able to contribute and support stable rumen environment which was the key to enhancing of good health and fertility to be better semen quality in bucks. All study parameters were showed in Table 1. Conclusion, dietary yeast *(S. cerevisiae)* probiotic has good efficacy to enhance growth performance, body weight and semen quality in Boer goats.

KEYWORD : Body weight, Boer goats, Probiotic, Saccharomyces cerevisiae, Semen quality

Table 1. The mean of gain:feed ratio (growth performance), body weight and semen quality parameters in bucks in all collecting periods of the present experiment

Parameters	The collecting period of the present experiment in bucks (n=5)			
	1^{st} month Without yeast probiotic feeding	2^{nd} month With yeast probiotic feeding	3^{rd} month With yeast probiotic feeding	4^{th} month Without yeast probiotic feeding
Gain:feed ratio	0.02±0.01[a]	0.06±0.01[b]	0.06±0.02[b]	0.04±0.01[c]
Body weight (kg)	44.30±4.58[a]	47.20±5.30[a]	49.70±6.05[a]	51.50±6.13[b]
Sperm concentration (X10^6) (cells/ml)	3,474±827.12	4,406±834.85	4,606±905.56	4,732±618.16
Progressive motility (%)	60.00±6.12[a]	74.00±9.62[b]	80.00±5.00[b]	78.00±5.70[b]
Sperm viability (%)	42.62±8.53[a]	81.45±6.86[b]	81.10±5.35[b]	75.20±5.60[b]
Positive sperm hypo-osmotic swelling test (%)	41.25±9.07[a]	77.30±7.25[b]	75.90±6.86[b]	71.40±8.32[b]

[a, b,c] Superscription in the same rolls as a significant difference ($p \leq 0.05$)

REFERENCES

Abd El-Ghani, A. A. 2004. Influence of diet supplement with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Rumin. Res. 52:223-229.

Jeyendran, R. S., H. H. Van der Ven and L. J. D. Zaneveld. 1992. The hypo-osmotic swelling test: An update. Arch. Androl. 29:105-116.

Khan, M. I. R. and A. Ijaz. 2008. Effects of osmotic pressure on motility, plasma membrane integrity and viability in fresh and frozen-thawed buffalo spermatozoa. Anim. Reprod. Sci. 2:548-553.

Moallem, U., H. Lehrer, M. Livshitz, M. Zachut and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency and digestibility. J. Dairy Sci. 92:343-351.

Piva, G., S. Belladonna, G. Fisconi anf F. Ssicbaldi. 1993. Effect of yeast on dairy cows performance, ruminanl fermentation, blood components and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Polyorach, S. and M. Wanapat. 2015. Potential of yeast as probiotics in ruminants. Khon Kaen Agr. J. 43(1):191-206.

Rameshwar, S., L. C. Chaudhary, D. N. Kamra and N. N. Pathak. 1998. Effect of dietary supplementation with yeast cell suspension (Saccharomyces cerevisiae) on nutrient utilization and growth response in crossbred calves. Asian-Aus. J. Anim. Sci. 11:268-271.

O-37-4 THE QUALITY AND Ca +2 INTENSITY CHARACTERS OF LOCAL INDONESIAN GOAT SPERM AFTER FREEZING BY NONCONVENTIONAL METHODS OF CRYOPRESERVATION

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Introduction

Artificial insemination (AI) is one way that is being implemented for local goats in Indonesia for the purpose of accelerating the improvement of the genetic quality and number of goat population. AI is done usually using frozen sperms (-196 ^{0}C) of local goat superior sires selected, that are generally conducted with slow freezing (conventional). The purpose of this study was to develop a modified simple method of freezing cells spermatozoa of Indonesia local goat known as Peranakan Etawah/Senduro breed in modified conventional system.

In general, cryopreserved animal semen is less fertile than fresh semen. Cytoplasmic factor in the spermatozoa has been used to activate mammalian M-II oocytes artificially. Ciptadi et al (2012), reported research that was be undertaken to characterize the Ca^{+2} phenotypic variation of intra cellular crude sperm extract (CSE) for improving activation rate of the M-II oocytes to mimicking fertilization. This research was attempted to study the characters of Ca^{+2} intensity of goat sperm after freezing by a modified conventional method. Cormier et al (1997) reported that calcium regulation by sperm from sires of low fertility appears to be deficient because their post-thawing sperms relative intracellular calcium level is higher than it is in bulls of good fertility.

The Cryopreservation is known to disrupt the sperm plasma membrane and it induces premature capacitation of a sperm subpopulation, which may be a result of the increased internal calcium levels after thawing (Collin et al, 2000). It is well established that calcium plays an important role in the capacitation and then fertilization. In this study, we would like to test whether goat fresh sperm have different calcium levels intensity than sperm from freezing (post- thawing) sperm. So the aims of this research were to determine the effect of this modified conventional freezing method (-1^{o}C/minute) both for fresh and freezing sperms with different commercial diluents on post- thawing sperm quality and also to study their pattern of Ca^{+2} intensity.

Material and Methods

Male animal was used in this study were male goats aged 4 years of PE and Senduro and has been trained for the storage of semen by artificial vagina with a standard method. Semen prior to freezing for research has previously been quality tested by Indonesian National Standart (SNI) with a minimum of 70% of individual motility. Each ejaculate was cryopreserved in commercial diluter according to standard procedures. For each experiment, 0.25 ml straws (40x10 ^{6} sperm/straw) were thawed in a 37^{o}C water bath for 60 seconds. Freezing is done with conventional modified methods with the speed (-1^{o}C/min) with the help of Mr. Frosty system ® and then immersed in liquid nitrogen (LN_2) at a temperature of - 196^{o}C, to do quality testing to post-thawing.

Method use is experimental design with commercial diluter (v/v). Freezing semen was cryopreserved in 2 main final temperatures of -45^{o}C and then -196 ^{o}C using Mr. Frosty (®) System. Early Sperm characters of Ca^{+2} Intensity was performed by Confocal Laser Scanning Microscope (CLSM) through Fluo-3 staining and analysis descriptively. At least 5 sperm each were selected to analyzed for each calcium intensity measure both for fresh and freezing sperms. The relative intracellular calcium intensity of each sperm was expressed as the line series of intensity pattern with the help of CLSM images.

Results and Discussion

In general reported that a freeze on modified conventional local goat semen showed good results and meet standard to implementation of Artificial Insemination (AI). Confirmation of the intensity of intra-cellular calcium spermatozoa with CLSM showed that there was no change in the pattern of fresh semen and results of modified conventional freezing, but different in the peak of Ca^{+2} intensity.

The result of sperm test quality in vitro showed that different diluents is influenced on viability, motility and

abnormalities of goat semen. Post-thawing qualities are considered as good as standard qualities, at least, more than 40 % (56.0+5.6 %). The different diluents of different level of cryoprotectant used were influenced highly significant ($P < 0.01$). However freezing sperm conserved in -196 ºC is better than -45 ºC (i.e. motility 39.3+.9.4 % vs. 56.0 + 5.6 %). Meanwhile, the sperm characters of two sperm conditions showed the important variation of Ca^{+2} intensity, with the length of region measurement of 39.06 + 4.595 *um* and 32.696 + 9.011 *um* each (Figure 1). Collin et al (2000), reported test result that sperm intracellular calcium level is correlated with in vivo fertility, with assess to intracellular calcium levels in frozen-thawed sperm from bulls of varying degrees of fertility. The ultra structural changes affect the ability of cells to control calcium flux across their plasma membranes.

Base on this research the calcium intensity pattern is varied more and higher in fresh sperm than freezing sperm (Figure 1). This result is in contrary with Bailey and Buhr (1994) that demonstrated that calcium levels are higher in cryopreserved sperm than they are in fresh semen.

An interesting report demonstrated that a sperm subpopulation is capacitated as a result of cryo-preservation even prior to artificial insemination (Cormier et al, 1997). Clearly, regulation of calcium plays an important during capacitation and the acrosome reaction. On the basis that calcium regulation plays an important role during capacitation and acrosome reaction, both of which are necessary for successful fertilization, it is reasonable to speculate that intracellular sperm calcium levels are associated with semen fertility

Conclusions and recommendations

The calcium intensity pattern is varied more and higher in fresh sperm than freezing sperm. The results of this study indicate that the local goat sperm frozen non conventional Indonesia could be used for the application of artificial insemination. However, this method needs to be enhanced by using diluter and cryoprotectant and more appropriate. It was necessary to study the relationship between the relative intracellular calcium intensity both fresh and freezing semen with their fertility in vitro.

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KEYWORD : Sperm, Local Goat, Viability, Calsium Intensity, AI

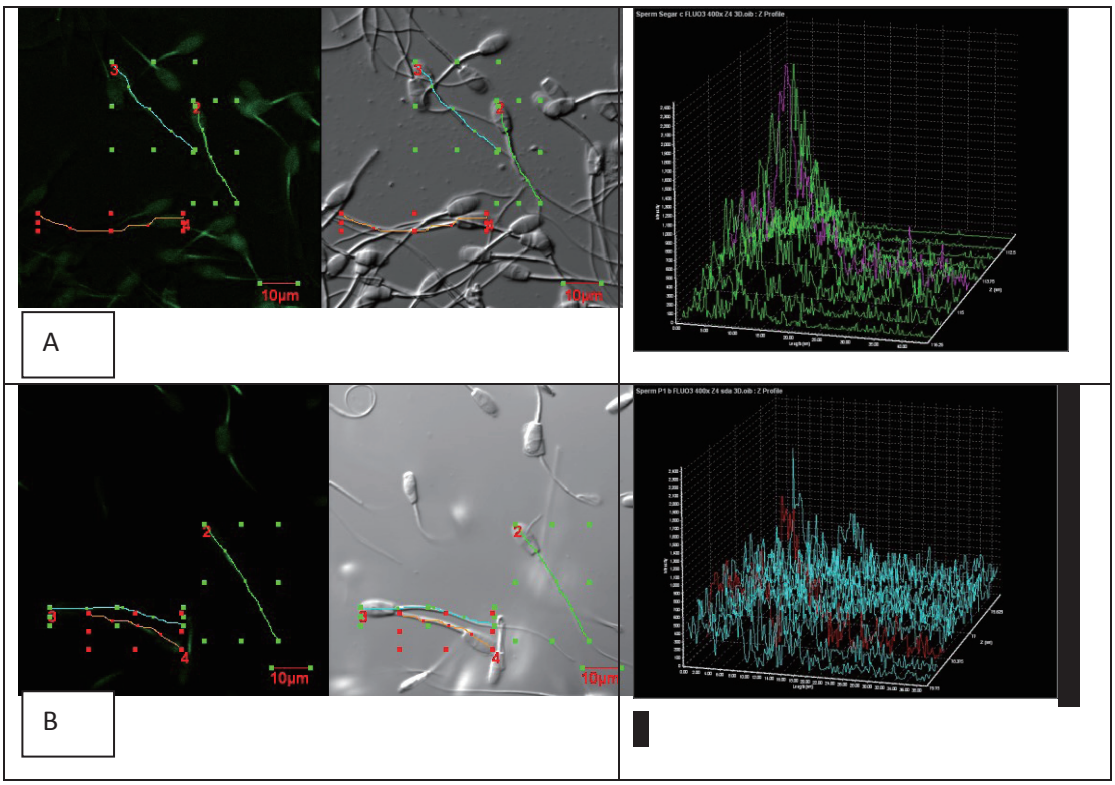

Figure 1. Different profile of Calcium intensity of local goat sperm : (a).Fresh sperm and (b). Freezing sperms (post- thawing) of local Indonesian Goat. It was performed by Confocal Laser Scanning Microscope CLSM : 400, Z.4 , 3 D.

REFERENCES

Bailey JL, Buhr MM. Cryopreservation alters the Ca2 flux of bovine spermatozoa. Can J Anim Sci.1994;74:45–51.

Ciptadi, G. S. Rahayu and B.Siswanto, 2012. The phenotype character of Ca+2 Intensity M-II OOcyte activated by Chemical and Crude Sperm Extract Stimulation. Paper presented in WASET Seminar International. Paris. France.

Cormier N, Sirard MA, Bailey JL. 1997. Premature capacitation of bovine sper-matozoa is initiated by cryopreservation.J Androl.1997;18:461–468.

Collin, S. , M>A. Sirard, M. Dufour and J.L, Bailey.2000. Sperm Calcium Levels and Chlortetracycline Fluorescence Patterns are Related to the In Vivo Fertility of Cryopreserved Bovine Semen .Journal of Andrology, Vol. 21, No. 6, November/December 2000. Copyright American Society of Andrology. J Androl 2000;21:938–943.

O-37-5 EFFECT OF DIFFERENT CRYOPROTECTANTS ON THE VIABILITY OF CRYOPRESERVED BOER GOAT SPERM CELLS IN THE PHILIPPINES

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INTRODUCTION

One of the important factors that determine the success of semen processing is the proper formulation of a medium or semen extender. Semen extender is used to extend the liveability of the spermatozoa once collected out from the body of the male animal. Inside the artificial environment, the spermatozoa should acquire all elements present in its natural place to sustain viability. The extender used in semen processing is composed of a buffer system, salt, sugar, cryoprotectants, which is either non-penetrating (milk or egg yolk), or penetrating (glycerol, ethylene glycol, or dimethyl sulfoxide) and antibiotics. These components interact with each other to create an environment suitable for the spermatozoa during storage until is it introduced to the reproductive tract of the doe.

Cryopreservation is a process whereinthe metabolic activities, either chemical or metabolic of cellsand tissuesis arrested under low temperature. Under this condition, the cell and tissues became susceptible to damage caused bychemical reactions from the time sperm are preserved from cooling to sub-zero temperaturestorage. Cryopreservation is also used to minimize the formation of ice crystal within the cell during freezing. Traditional cryopreservation has relied on its ability to coat the material to be frozen with a class of molecules termed cryoprotectants. Today, there are new methods constantly being investigated due to the inherent toxicity of many cryoprotectants.

In semen processing, a cryoprotectant is added into the extender to minimize the physical and chemical damage that can occur within the sperm during several stages of cooling, freezing and thawing. This process involves balancing many factor to obtain satisfactory results (Purdy, 2005). Knowledge on proper sperm dilution, sperm diluent, cooling and thawing rate and complex understanding on sperm physiology is required to attain maximum post-thaw motility and viability. Anghel et al. (2009) found out that freezing-thawing of spermatozoa is associated with a reduction in cell motility, viability and fertilizing capacity. Steps such as cooling and freezing-thawing carries both physical and chemical stress on the membranes (Beconi et al., 1993) as well as oxidative stress (Bilodeau et al., 2000).

The biophysical changes brought about by the transition of water to ice during cooling are the main cause of damage and not the low temperature present. As ice crystals grow first in the extracellular medium, there is an effective osmotic stress as the solute concentration surrounding the cells is excluded into decreasing solvent volume. On the other hand, there is an increase in the formation of intracellular ice crystals if the osmotic potential inside the cell becomes dislocated from that in the surrounding medium on a kinetic basis. This phenomenon is usually observed when cooling rate is faster than the time requires for water to move down the chemical potential gradient from the more diluted intracellular solution to the more concentrated extracellular medium. Cell's fluid dynamics should be maintained for the cells to be successfully cryopreserved. The most critical temperatures for sperm is during cooling and thawing between 0℃ to -35℃. Studies show that the addition of cryoprotectant (CPA) before freezing and can yield higher post- thaw survival and can also prevent cell aging.

The discovery of glycerol as cryoprotectant marked advancement in semen cryopreservation. Studies on the use of glycerol as CPA with concentration of 7% achieve maximum sperm motility. However, the study also revealed that glycerol can be toxic to the cell and it may induce osmotic damage and hence can lower motility of the spermatozoa (Holt, 2000). Membrane permeable cryoprotectant such as ethylene glycol, propylene glycol and dimethyl sulfoxide (DMSO) were used successfully in cryopreserving mammalian sperm, but there is no reports of their use in goat sperm in single-step addition. It was noted that DMSO makes the cell membrane porous which protects the cell from damage during thawing, while glycerol protects the membrane structure and maintains the nature of internal proteins.

OBJECTIVES

The study was conducted to determine which among cryoprotectant as such glycerol, ethylene glycol, propylene glycol and dimethyl sulfoxide (DMSO) will provide excellent freezing protection in goat sperm as presented by the post-thaw motility of sperm.

MATERIALS AND METHODS

Ten (10) Boer donor bucks of 2 years of age and under uniform production management at Cagayan Valley Small Ruminants Research Center- Isabela State University, Echague, Isabela, Philippines were used in the study from August to September 2015. Semen were collected twice in the morning starting 7 o'clock, and the second collection is done an hour after the first collection using artificial vagina. Semen included for processing should pass the standard quality on volume (0.5-1mL), concentration (3.5-6^9 cells/mL), and motility (75-90%) and without traces of urine or blood. For this study, motility of the spermatozoa was evaluated based on the wave motion characteristics of the sample. Ejaculates with dense, very rapidly moving waves or vigorous movement with motility score of 90 to 70% were further processed. On the other hand, individual motility was also conducted to evaluate the progressive forward movement of the sperm cells. Samples with 60% (ejaculate was observed to have only small, slow moving waves and individual spermatozoa can be observed moving) estimated progressively moving sperm cells were discarded to avoid decreased fertility.

Immediately after collection, the samples were evaluated under microscope. All samples that passed the evaluation will be diluted with semen extender composed of Tris-amino methane (3.028g), citric acid (1.675g), fructose (1.25g), egg yolk (10mL), soy bean lecithin (10g) and antibiotics such as penicillin (100IU) and streptomycin (100,000IU). Cryoprotectant (CPA) was added at the rate of 7mL. These components were diluted in 100mL of distilled water.

To study the effectiveness other CPA on the goat sperm, the following treatments were evaluated using soybean-based semen extender.
T_0- soybean lecithin based with glycerolT_1- soybean lecithin based with ethylene glycol as $CPAT_2$- soybean lecithin based with propylene glycol as $CPAT_3$- soybean lecithin with DMSO as CPA.
The dilution ratio used in the study was 1:6 following the proportion between the collected semen and semen extender. The diluted semen was plunged inside 0.5mL semen straw, and straws were sealed using polyvinyl powder. The processed goat semen were incubated for 4hours inside the refrigerator at 5 ℃ . After cooling, the straws were frozen using conventional, slow-freezing method. At this position, the semen straws filled with semen hang just above the liquid nitrogen gas and slowly being submerged into the gas. The frozen straws were stored for 24-hours, and post-thaw motility was evaluated. Three (3) straws per treatment per day of collection were evaluated. The mean of post thaw motility of the samples were analyzed using Analysis of Variance and treatment means were evaluated using Least Square Difference (LSD) at 1% level of significance.

Hypo-osmotic swelling test (HOST) was used to determine the functional integrity of the sperm membrane. The test is based on the presence of curled or swollen tails. The HOST is done by diluting 7.35g sodium citrate and 13.5g fructose, in 1000mL of distilled water. In doing HOST, 30µL of semen with 300µL of the HOST diluent. It is incubated at 37℃ for 60 minutes. After incubation, a drop of incubated mixture was spread over warm glass slide. A total of 200 spermatozoa were evaluated at 400 magnification. Number of coiled and swollen sperms cells for comparison.

RESULT

Result of the study showed that among treatment, T_0 has the highest post thaw motility at 55%, while the lowest was noted on T_2. Result of the ANOVA shows that treatments are significantly different (p=0.01). Moreover, T_0 was found to be significantly different from T1 and T2, and T3 was further different from T_0, T_1 and T_2.

Table 1. Analysis of variance of the post thaw motility on the treatments studied

Source of Variation	Degree of freedom	Sum of Squares	Mean Square	Computed f	Tabular f
Treatment	3	1545.84	515.28	22.9	1%= 2.38009
Experimental error	20	450	22.5		
TOTAL	23	1995.84			

cv= 11.16%

Table 2. Difference of means among treatment

Treatment	Mean post thaw motility
T_o- soybean lecithin based with glycerol	55^a
T_1- soybean lecithin based with ethylene glycol	38^b
T_2- soybean lecithin based with propylene glycol	33^b
T_3- soybean lecithin based with DMSO	42^{ab}

Letters above the mean post thaw motility are significantly different from the other

Using HOST, sperm stored under glycerol-based semen extender has the least number of observed coiled tails while the highest deformed cells was noted under T_2.

The study shows that glycerol provided the best protection to goat's sperm cells during freezing and post-thawing as presented by the motility. Several studies confirmed that the use of glycerol either for frozen or unfrozen (Davis et al., 1963) sperm gives better post thaw motility, hence this was used as medium worldwide for cryopreservation of bull sperm and sperm from several other species (Iritani, 1980). As noted in laboratory experiment, glycerol can induce osmotic damage to spermatozoa, however goat spermatozoa exhibits tolerance to osmotic conditions and can withstand rapid exposure to it (Purdy, 2005). The study further confirms that addition of glycerol at the rate of 1-8% in single-step at the temperature of 30℃ has resulted to more motile spermatozoa at the rate of 55% as compared to 2-step glycerolization after freezing and post- thawing. However, the result obtained on post-thaw motility in this study is better as compared previous published study conducted by Kunda et al. (2001) wherein post thaw motility is recorded at 13% and sperm cells processed with DMSO attained 21%.

CONCLUSION

Based on the result of the study, it was proven that the process of freezing and thawing can lead to the reduction of sperm cell's motility and viability as observed from the sudden decrease of sperm motility. The addition of a cryoprotectant that works best in the seminal fluid is important to ensure viability of the cells to be introduced into the female reproductive tract. The result further suggests that single-step addition of glycerol at 7% rate using slow-freezing method can lead to higher post-thaw motility for as high as 55% as compared to other commercially available cryoprotectant such as ethylene glycol, propylene glycol and DMSO. Furthermore, based on the protocol described in this study, cost of semen processing can be lowered as small amount of chemicals and other reagents is used for freezing. On the other hand, it is recommended that studies should also be conducted with focus on other factors that affects viability of the sperm cells to include cellular integrity as changes in osmotic pressure may lead to shrinkage or swelling of the cellular membrane.

KEYWORD : goat, semen processing, cryoprotectant, glycerol, sperm cell viability

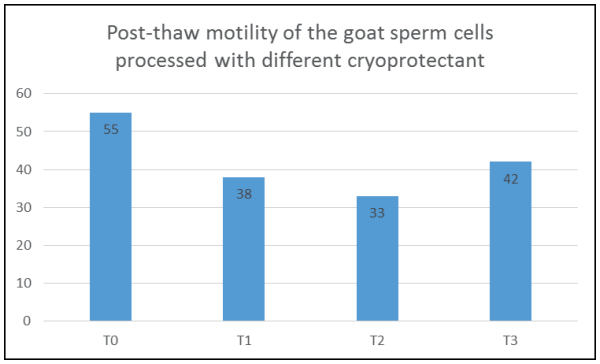

Figure 1. Result of post-thaw motility of the goat sperm cells processed with different cryoprotectant

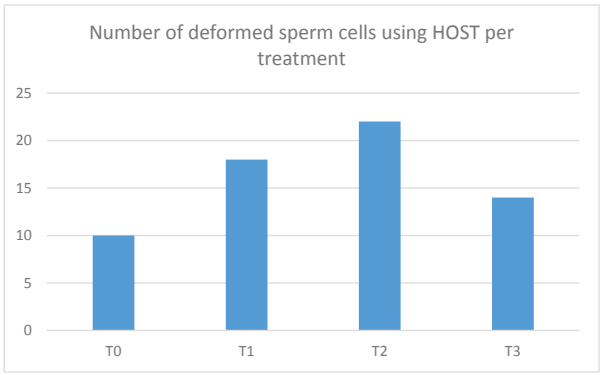

Figure 2. Result of HOST under different semen extender formulation

REFERENCES

Anghel, A., S. Zamfirescu, D. Coprean, and E. Sogorescu, 2009. The effects of cysteine, bovine serum albumin and vitamin E on the calitative parameters of frozen-thawed ram semen. RSCB, Vol. XIV, Issue 2.

Beconi, M.T., C.R. Francia, N.G. Mora, M.A. Affranchino, 1993. Effect of natural antioxidants on frozen bovine semen preservation. Theriogenology, 40:841-851.

Bilodeau, J.F.,S.Chatterjee, M.A.Sirard, and C. Gagnon, 2000. Levels of antioxidant defenses decreased in bovine spermatozoa after a cycle of freezing and thawing. *Mol. Repro. Dev., 55 (3), 282- 288.*

Holt, W.V., 2000. Basic aspect of frozen storage of semen. Anim. Reprod.Sci. 62, 31-32.

Davis, I. S., R. W. Bratton, and R. H. Foote, 1963. Livability of bovine spermatozoa at 5, — 25, and — 85℃ in tris-buffered and citrate- buffered yolk-glycerol extenders. J. Dairy Sci. 46:333–336.

Iritani, A, 1980. Problems of freezing spermatozoa of different species. In: Proc. 9th Int. Congr. Anim. Reprod. Artif. Insemin., Madrid, Spain. 1:115–131.

Kunda, C.N., K. Das, and G.C. Majumdes, 2001. Effect of amino acids on goat cauda epididymal sperm cryopreservation using a chemically defined model system. Cryobiology 41, 21-27.

Salamon, S., and A.J. Ritar, 1982. Deep freezing of Angora semen: effects of diluent composition and method and rate of dilution on survival of spermatozoa. Aust. J. Biol. Sci.35, 293-303.

Purdy, PH (2005). A review on goat sperm cryopreservation. Small Ruminant Research. www.elsevier.com/locate/smallrumres. Available online at www.sciencedirect.com

O-37-6

Major proteins in caprine seminal plasma

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Major proteins in caprine seminal plasma

Introduction

Seminal plasma is the fluid mainly secreted from accessory sex glands. The important of seminal plasma is that it contains the potential proteins which affecting male fertility. Some of seminal plasma proteins bind to ejaculated spermatozoa membrane and make spermatozoa to acquire the fertilization capacity with female gamete. For instance, in bovine, the heparin binding proteins (HBPs) are the other name of bovine seminal plasma proteins (BSP proteins) and largely secreted from the seminal vesicles (Manjunath et al., 1994). These proteins bind to spermatozoa membrane at ejaculation (Manjunath et al., 1994) and participate in the process of fertilization (Therien et al., 1995).

A few studies of seminal plasma proteins of goat have been reported. The isolation and characterization of gelatin-binding proteins from goat seminal plasma indicated that goat seminal plasma proteins (GSP proteins) homolog to BSP proteins which exist in several forms in each species and possibility play a common biological role (Villemure et al., 2003). In addition, Heparin-affinity proteins (HAPs) are under seasonal control and associated with sperm function during breeding and non-breeding seasons (Falci et al., 2002). The bodhesin-2, belong to a new family of spermadhesins, is the interested major seminal plasma protein in goat (Rucker et al., 2010).

Recently, proteomic approaches such 2D-PAGE and MS have been introduced to unveil the molecular basis of several physiological events and provide the information understanding the framework of biology. The underlying mechanism of fertility involved particularly with seminal plasma proteins would be a straightforward approach to improve buck fertility.

Objective

The objective of this study was to investigate the proteins in caprine seminal plasma using 2D-PAGE and MS techniques.

Methodology

Seminal plasma collection

Semen samples were collected from 5 Boer bucks with between 2 to 5 years of age. The first ejaculated semen was used in this study. Seminal plasma was obtained from the semen by centrifuging at 800×g for 5 min. The supernatant seminal plasma was then transferred to 1.5 ml tubes and centrifuged at 10,000×g for 60 min at 4°C. The supernatant of each sample was divided into 1.5 ml aliquots and stored at -70°C for further measuring protein concentration and performing the 2D-PAGE.

Two-dimensional polyacrylamide gel electrophoresis

Seminal plasma samples were assayed for protein concentration (Bradford, 1976) using bovine serum albumin as the standard before performing the 2D-PAGE. The 2D-PAGE was performed according to O'Farrell (1975). Briefly, the isoelectric focusing (IEF) was performed in Isoelectric Focusing System in 13 cm IPG dry strips with a pH range 3-10. Prior to the IEF, the seminal plasma sample was diluted to 200 µg of protein in 250 ml of re-hydration solution (7M urea, 2M thiourea, 4% w/v CHAPS, 2% w/v DTT, 0.5% v/v IPG buffer, 0.002% Bromophenol blue). This mixing solution was loaded to the Immobiline DryStrip Reswelling tray, the IPG dry strip was placed with the gel side down and overlayed with Immobiline DryStrip cover fluid. The IPG dry strip was allowed to re-hydrated overnight (10-20 h).

The focusing was performed on an Isoelectric Focusing Unit using the following 4 steps: (i) step and hold 500 V for 500 Vh (ii) gradient up to 1000 V for 800 Vh (iii) gradient up to 8000 V for 11300 Vh and (iv) step and hold 8000 V for 7400 Vh. The focused was equilibrated in a buffer (6 M urea, 2% w/v SDS, 75 mM Tris-HCl, pH 8.8, 29.3% v/v glycerol, and 0.002% Bromophenol blue) containing 1% w/v DTT for 30 min then changed to

equilibrate in a buffer containing 2.5% w/v Iodoacetate (IAA) for 30 min.
After being equilibrated, the strip was done in a second dimension of 12.5% SDS-polyacrylamide gel. The low molecular weight standard, range 14-97 kDa, was loaded to the gel. The equilibrated IPG gel strip was embedded in a sealing solution (0.5% agarose in 25 mM Tris-base, 192 mM glysine, 0.1% SDS and 0.002% bromophenol blue).
The vertical setup was used using 25 mA/gel. After that the gel was stained in colloidal Coomassie Brilliant Blue G-250 (0.08% Coomassie Brilliant Blue G-250, 8% ammonium sulfate, 0.8% phosphoric acid, 20% methanol) overnight. The gel was de-stained with de-ionized water for at least 24 h or until the background was clear. At least one duplicate gel was performed for each sample. Each gel was scanned with an ImageScanner System and analyzed spots by ImageMasterTM 2D Platinum software.
LC MS/MS
The major expression protein spots, with a large amount of relative protein content were cut from 2D-gel and identified using the LC MS/MS technique. Each spot sample was digested with trypsin enzyme then the digested peptides were analyzed by LC/MS/MS mass spectrometry, (Thermo Electron). The LC separation and MS analysis details were: HPLC system, Finnigan SurveyorTM MS pump with a flow splitter Column, 0.18 × 100 mm C18 (Thermo Electron) particle size 5µm Flow rate, 100µl/min Mobile phase A, water with 0.1% formic acid Mobile phase B, acetonitrile with 0.1% formic acid Gradients, 7-60 %B in 30 min 65-80 %B in 5min and hold 5min, 80-7 %B in 2min Mass Spectrometer, Finnigan LTQ Ionization mode, NanoSpray, positive ion Capillary temperature, 200 C Spray needle voltage, 1.8 KV Mass range, 400-1,600 m/z Scan sequence, Full-scan MS, MS/MS scan Acquisition modes, Normal, Data DependentTM and Dynamic ExclusionTM. The molecular weight values of the trypsinized peptides obtained by LC MS/MS were then used to identify the predicted proteins using MASCOT web-bases search engines.

Results and discussions

After performing the protein separating using 2D-PAGE and stained with colloidal Coomassie Brilliant Blue, more than 213 protein spots with a pI of pH 3-10, and M_r of 10-97 kDa could be detected in seminal plasma of an experimental buck (Figure 1). Of all these spots, there were proteins at molecular weight approximately 14-17 kDa expressed in a large amount on the gel. Identification using MS technique and MASCOT web-bases search engines found that the major seminal plasma proteins matched to PREDICTED: seminal plasma protein PDC 109-like (NCBI accession no. was gi|548504897) and PREDICTED: Spermadhesin Z13-like (NCBI accession no. was gi|548523829), in *Capra hircus*. The predicted means derived from a genomic sequence.
The major seminal plasma proteins of the buck which found in this study were PDC 109 and Spermadhesin Z13. These proteins expressed in a large amount on the 2D gel. For PDC 109, it expressed in 5 different spots of pI and MW. This, however, is the first study to report the presence of PDC 109 in different expression. The expression profile of PDC 109 of the buck was different to the bull (Thepparat et al., 2012).
PDC-109 is the BSP A-1/-A2, belongs to the BSPs proteins family, which has been reported in bovine. The function of PDC-109 involved in the fertilization process. In vitro study showed the functions of PDC-109 as a molecular chaperone, suggesting that it may assist the proper folding of proteins involved in the bovine spermatozoa capacitation pathway (Sankhala and Swamy, 2010). In addition, PDC-109 also plays a role in forming an oviductal spermatozoa reservoir by enabling sperm to bind to oviductal epithelium (Gwathmey et al., 2003 Ignotz et al., 2001).
Spermadhesin Z13 is a seminal plasma protein made up of two disulfide-linked 13 kDa subunits. The function of this protein is thought to play a role in fertilization (Tedeschi et al., 2000). Moura et al. (2007) also proposed that this protein potentially involved in sperm motility, but the mechanism for an inverse relationship with fertility is unclear.

Conclusions

It can conclude from this study that PDC 109 and Spermadhesin Z13 were the major proteins in seminal plasma of the buck. The expression of PDC 109 showed 5 different spots of pI and MW.

Acknowledgements

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KEYWORD : Caprine seminal plasma proteins, 2D-PAGE

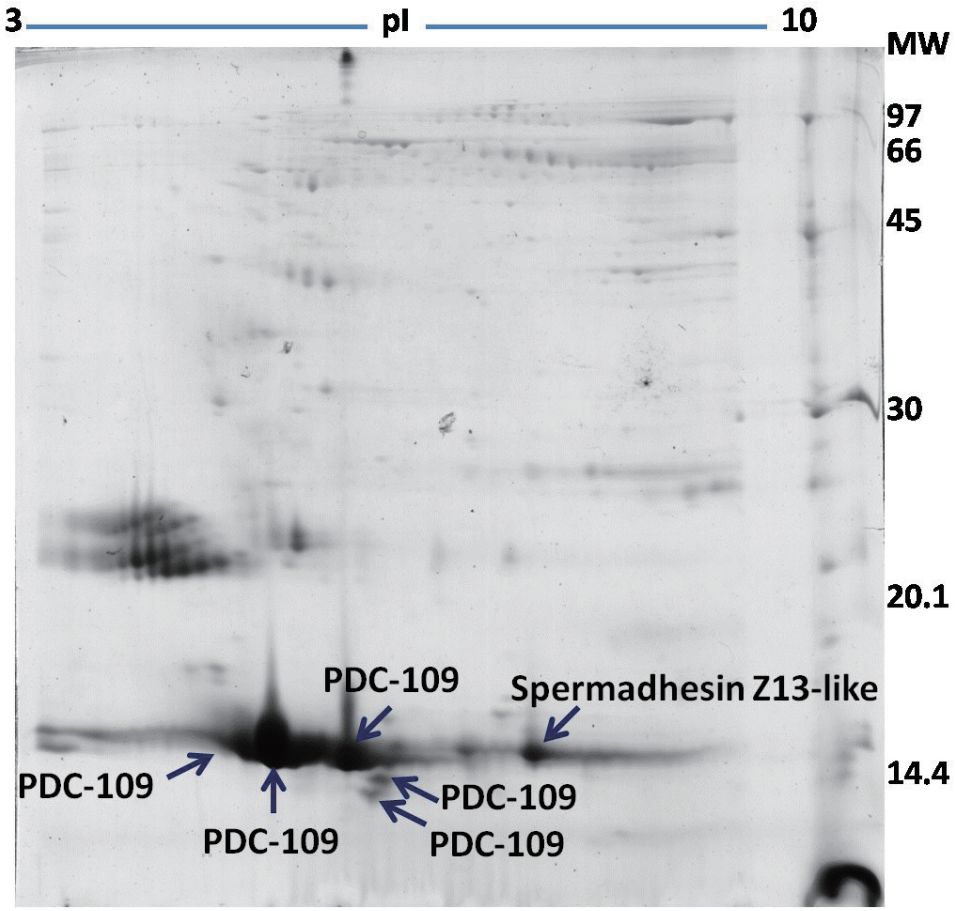

Figure 1 2D-gel of caprine seminal plasma protein. 200 μg of protein was electro-focused on 13 cm DryStrip gel (range, pH 3-10). SDS-PAGE was conducted on a 12.5% acrylamide gel plate. The M_r standard used ranged between 14.4-97 kDa. In this figure, M_r is on the Y-axis and pI (3-10) on the X-axis. Colloidal Comassie Brilliant Blue G-250 was used for the protein staining.

REFERENCES

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Falci, V. S., Tortorella, H., Rodrigues, J. L., and Brandelli, A. 2002. Seasonal variation of goat seminal plasma proteins. Theriogenoloy 57 (3): 1035-1048.

Gwathmey, T. M., G. G. Ignotz, and S. S. Suarez. 2003. PDC-109 (BSP-A1/A2) promotes bull sperm binding to oviductal epithelium in vitro and may be involved in forming the oviductal sperm reservoir. Biol. Reprod. 69: 809-815.

Ignotz, G. G., M. C. Lo, C. L. Perez, T. M. Gwathmey, and S. S. Suarez. 2001. Characterization of a fucose-binding protein from bull sperm and seminal plasma that may be responsible for formation of the oviductal sperm reservoir. Biol. Reprod. 64: 1806-1811.

Manjunath, P., Chandonnet, L., Leblond,E. and Desnoyers, L. 1994. Major proteins of bovine seminal vesicles bind to spermatozoa. Biol. Reprod. 50: 27-37.

Moura, A. A., D. A. Chapman, H. Koc, and G. J. Killian. 2007b. A comprehensive proteomic analysis of the accessory sex gland fluid from mature Holstein bulls. Animal Reproduction Science 98: 169-188.

O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007-4021.

Rucker, D., Baker, K., Tress, U., Louis, N., and Newton, G. R. 2010. Characterization of seminal plasma proteins from dairy and meat goat sire. Biology of Reproduction 83: 532.

Sankhala, R. S., and M. J. Swamy. 2010. The major protein of bovine seminal plasma, PDC-109, is a molecular chaperone. Biochemistry 49: 3908-3918.

Tedeshi, G., E, Oungre, M. Mortarino, A. Negri. G. Maffeo, and S. Ronchi. 2000. Purification and primary structure of a new bovine spermadhesin. European Journal of Biochemistry. 267: 6175-6179.

Therien, I., G. Bleau, and P. Manjunath. 1995. Phosphatidylcholine-binding proteins of bovine seminal plasma modulate capacitation of spermatozoa by heparin. Biology of Reproduction 52: 1372-1379.

Thepparat, T., S. Katawatin, T. Vongpralub, M. Duangjinda, S. Thammasirirak and A. Utha 2555. Separating of bovine seminal plasma proteins by using 2D-PAGE revealed the different profile of PDC-109. KHON KAEN AGR J. 40(2Suppl):387-391.

Villemure, M., Lazure, C., and Manjunath, P. 2003. Isolation and characterization of gelatin-binding proteins from goat seminal plasma. Reproductive Biology and Endocrinology 1:39.

O-38-1

Innovation Technology of Functional Chicken Sausage Formulated Using Microencapsulated Mixture of Selaroides spp) and Clarias sp Fish Oil

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Introduction

Chicken sausage containing animal fat is quite high, however these fats to function as forming the texture and aroma and attractive. Animal fats generally have relatively high in saturated fats and bad for human health, because this type of fat can trigger coronary heart disease. Several studies have been reported showing that five trademark sausage products, namely : the American Hotdog, Continental Frankfurters, Cheerios, Salami Austrian and Devon, proved to contain saturated fatty acids of 36.52 to 37.96 % (Imbeah, 2006). Among the various types of chicken meat available on the market, laying hens culled meat is one of the largest contributors of poultry meat, but chicken meat of this type has several drawbacks, among others, the nature of the meat is tough because of the age old chicken too. Therefore, it takes technology innovation poultry meat processing,among others, made of ground meat that is further processed into sausage. The meat of laying hens culled containing saturated fatty acids are quite high and unsaturated fatty acids omega-3 is very low (Ohtake, 1988). Fatty acids omega 3,6 (PUFA) and 9 (MUFA) long chain could be classified as critical nutrients for human health. These fatty acids were most minimal of all fatty acids in the human diet. Omega 9 has been shown to provide benefits for heart health (Herold and Kinsella, 1988). A study conducted in the U.S. found that giving a diet that has a high content of omega 9 can increase the good cholesterol (HDL-High Density Lipoprotein), lowers bad cholesterol (LDL), and also lowers blood pressure (Hu, *et al*., 2003). That can be ascertained cardiovascular health will be maintained. And in a study also proved that consuming a diet rich in omega 9, 5 times a week can lower the risk of heart attack by more than 15% (Kolanowski, 2005). The oil which is rich in omega-3 largely derived from cold water fish and sea food products (seafood) (Lee, *et al*.,2005). The previous study showed that tongkol (*Euthynnus* spp) fish oils contained 15,98% omega3, 11,62% omega6, while the Catfish (*Clarias* sp) fish oils contained 34.96% omega9. Therefore the aim of this study was to investigate the profile of fatty acids, omega3, 6, 9, cholesterol content and SFA, MUFA, PUFA chicken sausage functional added microencapsulated fish oil mixture tongkol (*Euthynnus* spp) and catfish (*Clarias* sp.) as a substituted chicken fat.

Materials and Methods

Sample preparation

Tongkol fish ((*Euthynnus* spp) and Catfish (*Clarias sp*) were obtained from sea water of Makassar or Tomini Gulf (Parimo Regency), Central Sulawesi Province and will be used as raw material. These fish oils were extracted by wet rendering method (AOAC, 2005). Mixing the oil with a ratio of 1: 1 using shaker for 20 minutes at 50℃, then in microencapsulation using freeze drying methods.

Breast meat and chicken thighs frozen,skin and fat is cut a small pieces, chicken fat substituted with microencapsulated fish oil according to treatment is : A= 0% MFO + 5% chicken fat B= 2,5% MFO + 2,5% chicken fat, and C=5% MFO + 0% chicken fat (MFO = micoencapsulation fish oil), as a prepared ingredients other formulations (salt, water and ice, phosphate, nitrite, red pepper, garlic, starch, protein isolates). The cuts of meat, skin and fat and all materials in milled together until smooth, mix the meat included in cellulose casing and tied both ends. Sausage steamed for approximately 1 hour to an internal temperature of 70℃, then cooled sausage in pieces of ice (Huda, *et al*., 2010).

Laboratory Analysis.

Sausage sample 25 g small pieces in blended, extracted using sokhlet with n-hexane 150 ml, homogenized for 30 seconds to produce a light colour filtrate, filtrate taken on evaporation to separate the solvent thus obtained fish oil with yield. Fish oil sample (0.3 ml) were methylated using 1.5 ml of Na-Metanolic and heated at 65℃ for 15 minutes in waterbath. 1.5 ml of BF_3-Methanol were added to the mixture, then heated at the same condition and the solution was allowed to cool down. The solution was extracted with 0.5 ml of N-Heptane and 1 ml of saturated NaCl, and the top-layer of solution (1µl) was injected to the Gas Chromatography (at the same condition with

standard) as described in AOAC (2005).

Result and Discussion

The fatty acid profile, omega-3,6,9, SFA,MUFA,PUFA and cholesterol content chicken sausage in a variety of treatments (% total fatty acid) on Table 1,

Table 1. Fatty acid profile, omega-3,6,9, SFA,MUFA,PUFA and cholesterol content chicken sausage

No	Fatty Acids	Sausage A (5% fat chicken + 0 % fish oil)			Sausage B (2,5% fat chicken + 2,5 % fish oil)			Sausage C (0% fat chicken + 5 % fish oil)		
		Average	std		Average		std	Average		std
1	C6:0 (Caproic Acid)	0,03	±	0,00	0.00	±	0.00	0,00	±	0,00
2	C12:0 (Lauric Acid))	0,03	±	0,00	0.24	±	0.00	0,16	±	0,00
3	C13:0 (Tridecanoic Acid)	0,61	±	0,01	0.02	±	0.00	0,03	±	0,00
4	C14:1 (Miristoleic Acid)	0,11	±	0,00	0.03	±	0.00	0,02	±	0,00
5	C14:0 (Miristic Acid)	1,68	±	0,00	2.42	±	0.00	2,81	±	0,00
6	C15:0 (Pentadecanoic Acid)	0,12	±	0,13	0.54	±	0.01	0,67	±	0,00
7	C16:1 (Palmitoleic Acid)	0,22	±	0,00	4.61	±	0.01	4,86	±	0,19
8	C16:0 (Palmitic Acid)	22,66	±	4,68	24.97	±	0.10	26,4	±	0,06
9	C17:0 (Heptadecanoic Acid)	0,23	±	1,28	0.73	±	0.03	0,88	±	0,01
10	C18:1 (Asam Oleic)	50,61	±	1,36	49.27	±	0.27	37,2	±	6,13
11	C18:0 (Stearic Acid)	18,37	±	1,21	7.61	±	0.02	7,89	±	0,02
12	C18:2 (Linoleic Acid)	6,88	±	0,00	0.09	±	0.09	0,16	±	0,07
13	C20:0 (Arachidonic Acid)	0,04	±	0,00	1.79	±	0.00	2,15	±	0,07
14	C20:3 (Eicosatrinoic Acid)	0,05	±	0,00	0.09	±	0.00	0,11	±	0,00
15	C18:3 (Linolenic Acid))	0,04	±	0,00	0.82	±	0.01	0,83	±	0,00
16	C22:1 (Euric Acid)	0,02	±	0,01	6.08	±	0.06	8,50	±	0,01
17	C20:4 (Asam Arachidonic Acid))	0		0	0.22	±	0.00	0,26	±	0,00
18	C24:0 (Lignoseric Acid)	0		0	0.04	±	0.03	0,07	±	0,00
19	C20:5 (Eicopentanoic Acid)	0		0	0.26	±	0.05	0,23	±	0,19
20	C24:1 (Tricosanoic Acid)	0		0	0.14	±	0.00	0,01	±	0,91
21	C22:6 (Docosaheksanoic Acid)	0		0	0.00	±	0.00	0.00	±	0.00
	Omega-3	0,29[c]	±	0.00	1,44[b]	±	0,04	1,51[a]	±	0,00
	Omega6	7,11[a]	±	0,01	2,03[c]	±	0,01	2,42[b]	±	0,00
	Omega9	50,85[b]	±	0,00	60,1[a]	±	0,02	50,53[b]	±	0,04
	Cholesterol	0,27[c]	±	0,00	0,08[b]	±	0,00	0,01[a]	±	0,02
	SFA	43,77			38,15			30,95		
	MUFA	50,96			60,1			50,53		
	PUFA	7,4			3,43			3,93		
	PUFA/SFA	0,17			0,08			0,12		
	Omega-6/omega-3	24,5			1,41			1,6		

[a,b,c] = the superscript on different the same line showed a highly significant (P > 0.01). All value were calculated 3 replication, ±std

Table 1 showed the profile of fatty acids, omega3,6,9, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFAs) and cholesterol in chicken sausages results of various treatments. Polyunsaturated fatty acids (PUFAs), sausage A (5% fat chicken+ 0% fish oil) was higher 7.4 % compared sausage B (2.5% fat chicken + 2.5% fish oil) and sausage C (0% fat chicken + 5% fish oil) respectively of 3.43% and 3.93%. On the other hand the percentage of saturated fatty acids (SFA) was very high for sausage A contained 43.77%, 30.95% sausage B and 38. % sausages C, which is dominated by fatty acids palmitic and stearic row for sausage A 22,66 % and 18.37%, sausage B 24.97% and 7.61%, sausage C 26.4% and 37.16%. As for the monounsaturated

fatty acids (MUFA) is almost the same for all treatments, but was a tend to be high on the sausage B 60.13%, 50.94% sausage A and 50.53 % sausage C. The results of the content omega3, 6 and 9 respectively tend to be lower on the sausage A (0.29%, 7.11% and 50.85%), sausage B (1.44%, 2.03%, and 60, 1%) and sausage C (1.51%, 2.42% and 50.53%). The ratio of omega-6 /omega3 was significant decreased from 24.5 (sausage A), 1.41 (sausage B) and 1.6 (sausage C). While the relatively high cholesterol content obtained on the sausage A 0.27%, while the sausage B and C decreased significantly 0.08% and 0.01%. Sausage cholesterol content of the experimental results of research significantly decreased with increasing levels of substituted of fish oil. According Reglero *et al.*, (2009) Stajic *et al.,* (2011) and Josquin *et al*., (2012) cholesterol is a compound that is not good for health because it can increase blood total and LDL cholesterol that can act as triggers of atherosclerosis, heart disease, asthma, and other patients. Therefore, it is important to minimize the cholesterol in food products. Conclusion The results of the research was concluded that chicken sausages substituted a microencapsulated mixture *Euthynnus spp* and *Clarias sp* fish oil was obtained in omega3, omega6, omega9 and cholesterol respectively sausage A 0.29%, 50.85% and 0.27%, and 7,11%, sausage B 1,44%, 2.03%, 60.1% and 0.08%, and sausage C 1.51%, 2.42% 50.53% and 0.01%. The addition of microencapsulated mixture *Euthynnus spp* and *Clarias sp* fish oil up to 5% instead of chicken fat could increased the proportion of unsaturated fatty acids omega3 PUFA and lower percentage of saturated fatty acids (SFA) and cholesterol content of chicken sausage products functional.

KEYWORD : Fish oils mixed, n3,n6,n9,SFA,MUFA,PUFA

REFERENCES

AOAC, 2000. Official Methods of Analysis of The Association of Official Analysis.18th Edn. Washington D.C. Association of Official Analytical Chemists.

Herold and Kinsella. 1988. Fish oil Consumption and Decreased Risk of Cardiovascular Disease: a Comparison of Findings from Animal and Human Feeding Trials. American Journal of Medicine, Waltham 318 (9): 549-557.

Huda, N., Lim, H. W.,Alistair, T. L., Jean and Ishami, I. 2010. Physicochemical Properties of Malaysian Commercial Chicken Sausages. International Journal of Poultry Sciences 9 (10) 954-958.

Hu, F, B., Cho, E., Rexrod, K, M., Albert, C. M., Manson, J. E. 2003. Fish and Long chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic woman, Circulation, 107, 1852-1857.

Imbeah, M. 2006. Fatty Acid composition of five sausages in Australia. Research Paper. Journal For Meat Production and Meat Processing. Fleisch Wirtschaff International : 35-37.

Josquin, N.M., Linssen, J.P.H., and Houben, J.H. 2012. Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil. Meat Science 90, (1), 81–86

Kolanowski, W. 2005. Bioavailability of omega-3 PUFA from foods enriched with fish oil - A mini review. Polish Journal of Food And Nutrition Sciences, 14/55, (4): 335–340

Lee, M.R.F., Tweed, J.K.S., Moloney, A.P., Scollan, N.D. 2005. The effects of fish oil supplementation on rumen metabolism and biohydrogenation of unsaturated fatty acids in beef steers given diets containing sunflower oil. Animal Science, 80 : 361-367.

Ohtake, Y. 1988. Fatty acids composition of lipids of meats and various internal organs from culled laying hen. 1988. Journal of Japanese Society for Food Science Technology (Nippon Shokuhin Kogyo Gakkaishi): 204-209.

Reglero, M. M., Taggart, M. A., Castellanos, P., & Mateo, R. (2009). Reduced sperm quality in relation to oxidative stress in red deer from a lead mining area. Environmental pollution, 157(8), 2209-2215.

Stajic, S., Zivkovic, D., Perunovic, M., Sobajic, S., Vranic, D. 2011. Cholesterol content and atherogenicity of fermented sausages made of pork meat from various breeds. Procedia food sciences, Elsevier 1:568-575

O-38-2

The Physical and Chemical Properties of Gelatin Extracted From Chicken Leg Skin

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ABSTRACT

Gelatin is a denaturized protein that is derived from collagen by acidic or alkaline hydrolysis and is an important functional biopolymer that has a very broad application in many industrial fields. Chicken leg is a by product of cutting chicken limited use because of the high content of skin and bones that rich in collagen. The aim of this research was to determine the effect of interaction between temperature and time of extraction on physical and chemical properties of chicken leg skin gelatin. This study used Completely Randomized Design (CRD) with two factors and three replications of treatment. The first factor was extraction temperature (A1 = 50⁰C, A2 = 55^{0}C, A3 = 60^{0}C) and the second factor was extraction time (B1 = 3 hours, B2 = 6 hours, B3 = 9 hours). Data were analyzed by ANOVA and continued with Multiple Range Duncan Test. The results showed that the interaction between extraction temperature and time of extraction had significant effect ($P<0.05$) on gel strength and protein content but had no significant effect ($P>0.05$) on the yields, viscosity and pH value of chicken leg skin gelatin. It was concluded that the chicken leg skin gelatin produced from extraction temperature 55^{0}C and 6 hours extraction time had the best physical and chemical properties.

INTRODUCTION

Gelatin is a protein of animal origin, that can be obtained from collagen by acidic or alkaline hydrolysis. Gelatin production required a curing step to improve quality of (Sompie et al., 2015). Gelatin is a denaturalized protein that is derived from collagen and is an important functional biopolymer that has a very broad application in many industrial fields. Its functional properties depend on processing conditions as well as the raw material (Sobral and Habitante, 2001). Curing materials from the group of acids have been widely applied in gelatin production. Effect of Acetic Acid concentration and curing time to produce gelain from native chicken legs skin was limited information. The quality of gelatin depends on its physichochemical properties, rheological properties and manufacturing method. Gelatin has been applied within the food, pharmaceutical, medical, cosmetic and photographic industries because of its unique functional (Karim and Bath, 2008). Most gelatins are currently made from beef bone and hide, different species of fish (Gomez-Estaca et al., 2009). Scientists has been doing many research of gelatin from pigskin (Sompie et al., 2012), from goat skin (Said et al.,2011), from native chicken legs skin (Sompie et al., 2015) and gelatin from tunafish skin (Gudmundsson, 2002). Gelatin production required a curing step to improve quality of gelatin. Curing materials from the group of acids have been widely applied in gelatin production. Gelatin quality is influenced by the stages of the process of making gelatin such as swelling (swelling), extraction, precipitation (filtering extracted) and drying. Swelling typically using acid, alkaline or acidic and alkaline. The type and concentration of the acid solution affects the properties of the resulting gelatin. Kolodziejska et al. (2004) stated that the chemicals used prior to treatment and the extraction conditions (temperature and time) may affect the long-chain polypeptides and functional properties of gelatin. Effect of temperature and time extraction to produce gelatin from chicken legs skin was limited information. Thus, this research was conducted to study the effect of combination between different level of temperature and time extraction on the physical and chemical properties of chicken leg skin gelatin.

MATERIALS AND METHODS

Materials. Four thousand g chicken leg skin were used as a raw material, acetic acid, and distilled water.

Preparation of gelatins. Gelatine was prepared by the acid extraction method (Sompie et al., 20015). Acetic acid (CH_3COOH 0.5M) concentrations of 3%. The raw material were soaked in acetic acid solution at 24 hours. After soaked, samples were neutralized to pH 6, weighed and extracted. The extraction process were performed (temperature at 50^{0}C, 55^{0}C and 60^{0}C were used as a treatments. Solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at 70^{0}C for 5 hours and it was stored in the refrigerator 5-10^{0}C for 30 minutes, then dried at 60^{0}C for 24-36 hours until the gelatin

sheet solid. Gelatin sheets were milled and packaged in vacuum plastic and stored in a desiccator for subsequent process.

Experimental Design and Data Analysis. The experiment were determined by analysis of Completely Randomized Design (Steel and Torrie, 1991) with two factors and threereplicates of treatments. The first factor was extraction temperature consisting of 3 levels (50^{0}C, 55^{0}C and 60 ^{0}C). The second factor was extraction time consisting of 3 levels (3, 6 and 9 hours). The significant differences of the average were determined using Duncan's new multiple range test.

Parameters. The characteristics parameters of this research were yield, gel strength, viscocity, protein content, and pH value gelatin. The yield obtained from dry weight ratio of raw material and the weight of the extracted pigskin multiplied by 100% (AOAC, 2005). Gel strength was determined with a Universal Testing Machine (Zwick/ Z.0,5). Gelatin solution 6,67% w/v (6,67 grams to 100 ml distilled water) was heated at 60^{0}C to dissolve the particles. Solution in the container Ø5 cm and height 6 cm was stored at 5^{0}C for 16-18 hours. Gelatin was placed at the bootom of the plunger (Ø=13mm). Measurement was conducted at the temperature of 10^{0}C and the speed 10 mm/min as deep as 4 mm was used as plunger. The value of gel strength (g Bloom) use the formula = 20 + 2,86 x 10^{-3}D, where D = F/G x 980 F = height chart before fracture G = constant (0,07) (Said *et al*., 2011). Viscocity was measured by gelatin powder dissolved in distilled water at a temperature of 40℃ with a solution concentration of 6.67%. The values was measured by Stromer Viscosimeter Behlin CSR-10, It was obtained by expressed in centipoise according to the method Gomez (Guillen *et al.,* 2002). FOSS Kjeltec 2200 was used to determine protein content. A total of 0,5 g of sample + ¼ bussino tablet + 12 ml H_2SO_4 was concentrated in the destruction of the tube FOSS at ± 410^{0}C for 1 hour. The results of destruction was distilled with thio-NaOH 40% + H_3BO_4 4% + BCGMR indicators. A total of 150 ml was destilated in Erlenmeyer disk and titrated with 0,099 N HCl until the color changed from blue to pink. Five point fifty five was used as the conversion factor of gelatin protein. The protein content (%) was calculated using the formula (ml HCL - ml Blanko) x N HCL x 14,0008 x 100 x 5,55)/g sample x 1000 (AOAC, 2005).

RESULTS AND DISCUSSION

Yield

Yield is amount of dry gelatin produced from a number of raw materials with extraction process (Said, 2011 and Sompie et al., 2015). The average gel yield of chicken legs skin gelatin was displayed in Table 1. Statistical analysis showed that the interaction between extraction temperature and extraction time had no significant (P> 0.05). Duncan test resulted that the yield of gelatin tended to rise with increasing the level of extraction temperature. Chamidah and Elita (2002) and Sompie et al. (2015) reported that acetic acid solution used to hydrolyze collagen making it easier solubility in hot water when the extraction of gelatin. The collagen structure is open due to several bond in protein molecules apart. Several author have reported different gelatin yield, from the fish skin was 16 % (Binsi et al., 2009), the skin of goat was 6,32% (Said et al, 2011), the skin of native chicken was 13.01 to 14.42 % (Sompie et al., 2015). The yield of these results were 13.22 to 15..62 % and it was included in the range of Indonesian National Standard of gelatin.

Gel strength

Gel strength of gelatin is very important on physical properties of gelatin. The average gel strength of chicken legs skin gelatin was displayed in Table 1. Statistical analysis indicated that the interaction between the level of extraction temperature and time temperature had significant effect (P<0.05) while the level extraction time had no significant effect (P>0,05) on gelatin. Arnesen and Gildberg (2002) reported that a high content of hydroxyproline caused the gel strength increased. The presence of hydroxyproline caused the stability of the hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin, it is very important for gel strength. Furthermore Sims et al. (1997) reported that the gel formation of a stable condition that ability of a free chain to form a lot of crosslinking. Gel strength values from pigskin gelatin was ranged 134.77 - 143.12 g Bloom (Sompie et al., 2012), from native chicken leg skin was ranged 64.12 to 67.09 g Bloom (Sompie et al., 2015), and gel strength values from these research was ranged 61.15 to 69.42 g Bloom.

Viscocity

The average viscocity of pigskin gelatin is displayed in Table 1 Statistical analysis indicated that the interaction beetwen level of extraction temperature and extraction time had no significant effect (P>0,05) on chicken legs skin gelatin. The value of viscocity tended to decreased at the extraction temperature increased. This is because the curing material has been breaking the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. This is because the viscosity of gelatin is directly proportional to the gel strength that was not significantly different between treatments (Astawan et al., 2002). Ulfa et al. (2011) reprteded that viscosity is affected by molecular weight and amino acid chain length. Increased concentrations of CH_3COOH in the gelatin production process can reduce the viscosity. This is because the curing material has been breaking the peptide bonds of amino acids into short-chain molecule so that its viscosity decrease. Viscocity values from native chicken leg skin gelatin was ranged 4.27 to 5.70 cP (Sompie et al.. 2015), and from these research was ranged 4.01 to 6.70. It values is included in the ISO range 2.0 to 7.5 cP (Said, 2011).

Protein Content.

Gelatin is the collagen protein (Said et al., 2011 and Sompie et al., 2012)**).** The average protein content of chicken leg skin gelatin was presented in Table 1. Statistical analysis indicated that the interaction of level temperature and time extraction had significant effect (P<0.05) on protein content of chicken leg skin gelatin. Duncan test results showed that protein content of gelatin had a tended to increase with increasing level of extraction temperature and extraction time. According to Swatland (1984), age slaughter affect the content of collagen in the skin, increasing age increased collagen protein. Protein content from native chicken legs skin gelatin ranged 88,10 to 89,92 %. Protein content from these research was ranged 87.53 to 90.43 %. that it was not different with commercial gelatin (Said et al., 2011 and Taufik, 2011).

pH Value

The pH value of gelatin is very important on chemical properties. The average pH value of chicken leg skin gelatin is ranged between 5.03 to 5.60. Statistical analysis indicated that interaction between level of extraction temperature and extraction time.had no significant effect (P>0.05). This is because the raw materials that have been in curing skin with acetic acid before undergoing a process of neutralization and washing before further processing so that the acid molecules that are bound to skin protein amount is very small. Conditions in the range of neutral pH values indicate that the process of neutralizing and washing the raw material before the extraction process is running perfectly so that contamination can be minimized. Therefore, the neutralization process plays an important.

CONCLUSIONS

Chicken leg skin gelatin produced from extraction temperature 55^0C and 6 hours extraction time had the best physical and chemical properties.

KEYWORD : Acetic acid, Chicken leg skin, Extraction temperature, Gelatin

Tabel 1. The Physical and Chemical Properties of Chicken Legs Skin Gelatin

Parameters	Time (hours)	Extraction temperature (^{0}C) + Sd			Average
		50	55	60	
Yields (%)	3	13.22±0.03	14.42±0.22	14.22±0.11	13.95±0.01^b
	6	14.41±0.05	14.52±0.01	15.62±0.10	14.85±0.02^b
	9	15.01±0.07	15.11±0.12	15.21±0.24	15.11±0.03^a
	Average	14.21±0.02^c	14.68±0.05^c	15.01±0.23^d	
Gel Strenght (g/Bloom)	3	64.64±0.22	65.91±0.06	66.06±0.55	65.53±0.21^a
	6	63.26±0.30	66.22±0.04	67.39±0.71	65.62±0.11^a
	9	61.15±0.16	67.09 ±0.91	69.42±0.21	65.88±0.01^a
	Average	63.01±0.15^b	66.41±0.02^c	67.62±0.22^d	
Viscocity (cP)	3	6.70±0.37	5.43±1.04	4.28±0.02	5.47±0.16^b
	6	5.50±0.12	4.40±0.11	4.20±0.10	4.70±0.31^a
	9	4.52±0.16	4.01±1.14	4.07±0.07	4.20±0.01^a
	Average	5.57±5.15^c	4.61±0.79^d	4.18±0.72^d	
Protein Content (%)	3	87.53±0.07	88.01±0.12	89.10±0.06	88.21±0.22^a
	6	88.50±0.10	89.33±0.17	89.60±0.46	89.14±1.02^b
	9	88.82±0.16	90.43±0.02	90.79±0.06	90.01±0.20^c
	Average	88.28±0.62^d	89.32±0.62^e	89.83±0.63^c	
pH Value	3	5.06±0.03	5.18±0.15	5.31±0.02	5.18±0.44^a
	6	5.03±0.10	5.16±0.12	5.23±0.45	5.14±0.33^a
	9	5.23±0.33	5.25±0.26	5.60±0.13	5.36±0.43^a
	Average	5.11±0.22^b	5.19±0.06^b	5.38±0.62^b	

Different letters in the same row and colum indicated the significant differences (P<0,05)

REFERENCES

AOAC, 2005. Official Methods of Analyses Association (18th Ed), Official Analytical Chemists. Association Analytical Chemist, Washington, D.C.

Arnesen, J.A., and A. Gildberg. 2002. Preparation and characterization gelatin from the skin of Harp seal (Phoca groendlandica) J. Bioresource Technology. 82 : 191-194

Astawan, M., P. Hariyadi dan A. Mulyani, 2002. Analisis sifat reologi gelatin dari kulit ikan cucut. Jurnal Teknologi dan Industri Pangan 13 : 38-46.

Binsi, P.K., B.A. Shamasundar., A.O. Dileep., F. Badii and N.K. Howell. 2009. Rheological and functional properties of gelatin from the skin of bigeye snapper (Priacanthus hamrur) fish influence of gelatin on the gel-forming ability of fishmince. Food Hydrocolloids, 23(1):132-145.

Chamidah A, Elita Ch. 2002. Pengaruh pengolahan terhadap kualitas gelatin ikan hiu. Seminar Nasional PATPI. Malang.

Gomez-Estaca, J;Montero, P;Fernandez-Martin, F; Gomez-Guillen MC, 2008. Physico-chemical and film-forming properties of bovine-hide and tuna skin gelatin: A comparative study. J. food Engineering 2008; 90:480-486.

Gugmundsson, M. 2002. Pheological properties of fish gelatin. J. Food Sci. 67(6):2172-2176.

Karim, A.A and R, Bhat. 2008. Fish gelatin : properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids. 23(3) : 563-576.

Gennadios, A., A. H. Brandenburg, C.L. Weller, and R.F. Testin. 1993. Effect of pH on properties of wheat gluten and soy protein isolate flms. J. Agr. Food Chem. 1835–1839.

Ockerman, H. W dan Hansen, C. L. 2000. Animal by product processing and utilization. CRC Press, USA.

Said, M.I., S. Triatmojo., Y. Erwanto dan A. Fudholi. 2011. Gelatin Properties of Goat Skin Produced by Calcium Hydroxide as Curing Material. Media Peternakan. 34(3): 184-189.

Sims, T.A., A.J.Bailey., and D.S. Field. 1997. The chemical basis of molecular weight differences in gelatins. The Imaging Science Journal, 45 : 171-177.

Sobral, P,J,A., and A,M,Q,B, Habitante. 2001, Phase transitions of pigskin gelatin, Food Hydrocolloids, 15: 377-382.

Sompie, M., S. Triatmojo., A. Pertiwiningrum and Y. Pranoto. 2012. The effects of animal age and acetic concentration on pigskin gelatin characteristics. Journal of the Indonesian Tropical Agriculture Animal, 37:3(176-182)

Sompie, M., S.E. Surtijono, and J.H.W. Pontoh. 2015. The effect of acetic acid concentration and curing time on the characteristics of native chicken legs skin gelatin. Proceeding. The 6th Internasional Seminar on Tropical Animal Production.

Steel, R. G. D. and J. H. Torrie. 1991. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. New York..

Swatland, H.J.1984. Structure and development of meat animals. Prentice-Hall Inc, Englewood Cliffs, New Yersey

Taufik.,M. 2011. Kajian potensi kulit kaki ayam broiler sebagai bahan baku gelatin dan aplikasinya dalam edible film antibakteri. Disertation. Universitas Gadjah Mada.

Ulfah, M. 2011. Pengaruh konsentrasi larutan asam aseta dan lama waktu perendaman terhadap sifat-sifat gelatin ceker ayam. J. Agritech. 31(3) : 161-167.

O-38-3 EFFECT OF 2% SODIUM LACTATE ON MEAT QUALITY AND MICROBIOLOGICAL PROPERTIES OF BROILER CHICKEN BREAST MEAT

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INTRODUCTION

The factors affecting on shelf life and keeping quality of meat can result detrimental changes in the physico-chemical, organoletic and microbiological properties. Meat preservation plays an important role in this situation and marination of raw meat is one of preservation method which is done by immersion, injection and vacuum tumbling. Sodium lactate: application on meat processing ensures quality, food safety and health by reducing excess sodium intake and is recommended as a good manufacturing practice of meat processing plant.

OBJECTIVES

To evaluate effect of 2% sodium lactate and dipping time on broiler breast meat qualities (pH, color, marinade uptake, drip loss, cooking loss, cooking yield and sensory characteristics) To evaluate effect of 2% sodium lactate and dipping time on broiler breast meat microbiological properties (Aerobic Plate Count, *Salmonella* and *Staphylococcus* spp.) To evaluate the effective dipping time, while preserving better meat qualities and microbiological properties

METHODOLOGY

Sample preparation

Twenty Cobb 500 broiler chicken (38 days old, 1.9+ 0.1kg) were obtained from a same flock from Nelna Farm Pvt Ltd, Eheliyagoda, Sri Lanka. The animals were manually slaughtered, bled out (3 minutes), scalded (58^0C for 1 minute), and picked in line using commercial defeathering equipment. The carcasses were manually eviscerated and washed. Then skinless, boneless breast meat was obtained from each twenty carcasses for the study.

The breast meat samples were treated with 2% Sodium Lactate solution at two dipping periods as 10 and 5 minutes and were compared with a control (only dipped in 9^0C chill water). Five replicates were used for each level.

Sample treatment

Immediately after 2% Sodium Lactate or chilled water application, samples were separated for pH, color, moisture changes after dipping and cooking, and microbial tests for APC, *Staphylococcus* spp. and *Salmonella* spp. detection from each replicates. All the samples were sealed in autoclaved polypropylene bags separately and stored at -18^0C for 14 days for further analysis.

pH analysis

Ten grams of muscle was homogenized in 50 ml of distilled water and measured by using a pH meter (Smaoui *et al*., 2011).

Color analysis

The CIE (1976) system color profile of lightness (L*), redness (a*) and yellowness (b*) was measured by a Chroma meter (CR-400, Konica Minolta, INC, Japan) at 0 and 7 day of storage.

Weight changes during dipping and cooking

The weight of the individual samples were recorded before dipping (W_1), after dipping (W_2), after 7 days of storage at -18^0C (W_3) and after cooking at 170^0C for 15 minutes at 7 days of storage (W_4). The following calculations were carried out according to Petracci *et al*. (2011).

Marinade uptake % = $[(W_2 - W_1) / W_1] * 100\%$

Drip loss % = $[(W_2 - W_3) / W_2] * 100\%$

Cooking loss % = $[(W_3 - W_4) / W_3] * 100\%$ and

Cooking yield % = $(W_4 / W_1) * 100\%$

Sample preparation for the microbial tests

Twenty five grams of thawed (at 4^0C) meat sample was blended aseptically. The blended meat sample was mixed with 225ml of Buffered Peptone Water (BPW). As soon as possible the initial suspension (10^{-1}dilution) was used

to conduct the microbial test on Aerobic Plate Count (APC) and *Staphylococcus* spp. detection. After incubation at 37^{0}C for 24 hours, the initial suspension was used for *Salmonella* spp. detection and confirmation.

Aerobic Plate Count (APC)

Fifty milliliters of Plate Count Agar (PCA) medium at 45+0.5^{0}C was poured into the petri dish as the pour plate method. After complete solidification, the dishes were inoculated with the sample and incubated at 37^{0}C for 24 hours and the colonies were counted.

Salmonella spp. detection and confirmation

Twenty five grams of meat sample with BPW was pre enriched at 37^{0}C for 24 hours. Then 1ml of diluent was cultured in 10ml Selenite Cysteine (SC) medium and incubated for 24 hours at 37^{0}C. After confirmation of color change of SC, a loopful of diluent was cultured on Xylose Lysine Deoxycholate (XLD) agar using Streak Plate method and incubated at 37^{0}C for 24 hours. Suspected typical colonies of *Salmonella* spp. were used for biochemical confirmation using Triple Sugar Iron (TSI) agar, SIM (Sulfide, Indole, Motility) medium and Urea agar.

Staphylococcus spp. detection and confirmation

One loopful of diluent was streak plated on Baird Parker Agar and were incubated at 37^{0}C for 24 hours. *Staphylococcus* spp. was detected by using available pure culture.

Sensory evaluation

Sensory evaluation was conducted by after confirmation of microbial tests (APC, *Staphylococcus* spp. and *Salmonella* spp.) on seven days storage. Marinated samples were oven dried at 170^{0}C for 15 minutes to reach an internal temperature of 75^{0}C, as measured by an internal temperature probe. All the test samples were cooked same time without mixing. Breast meat samples were kept warm in an oven at 40+5^{0}C until served to panelists within an hour after cooking (Smaoui *et al.*, 2011).

Thirty untrained panelists were participated for sensory evaluation. Each person had to mention preference level of color, odor, flavor, texture and overall acceptability with remarks on given nine point hedonic scale sensory evaluation sheet. Panelists were presented with all four treatments at a time (consisting 1cm cube of breast meat) with randomized labeling.

RESULTS

Results were determined on two treatments (10T: 10 minutes sodium lactate treated sample and 5T: 5 minutes sodium lactate treated sample) and two control samples (10C: sample without 10 minutes sodium lactate treatment and 5C: sample without 5 minutes sodium lactate treatment) of breast meat.

pH

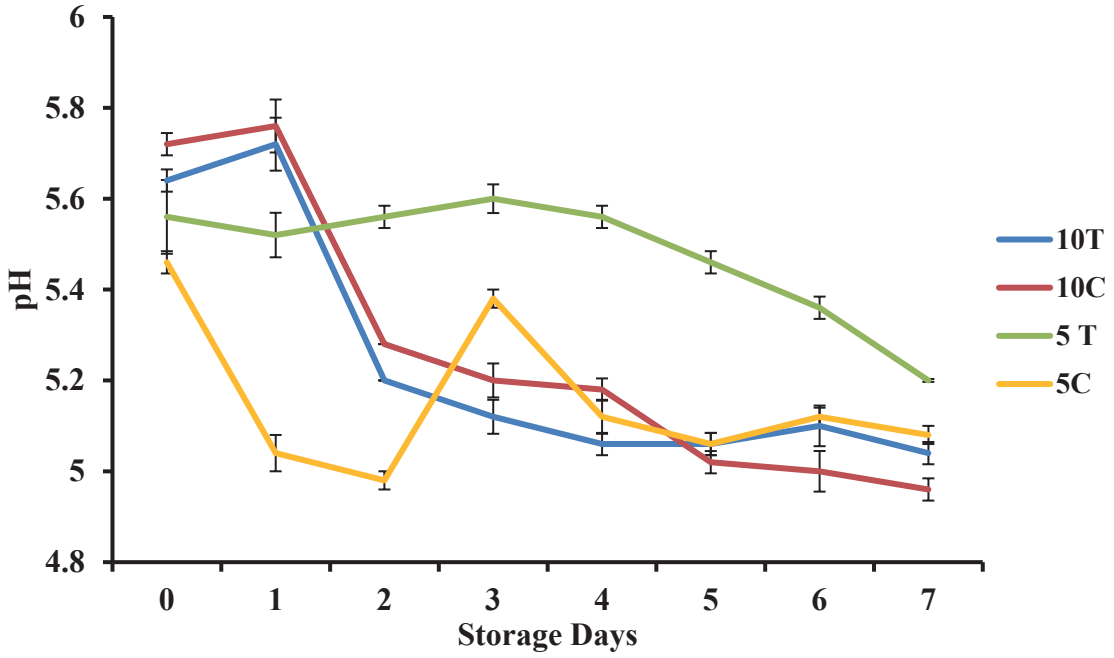

Figure 1: pH value changes of 2% sodium lactate treated and control samples with storage

The pH range is between 4.8 -5.8 for all samples within 7 days storage period (Figure 1). Reduction of pH values after treatment was observed in all samples comparatively to the initial pH value. Because the buffering capacity of the acid system seems to be sufficient to maintain a low pH in the meat (Smoui *et al*., 2011).

Weight changes during Sodium Lactate application and cooking

Marinade uptake percentage

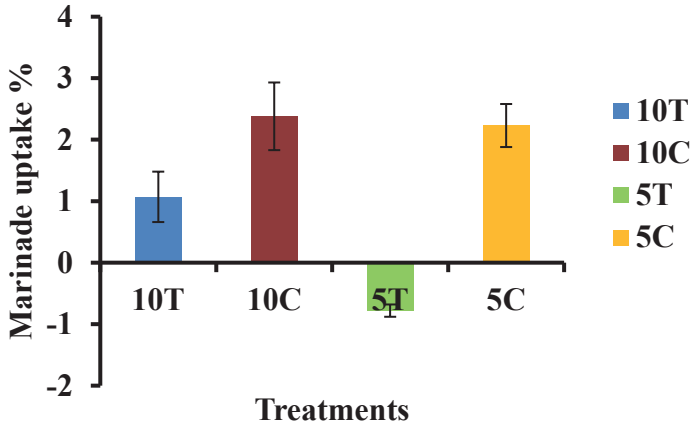

Figure 2: Marinade uptake percentages of samples at different dipping time

The 5 minutes sodium lactate dipped sample showed -0.78 +0.1% marinade uptake (Figure 2). Normally the meat pH is an influencing factor for water binding (Alvarado and Mckee, 2007). The rate of pH declines during rigor development in muscle can influence myofibrillar protein functionality, by altering meat tenderness, color, water holding capacity and meat protein binding ability (Xargayo *et al*., 2001). When rigor development is occurring, the pH drops to 5.6 or 5.7 in normal tissue as result of accumulation of lactic acid (Alvarado and Mckee, 2007).

An efflux of fluid from the myofibrillar space is caused by the decrease in negative electrostatic repulsion between filaments when pH declines during rigor development (Alvarado and Mckee, 2007). When rigor is resolved, the muscle pH approaches the isoelectric point (5.1) for the myofibrillar proteins. Water holding capacity of meat is decreased when actin and myosin are near their isoelectric point in post rigor and the net charges on the protein will be a minimum, as will space between filaments for water to be held or bound (Alvarado and Mckee, 2007). Therefore the above reason might be cause to minus marinade uptake of 5 minutes sodium lactate dipped sample which had 5.5 pH at the time.

Drip loss percentage

The drip loss percentage is high in 5 minutes chilled water dipped sample (15.58+0.45%) and the least drip loss percentage was recorded in 5 minutes sodium lactate dipped sample (7.56+0.84%). There is no significant changes of mean drip loss percentages of 10 minutes sodium lactate dipped sample (10.81+0.98%) and 10 minutes control sample (10.11+0.29 %). Therefore the drip loss percentages of sodium lactate dipped samples are lower than control samples.

The drip loss gradually increases with storage time (Afisc, 2002). According to Alvarado and Mckee (2007) free or loosely bound remaining water is lost by processing procedures such as cutting, grinding and storage.

Cooking loss percentage

The highest mean cooking loss percentage showed in 10 minutes chilled water dipped control sample (37.2+0.99%) and lowest was showed by 5 minutes sodium lactate dipped sample (21.92+0.42%).

The immobilized water can be removed by cooking and accounts for 10-15% in sodium chloride solution (Alvarado and Mckee, 2007). But in here the cooking loss percentage range is around 21-31% in all the samples.

Cooking yield percentage

The highest mean yield percent was obtained by 5 minutes sodium lactate dipped sample (71.62+0.94%) and the least was showed by 10 minutes chilled water dipped meat sample (57.33+0.69%).

According to Williams and Phillips (1998) the cooking yield is 82.45% of sodium lactate treated meat sample with 76.43% cooking yield for the control. So this research also proved cooking yield of sodium lactate treated samples

has higher values than cotrol.

Color

The CIE (1976) color profile consists with three dimensions. They are L*(lightness), a*(redness) and b*(yellowness).

L* value

The lightness of 10 minutes sodium lactate dipped and 10 minutes control samples stored for 7days were higher than the dipping day. But it was negative for 5 minutes treated samples (5T and 5C). All the samples except 5 minutes control (5C) samples were showed significant difference ($p<0.05$) for L* value of color before and after treatment.

a* value

Dipping solution, storage days showed significant difference ($p<0.05$) for a* value except dipping time. Also the interaction effects of dipping solution and dipping time, dipping solution and storage days and, dipping time and storage days are statistically significant ($p<0.05$) for a* value. The redness is increasing with the storage in all the samples except 5 minutes control sample.

b* value

The dipping time, storage days and all interaction effects are significantly affected for b* value except dipping solution ($p<0.05$). The yellowness of all samples were increased with storage time.

Normally ultimate perceived color is affected by many factors such as species, animal genetics and nutritional background, postmortem changes in muscle (especially the dynamics of pH and meat temperature decline), inter- and intramuscular effects, postmortem storage temperatures and time, and a whole host of processing (including antimicrobial interventions), packaging, and display and lighting variables (AMSA, 2012).

According to Allen *et al*. (1997) dark fillets had lower lightness values (L*), higher redness values (a*), lower yellowness values and higher pH values. The fillet color and pH were highly correlated with water holding capacity and percentage of brining pick up and retention. When the meat sample characterized as lighter in color had an initial lower pH, lower brine pick up and higher drip and cook loss compared with meat samples that were characterized as dark (Alvarado and Sams, 2003 Woelfel and Sams, 2001). But at this study the correlation of each meat qualities are not related to previous studies.

Microbiological evaluation

Aerobic Plate Count (APC)

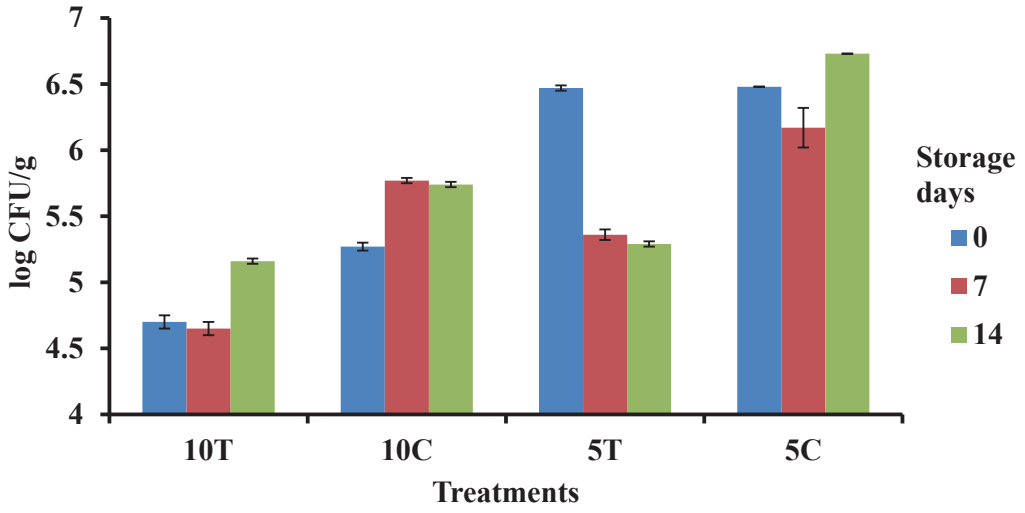

Figure 3: Aerobic Plate Count (APC) of samples with storage

At 7th day of storage, the APC values of 10T, 5T and 5C were lower than dipping day. However, at 14th day of storage the APC values of sodium lactate dipped samples were lower than control.

According to Williams and Phillips (1998) the APC value of the control has gone over 7$\log_{10}$ CFU/g at 7th day of storage from 4.99 $\log_{10}$ CFU/g of first storage day where in this research the APC value range is between 5-6.5 $\log_{10}$ CFU/g at 7 day of storage period. The unacceptable limit of APC of meat samples at 7th day was indicated

as <7log$_{10}$ CFU/g (Williams and Phillips 1998) however, all the samples were below the unacceptable APC limit in this research. The treatments of Williams and Phillips (1998) showed spoilage condition though the APC value range of treatments is between 5-5.5 log$_{10}$ CFU/g even up to 14th day of storage.

Staphylococcus spp. detection

Table 1: *Staphylococcus* spp. detection of breast meat samples

Treatments	Storage days		
	0	7	14
10T	No	No	No
10C	No	Yes	Yes
5T	No	No	Yes
5C	No	No	Yes

There was no *Staphylococcus* spp. detection in all the samples at first day of storage. Except 10C sample, there was no *Staphylococcus* spp. detection up to 7th day of storage.

Houtsma *et al*. (1993) explained that gram-positive bacteria were more sensitive towards lactate than gram-negative bacteria. It was shown especially, that strains that were able to grow at water activities of 0.95 and below in the presence of sodium chloride (*Staphylococcus aureus, Listeria monocytogenes, and Brochothrix thermosphacta*) were inhibited by sodium lactate.

Salmonella spp. detection and confirmation

Salmonella spp. was absent per 25g of all samples from 0 to 14 days of storage. This research is proved that meat samples are safe to consume up to 14 day of storage even using sodium lactate or not. It is clearly mentioned that 3-4% sodium lactate in cooked beef is effective in limiting proliferation of *Salmonella typhimurium* (Bolton, 2002).

Sensory evaluation

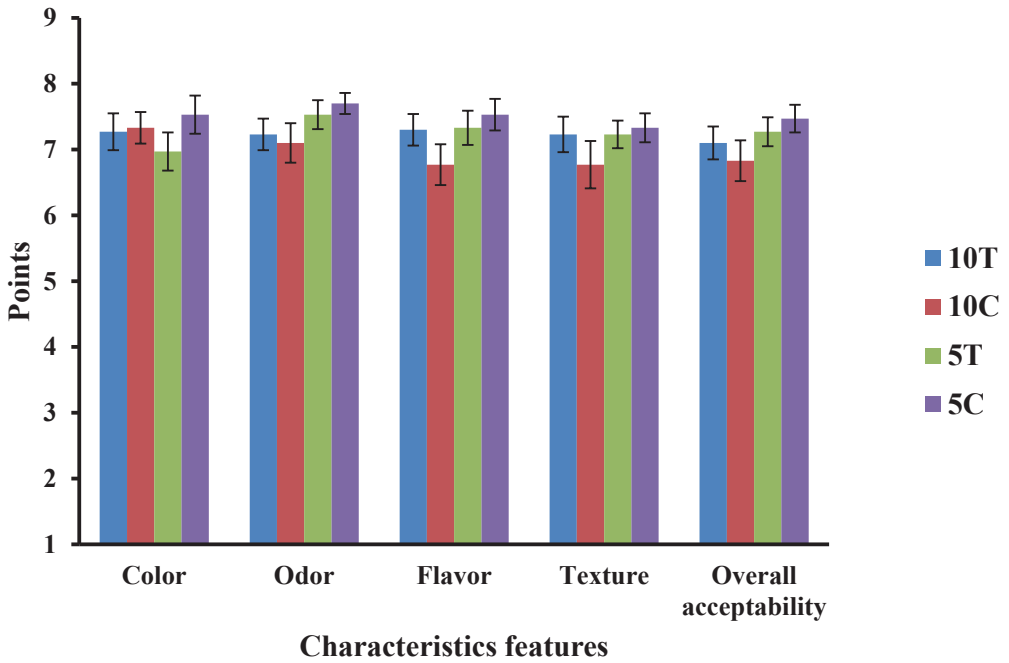

Figure 4: Mean sensory attribute scores for each characteristic features of samples

Smaoui *et al.* (2011) explained that, the scores for odor, texture, flavor and overall acceptability had obtained more scores for 2% sodium lactate treated meat samples than the control where score range is between 5.5-6.5 for the control and 6-7 score range for sodium lactate treated meat sample under 10 point hedonic scale test. Williams and Phillips (1998) was proved that flavor and overall tenderness of 2% sodium lactate treated meat samples are better than the control. But according to this research all the treated and control samples were between 6.5-8 score range.

CONCLUSIONS

This study was performed to evaluate effect of 2% sodium lactate and dipping time on meat quality and microbiological properties of broiler chicken breast meat. It is concluded that 2% sodium lactate affects for pH, increasing marinade uptake, reducing cooking loss, increasing cooking yield, reducing Aerobic Plate Count without interfering sensory characteristics. If 2% sodium lactate 5 minutes dipped meat samples showed minus marinade uptake, it proved the lowest drip loss, the lowest cooking loss and the highest cooking yield with reducing microbial growth. Five minutes dipping time of 2% sodium lactate breast meat sample is effective to preserve meat qualities while 10 minutes dipping time affects to keep better microbiological properties. Therefore, it is worth to conduct further researches on 2% sodium lactate application between five to ten minutes dipping time.

KEYWORD : Dipping Time,, Meat Quality,, Microbiological Properties,, Sensory Characteristics,, Sodium Lactate

REFERENCES

Allen, C. D., Russell, S.M. and Fletcher, D.L. 1997. The Relationship of Broiler Breast Meat Color and pH to Shelf-Life and Odor Development. *Poultry Science*. 76:1042-1046.

Alvarado, C. Z. and Sams, A.R. 2003. Injection marination strategies for remediation of pale, exudative broiler breast meat. *Poultry Science*. 82:1332–1336.

Alvarado, C. and Mckee, S . 2007. Marination to Improve Functional Properties and Safety of Poultry Meat. *The Journal of Applied Poultry Research*. 16:113-120.

American Meat Science Association (AMSA) 2012. Meat Color Measurement Guidelines. 201 West Springfield Avenue, Suite, 1202. Champaign, Illinois. USA.[23.12.2015]http://www.meatscience.org/docs/default-source/publications-resources/Hot-Topics/download-the-ebook-format-pdf-of-the-meat-color-measurement-guidelines.pdf?sfvrsn=0

Australian Food Industry Science Centre (Afisc) 2002. The causes of drip in meat. Meat Technology Update. Australian Meat Processor Corporation [23.12.2015] http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_02-6.pdf

Bolton, D. J., Catarame, T., Byrne, C., Sheridan, J. J., McDowell, D. A. and Blair, I. S. 2002. The ineffectiveness of organic acids, freezing and pulsed electric fields to control Escherichia coli O157:H7 in beef burgers. *Letters in Applied Microbiology* 34:139–143.

Houtsma, P. C., Wit, J. C. D., and Rombouts, F. M. 1993. Minimum inhibitory concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring in meat products. *International Journal of Food Microbiology* 20: 247 — 257.

Petracci, M. and Cavani, C. 2011. Muscle Growth and Poultry Meat Quality Issues. *Nutrients* 4(1) : 1-12.

Smaoui, S., Hlima, H. B., Salah, R. B. and Ghorbel, R. 2011. Effects of sodium lactate and lactic acid on chemical, microbiological and sensory characteristics of marinated chicken. *African Journal of Biotechnology* 10(54) :11317-11326.

Williams, S. K. and Phillips, K. 1998. Sodium Lactate Affects Sensory and Objective Characteristics of Tray-Packed Broiler Chicken Breast Meat. *Poultry Science* 77:765-769.

Woelfel, R. L. and Sams A. R. 2001. Marination performance of pale broiler breast meat. *Poultry Science* 80:1519–1522.

Xargayo, M., Lagares, J., Fernandez, E., Ruiz, D. and Borrell, D. 2001. Fresh meat spray marinating: The influence of spray injection on the quality of marinated products. [21.01.2016] http://www.metal quimia.com/images/doctecnologic/art13.pdf

O-38-4

The Physicochemical Properties of Refined Duck Fat in Taiwan

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INTRODUCTION

After duck was slaughtered, there were lots of skin and fat tissue had been discarded. According to the calculation of the Agricultural Statistics Annals in 2014 in Taiwan, there were more than 5.5 million heads of Pekin ducks had been slaughtered. The dividing and cutting percentage of the heads of Pekin ducks was 35%, and each head could get 0.4 kg fat, which means that there were almost 8 hundred tons fat were been discarded.

The duck fat could be useful and valuable if it was suitable for usages. The researches of animal fats were almost studied in healthy diet or good flavor (Chen and Hwang, 1994), but not in resources re-use. This experiment was conducted to compare the physicochemical properties of duck fat in dry rendering method and in refined method in Taiwan. The acidic value (AV), iodine value (IV), saponification value (SV), peroxide value (POV), the components of fatty acid (FA), and some other physicochemical properties were measured to know if we could take advantage of this discarded material and turn into high value products, and also try to increase the competitive ability in the agricultural industry.

MATERIALS AND METHODS

The skin fat and abdominal fat of duck were bought from the Shin-Sei Frozen Foods in Pingtung, the south part of Taiwan. After removing the impurity and mincing, the duck fat was rendered in the dry rendering method and was refined by the vacuum double layer steam clave with reduced pressure and low temperature. The refining steps were the deacidification with NaOH and the decoloration with activated clay. AV, IV, SV, POV, the components of FA, and some other physicochemical properties were measured including the rendered fat and the refined fat.

RESULTS AND DISCUSSION

The duck fat was rendered in the dry rendering method could get the batter taste, color and flavor (Pereira *et al*., 1977). Rendering and Refining with reduced pressure and low temperature could reduce the duck fat be contacted with oxide, heat and light to avoid oxidation and photo-oxidation. The components of fatty acid in duck fat were showed in Table 1. Unlike other kinds of animal fat, the content of the unsaturated fatty acid (UFA) of duck fat was much higher. There was almost 70% UFA in duck fat, including over 50% mono-unsaturated fatty acid (MUFA) which could reduce the low-density lipoprotein (LDL). The most content of saturated fatty acid (SFA), MUFA and poly-unsaturated fatty acid (PUFA) in duck fat were the palmitic acid, oleic acid, and linoleic acid. Linoleic acid is a kind of essential FA that animals couldn't synthesis by themselves.

The physicochemical properties of duck fat were showed in Table 2. The AV represents the amount of free FA in fat or oil. The higher the AV, the worse of the oil quality (Lee *et al*., 2012). According to Table 2, the refining step could increase the AV of duck fat from 1.1 to 0.4 mg KOH/g. The more amount of the double bonds in oil or fat, the more IV. Decreasing the IV could stabilize the oil property and avoid oxidation. Refining could also decrease the IV from 87 to 78 g I_2/100 g, decrease the unsaponifiables matter (USM), the purity index in oil or fat, from 0.78 to 0.21 g/100 g. Fat with high smoke point property is good for any kinds of cooking style, even though frying. The refining could increase not only the smoke point of duck fat from 202 to 230 degrees Celsius but also the content of MUFA which is more healthful to human. The result shows that the refining method with dry rendering in low pressure and low temperature could promote the quality of duck fat efficiently.

KEYWORD：Duck fat, Rendering, Refining, Fatty acid

Table 1. The components of fatty acid in the rendered and refined duck fat.

		Rendered fat	Refined fat
Lauric Acid	C 12:0	0.04 ± 0.00	-
Myristic Acid	C 14:0	0.60 ± 0.00	0.71 ± 0.02
Myristoleic Acid	C 14:1	0.07 ± 0.01	0.12 ± 0.03
Pentadecanoic Acid	C 15:0	0.07 ± 0.01	0.08 ± 0.02
Palmitic Acid	C 16:0	23.1 ± 0.01	23.5 ± 0.09
Palmitoleic Acid	C 16:1	3.00 ± 0.01	3.21± 0.03
Margaric Acid	C 17:0	0.13 ± 0.00	0.13 ± 0.01
Stearic Acid	C 18:0	5.76 ± 0.01	5.69 ± 0.05
Oleic Acid	C 18:1	47.2 ± 0.01	48.0 ± 0.09
Linoleic Acid	C 18:2	17.9 ± 0.02	16.7 ± 0.11
α-Linolenic Acid	α-C 18:3 (ω-3)	1.11 ± 0.03	1.02 ± 0.03
γ-Linolenic Acid	γ-C 18:3 (ω-6)	0.06 ± 0.00	0.06 ± 0.01
Arachidic Acid	C 20:0	0.11 ± 0.01	0.11 ± 0.01
Gadoleic Acid	C 20:1	0.35 ± 0.01	0.38 ±0.02
Eicosadienoic Acid	C 20:2	0.09 ± 0.00	0.10 ±0.02
cis-8,11,14-Eicosatrienoic Acid	C 20:3 (ω-6)	0.09 ± 0.00	0.09 ± 0.02
Archidonic Acid	C 20:4 (ω-6)	0.12 ± 0.00	0.12 ± 0.00
Eicosapentaenoic Acid (EPA)	C 20:5 (ω-3)	0.03 ± 0.00	-
Behenic Acid	C 22:0	0.03 ± 0.01	-
Erucic Acid	C 22:1	0.04 ± 0.00	0.07 ± 0.01
Docosatetraenoic Acid	C 22:4	0.09 ± 0.01	-
Docosapentaenoic Acid	C 22:5 (ω-3)	0.03 ± 0.00	-
MUFA		**50.7 ± 0.00**	**51.7 ± 0.10**
PUFA		**19.5 ± 0.03**	**18.1 ± 0.11**
FA		**29.8 ± 0.03**	**30.2 ± 0.08**
ω-3 FA		**1.19 ± 0.03**	**1.02 ± 0.03**
ω-6 FA		**0.27 ± 0.00**	**0.28 ± 0.01**

Table 2. The physicochemical properties in the rendered and refined duck fat.

		Rendered fat	Refined fat
AV	mg KOH/g	1.08 ± 0.09	0.36 ± 0.03
IV	g I_2/100g	87.1 ± 2.63	78.2 ± 1.26
POV	meq/kg	3.50 ± 0.04	3.56 ± 0.11
USM	g/100g	0.78 ± 0.24	0.21 ± 0.02
SP	℃	202 ± 3.51	230 ± 2.52

REFERENCES

Chen, C. W. and L. S. Hwang. 1994. Volatile Compounds of Lards from Different Treatments. American Chemical Society (ACS) Symposium Series 558:244-255.

Lee, H. J., M. R. Lee and H. Y. Wu. 2012. Application of poultry fat in cosmetics researches. Taiwan Livestock Res. 45(3): 227-238.

Pereira, A. S., Evans, R. W. and Stadelman, W. J. 1977. Stabilization of chicken fat. Poultry Sci. 56(1): 166-173.

O-38-6

The Effect of Super Red Dragon Fruit (*Hylocereus costaricensis*) Peels on Physico Chemical, Antioxidant y and Microstructure of Chicken Saussage*

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ABSTRACT

The purpouse of this reaseach was to determine SRDF (Super Red Dragon Fruit) peel concentrate added on Physico-chemical quality, antioxidant activity and microstucture by of chicken sausages. The experimental method was designed by completely randomized design (CRD), which consists of 5 treatments and 5 replications. The treatments were T0: Chicken Sausage + 0% of SRDF peels concentrate (control), T1: ChickenSausage + 5% SRDF, T2: Chicken Sausage + 10%, T3: Chicken Sausage + 15%, T4: Chicken Sausage + 20% of SRDF peels concentrate. The variables measured were moisture, fat and protein content, pH value, color measure L*a*b*, texture, microstructure with SEM (Scanning Electron Microscope) and antioxidant activity. Data were analyzed using one-way Anova, if there were a significant effect between the treatments then continued with Duncan's Multiple Range Test. The research result showed the significant effect ($P < 0.01$) of SRDF peels concentrate on protein, pH, texture, antioxidant activity and color L*a*b*, but there was no significant effect ($P > 0.05$) on moisture and fat content. It can be concluded increase the color of sausage from the normal, moisture and fat content value similar with control and different effect for texture.

INTRODUCTION

Research on vegetable and fruit juice attracts more attention due to the nutritional and phytochemical value (Huda et al, 2010). Dragon fruit is part of the Cactaceae family and order of Caryophyllales (Rebecca et al. 2010). Originating in CentralAmerica and Mexico, this plant is now widely grown incountries like Taiwan, Vietnam and Malaysia (Nurliyana et al. 2010), Ones types of dragon fruit are commonly found in Indonesia with pink peel and purple flesh (Hylocereus*costaricensis*) used in this study. It has an appealing deep purple or red coloured flesh, which is interspersed with tiny black seeds. The flesh is juicy with a slightly sweet flavour and the pigments known as betalains (Rebecca et al. 2010 Faridah, 2015).

Super red dragon fruit (SRDF) besides consumed in the fresh form also processed into several processed products (Dodol, Dry Noodle Product, syrup) (Wahyuni dan Nugroho, 2014), while peel which has a weight of 30% - 35% of the weight of the fruit yet unexploited and discarded only as garbage, so it can cause pollution environment (Jamilah et al, 2011). It is rich in polyphenols and a source of antioxidants (Wu, 2005). According to Kanner et al., (2001) anthocyanin can serve to degrading cholesterol in the blood.

Sausage is one of the major concern related to poultry processing. The raw material of sausage product is meat contain unsaturated lipid as pro-oxidant components, are prone to lipid oxidation (Muthia, et al 2010). Lipid oxidation is one of the main factors that determine quality loss and shelf lifereduction of meat and meat products owing to their high fat content. Because of itsnegative effects on organoleptic qualities, it is a major cause of the deterioration of meatand meat products during production, processing, distribution and storage. Oxidationof lipids can also have a marked negative effect on nutritional value and could beresponsible for the production of toxic compounds (Kenawi *et al.*, 2011). Some poultry processors are now looking for alternatives natural antioxidants. The SRDF peel is very worthy to be raw materials processed products, one which is used as chicken sausage additive. The SRDF peel meet the criteria of making sausage because it has color red light as natural dye, contain macronutrients and pectin, be expected encourage the diversification of food processing. Based on this background, it was required to investigate the effect of SRDF peel concentrated on physico-chemical, antioxidant activity and microstructure of chicken saussage. A new approach to maintain the poultry product quality and to satisfy the expectations of healthy conscious consumers is necessary.

MATERIALS AND METHODS

Materials

The SRDF peel concentrate was obtained from the peel of SRDF grinded without solvents. The goal is to get the

pure essence of dragon fruit peel without removing or damaging the active substance content. The SRDF was obtained from traditional market with characteristic has a red peel, and harvesting around 50-55 days from the bud. The SRDF composition was protein 7,41 %, pH 4.95, Antioxidan activity 54,63%. The sausage chicken made of 20% chicken meat , 20% of tapioca flour, 2% of garlic, 3% of onions, 0.25% of pepper, 0.25% of nutmeg, 2% of sugar, 2% of salt, 20% of oil, 5% of egg, 1% of STPP, 20% of ice cubes.

Research Method

The research was experimental method used a completely randomized design (CRD), which consists of 5 treatments and 5 replications, follows:T1 = Chicken Sausage + 0% as controls T2 = Chicken Sausage + 5% T3 = Chicken Sausage + 10% T4 = Chicken Sausage + 15% T5 = Chicken Sausage + 20% of SRDF peels concentrates. Data from this research were analyzed by using one-way Anova (Analysis of Variance). If appear significant effect then continued with Duncan Multiple Range Test. The research variables were moisture, protein, fat, pH, microstructure and atioxidan activity of chiken sausage

Research Variable

The variables observed in this research were the physical quality of chicken sausage included :Moisture content measurement was done by method of AOAC (1995). Texture was observed by method of AOAC (1995) Color test was observed by method of Siregar (2006) Microstructure was observed by SEM. pH measurement. A slurry was prepared by blending the beef product (5g/50ml distilled water). The pH of this slurry was measured by using the glasselectrode method according to the AOAC (AOAC, 1975).

Color Measurement

The surface color of turkey sausages was measured in the package using a Hunter LabScan colorimeter and expressed as color L* (lightness), a* (redness), and b* (yellowness) values. One colorimeter reading was made on each side of the sample.

Antioxidant activity

DPPH radical scavenging assay

Radical scavenging activity in samples was determined according to Beretta *et al*. (2005) Stable DPPH radical reaches the absorbance maximum at 517 nm and its colour is purple. The change of this colour into yellow is a result of pairing of an unpaired electron of a DPPH radical with the hydrogen of the antioxidant, thus generating reduced DPPH-H. Adding an antioxidant results in the decrease of absorbance, which is proportional to the concentration and antioxidant activity of the compound. The absorbance was measured in spectrophotometer at 517 nm. Percentage of the remaining DPPH was calculated from the following equation: DPPH=(*A*sample-*A*blank)/ *A*control x 100 %.

RESULTS AND DISCUSSION

Sousage Quality

The sugar, betalain and dietary fiber content in SRDF peels was expected to improve the quality of chicken sausages in term of the moisture, fat, protein content, texture and color L*a*b*. The SRDF peel concentrated used 0 %, 5%, 10 %, 15%, and 20% , mean value of the quality of chicken sausages such as moisture, fat, protein content and texture Table 1.

Table 1. The average test results of moisture, protein, fat content, texture and pH

Treatment	Moisture	pH value	Protein (%)	Texture	Fat Content	Antioxidan Activity (%)
T0	39.83 ± 4.8	6.29^{a} ± 0.20	$21,61^{a}$ ±0.52	3.95^{b}± 0.19	42.36 ± 0.66	$21,49^{a}$ ± 1.20
T1	39.58 ± 0.5	$6,38^{c}$ ± 0.05	$23,96^{b}$ ±0.54	4.32^{b}± 0.46	40.95 ± 1.1	$20,35^{a}$ ± 2.01
T2	41.38 ± 0.3	$6,34^{b}$± 0.10	$23,81^{b}$± 0.42	3.68^{a}± 0.30	40.53 ± 1.6	$29,19^{b}$ ± 0.60
T3	43.51 ± 2.8	$6,29^{a}$± 0.10	$23,75^{b}$± 0.34	3.76^{a}± 0.77	40.97 ± 0.66	$27,25^{b}$ ± 0.56
T4	45.85 ± 5.7	$6,31^{ab}$ ± 0.06	$23,98^{b}$± 0.13	3.66^{a}± 0.35	40.87 ± 0.68	$31,81^{b}$ ± 0.42

Notes: The different superscript letters (a, b, c, d) in the same coloumn show highly significant ($P<0.01$).

Significant effect (P>0.05) on the moisture of chicken sausages. The lowest moisture was obtained from T0 39.83 ± 4.8 % and the highest value come from T4 45.85 ± 8.7 %. Mean value among the treatments were relative similar, although increased from T0 to T4. Moreover, all of moisture values of sausage were lower when compared to the moisture of chicken sausage with the addition of red pumpkin was 65.78-68.43% (Khotimah, 2013). This might be due to moisture can also be affected by moisture of ingredients used. Higher concenration of SRDF peels concentrate due to increase moisture content in chicken sausage.

Proteins also plays important role in the product. As known, SRDF peels has 9.26% of protein content, while chicken meat has 13% of protein content, due to the protein contain similar. Betasianin a nitrogenous pigment group, it is nitrogen in the ring structure (Mohammer et al, 2005) causing the nitrogen can be measured at the time of testing for protein content by the method Kjeldal rough, resulting in high crude protein content.

pH value

The pH value of the red dragon fruit peel 4.95. The pH value can be concluded that the pigment responsible for the red color of the red dragon fruit is betasianin not anthocyanins (Stintzing et al., 2002) because of anthocyanin stable at pH 1-3 and was almost colorless at pH 4-5 While betasianin has the appearance that is stable at a pH range wider, ie 3-7 (Stintzing, 2002). The average pH values of chicken sausage research results ranged from 6.29 to 6.38. Results of analysis of variance showed that the SRDF peel provides a highly significant difference (P <0.01) the pH value of chicken sausage. The highest pH value obtained in chicken sausage with the addition of 5% SRDF peel concentrated. The addition 20 % SRDF Peel causes the dough lowest pH. Havlikova 'et al. (1983) reported that high temperature shifts the pH optimum for stability betasianin heating .

Texture

The result showed highly significant (P<0.01) on chicken sausage texture. The results were T0 3.92±0.19, T1 4.32 ± 0.46, T2 3.68 ± 0.30, T3 3.76 ± 0.77, and T4 3.66 ± 0.35. T1 as the highest value and T4 as the lowest value. Decrease in the texture value might be caused by the addition of SRDF peels concentrate, cause the reducing of compactness. According to Daniel (2014) that moisture in the peels of fresh dragon fruit was still high and the crude fiber content of approximately 23-26%. This is proven the softest texture with the higher SRDF peel concentrated. The presence of crude fiber in food products increased the compactness, so the tenderness will adequately acceptable (Ambasari, 2009), but in this study the SRDF peel concentrated has high moisture contain. The changes texture are also due to heating temperatures. Gelation involving the starch molecules, proteins, and water occured during the boiling process with temperature of 70ºC, at 40ºC protein myofibril began to coagulate and be denatured at 55ºC perfectly. So, the starch gel in sausage will form a matrix between actin and myosin of meat.

Color L* a* b*

The color of sausage products is one of the parameter which can be evaluated by consumers physicallly. Mean value of the color L*, a*, b* test Table 2. Based on the testing process, color L* (brightness), a* (redness), b* (yellowness) of chicken sausage with the addition of SRDF peels showed highly significant (P<0.01).

According to Daniel (2014) there was 0.45 ± 0.26 ppm natural anthocyanin substances in SRDF peels which was higher than the other natural sources of anthocyanins. The SRDF peel contains active compounds including alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and fitoalbumin (Jaafar et al. 2009).

Data analysis of color L* (brightness) . The treatment had highly significant (P<0.01) on color L* (brightness) of chicken sausage. The color L* resulted in this research was T0 57.82 ± 0.63, T1 50.54 ± 0.44, T2 47.92 ± 0.74, T3 46.42 ± 1.03, and T4 (43.56 ± 1.30). The highest brightness level was obtained from T0 57.82 ± 0.63 and the lowest value from T4 43.56 ± 1.30.

Table 2. The average color of the L * a * b *.

Treatment	Color L* (Brightness)	Color a* (Redness)	Color b* (yellowness)
TO	$57.82^{e} \pm 0.63$	$14.94^{a} \pm 0.18$	$17.42^{d} \pm 0.37$
T1	$50.54^{d} \pm 0.44$	$22.32^{b} \pm 0.72$	$15.12^{c} \pm 0.35$
T2	$47.92^{c} \pm 0.74$	$27.24^{c} \pm 0.81$	$14.94^{b} \pm 0.61$
T3	$46.42^{b} \pm 1.03$	$30.86^{d} \pm 2.49$	$13.14^{a} \pm 1.26$
T4	$43.56^{a} \pm 1.30$	$34.78^{e} \pm 2.36$	$12.96^{a} \pm 0.48$

Notes: The different superscript letters (a, b, c, d) in the same coloumn show highly significant (P<0.01).

Notes: The different superscript letters (a, b, c, d) in the same coloumn show highly significant (P<0.01).

The antioxidant activity

The results showed highly significant difference (P <0.01) of antioxidant activity of chicken sausages produced ranged from 20.35 to 31.81%. Table 1. The greater the percentage of 20% of SRDF was the higher the antioxidant activity. Antioxidant activity is increased when the concentration of plant samples also increased. This is because the SRDF has a higher antioxidant activity than the commercial antioxidants such as BHT and α -tocopherol. As well as the percentage difference between the inhibition of linoleic acid red dragon fruit with BHA 0.24%.Table 3 shows that the antioxidant activity once applied to chicken sausage products is lower than the SRDF. The decrease is due betasianin on chicken sausage products are not stable at high temperatures of 70℃ for 45 minutes. Temperature is the most important factor in the stability of betalain during food processing and storage. Several studies have reported an increased level of betalain degradation resulting from increased temperature (Havlikova et al., 1983).

Microstructure by SEM

The microstructure of the chicken sausage with or without SRDF peel concentrate addition Figure 1.

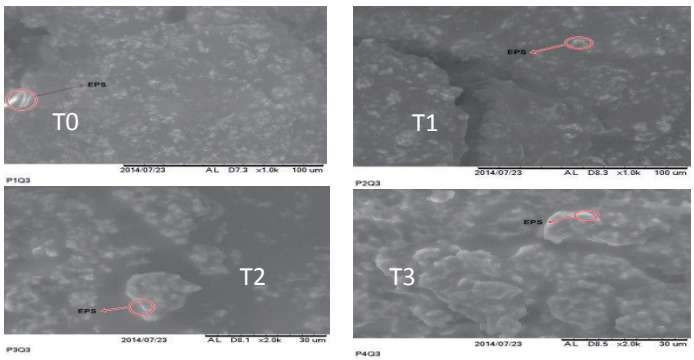

Figure 1. Scanning electron micrograph at 500x magnification of chiken sausage : T0 sample without SRDF peel concentrate, T1 (5%) T2 (10%), T3 (15%) T4 (20%) SRDF peel concentrate

Micrograph demonstrated a difference in chicken sausages of T0 as control and T1-T4 with SRDF peel concentrate added. The T0 has more protein and fat globula fiiling the spaces of the large voids. In these micrograph (Figure 1) show the gelling process, were suspected to be caused by an interruption of myofibrillar gelation. Water holding will be change at 60°C, caused of denaturing the myofibril on the meat so can increase the displacement of water into the extracellular. The high temperature can led to the gel formation by the presence of protein and starch. The gel formation caused a decrease the amount of water bound. The establishment of a gel involving protein.

The fat of T0 chicken sausages was high with 42.36 ± 0.66% showed compact structur. The T4 micrograph show the porus structure . This is proven by 20% addition of SRDF peel concentrate effect due to fill SRDF compound in chicken sausage. In these micrograph apperance total solid such as the fiber.

CONCLUSION

The addition 20% of SRDF peels paste on chicken sausage could decreasing color L* 43.56 ± 1.30, increasing the color a* 33.78 ± 2.36, decreasing color b* 12.96 ± 0.48, decreasing fat content 40.87 ± 0.68, decreasing texture 3.16 ± 0.35, and increasing moisture 44.75 ± 8.7. The advantages from this reseach color red sausage increase to consumen attractiveness and the content of fat and moisture appropriate the satndart T0, disadvantages of the result the texture decrease cause the concentration of SRDF concentrate.

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KEYWORD : Anthocyanin,, Natural Dye, Microstructure

REFERENCES

Ambarsari, I., Sarjana, dan C. Abdul. 2009. Rekomendasi dalam penetapan standar mutu tepung ubi jalar. Jurnal Standarisasi 11 (3): 212-219.

Andersen, O.M. and Bernard, K. 2001. Chemestry, Analysis and Application of Anthocyanin Pigment from Flowers, Fruit and Vegetables. Available at http://www.Uib.no/makere-uib/subproject.htm-18.

Bali, A. S. K. D., Anupam, K., Dipanwita, P., Biswas, S. and Bhattacharyya, D. 2011. A Comparative Study on the Antioxidant and Antimicrobial Properties of Garlic and Coriander onChicken Sausage. International Journal of Meat Science, 1: 108-116.

Budianta, T. D. W., H. Purnama dan Natalia. 2001. Pembuatan Dendeng GilingDaging Kambing yang Diperkaya dengan Buah Nangka Muda (*Artocarpusheterophyllus Lamk*). Buletin Peternakan . Edisi Tambahan: 194-204.

Beretta, G, P. Granata, M. Ferrero, M. Orioli, R.M. Facino, 2005. Standardization of antioxidant properties of sausage by a combination of spectrophotometric/fluorimetric assays and chemometrics. J. Anal. Chim. Acta, 533 (2005) 185–191.

Daniel R. S. 2014. Kajian Kandungan Zat Makanan dan Pigmen Antosisanin Tiga Jenis Kulit Buah Naga Sebagai Bahan Pakan Ternak. Fakultas Peternakan, Universitas Brawijaya. Malang.

Darojat, D. 2010. Manfaat Penambahan Serat Pangan pada Produk DagingOlahan. Majalah Food Review. 5 (7): 52-53.

Faridah, A. R. Holinesti and D. Syukri. 2015.Betalains from Red Pitaya Peel (Hylocereus polyrhizus):Extraction, Spectrophotometric and HPLC-DAD Identification,Bioactivity and Toxicity Screening.Pakistan Journal of Nutrition 14 (12): 976-982, 2015

Havlikovă, L. Mikova, K. and Kyzlink, V. 1983. Heat Stability of Betacyanins. Zeitschrift fűr Lebensmittel-Untersuchung und –Fors- chung, 177: 247-250.

Huda. N, Wei. L.H, Jean. A.T.L. and Ismail. I. 2010c. Physicochemical properties of Malaysian commercial chicken sausage. International Journal of Poultry Science, 9 (10), 954-958. ISSN 1682-8356.

Jaafar F.M, C.P. Osman, N.H. Ismail, dan K. Awang.2007. Analysis of Essensial Oils of Leaves, Stems, Flowers dan Rhizomes of Etlinagen Elatior (JACK) R.M. SMITH. The Malaysian Journal of Analytical Science, 11(1): 269-273.

Jamilah ,B ., Kharidah, M ., Dzulkifly., Noraniza. 2011. Physco-Chemical Characteristic Of Dragon Fruit (*Hylocereus Polyrhizus*) Peel. Journal Reasearch Food International. 18 : 279-286. Departement Of Food Technology. Malaysia.

Jamilah, B., Shu, C. E., Kharidah, M., Dzulkifly, M. A. and Noranizan, A. 2011. Physico-chemical Characteristics of Red Pitaya (*Hylocereus polyrhizus*) Peel. International Food Research Journal, 18: 279-286.

Kanner, J., Harel, S. and Granit, R. 2001. Betalains - a New Class ofDietary Cationized Antioxidants. Journal of Agricultural and Food Chemistry, 49: 5178–5185.

Kenawi, M.A., M. M. A. Zaghlul, R. R. Abdel-Salam. 2011. Effect of Two Natural Antioxidants in Combination With

Edible Packaging on Stability of Lw Beef Product Stored Under Frozen Condition. J. Biol in Anm. Husband 27 (3), p 345-356

Kotimah, Khusnul., Hartatie. 2013. Kualitas Fisik Kimia Sosis Ayam dengan Penggunaan Labu Merah Sebagai Alternatif Pengganti Pewarna dan Antioksidan. Jurnal Ilmu Ternak. Vol. 13, No 1.

Muthia. D , N. Huda , A.M. Easa. 2014. Effect of Garlic (Allium Sativum) on Duck Sausage Quality during Refrigerated Storage. International Journal on Advand science Engoinering Information Technology. Vol.4 No. 3

Nurliyana, R. , S. Zahir, K. M. Suleiman, M. R. Aisyah, K. K. Rahim. 2010. "Antioxidant study of pulps and peels of dragon fruits: a comparative study," *International Food Research Journal* , vol. 375, pp. 367-375

Rahmaniar,A. Rejo, G. Priyanto dan B. Hamzah. 2014. Pemanfaatan Tepung Dari Kulit Secang, Kunyit, dan Kulit Manggis untuk Kompon Karet. Jurnal Dinamika Penelitian Industri 25(1) :71-78.

Rebecca OPS, Boyce AN, Chandran S. 2010. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). Afr. J. Biotechnol 9:1450–1454

Stintzing, F. C., Schieber, A. and Carle, R. 2002. Betacyanins in Fruits From Red-purple Pitaya, *Hylocereus polyrhizus* (Weber) Britton and Rose. Food Chemistry, 77: 101-106.

Wahyuni, R. 2011. Pemanfaatan Kulit Buah Naga Supermerah (*Hylicereus costaricensis*) sebagai Sumber Antioksidan dan Pewarna Alami pada Pembuatan Jelly (Use Super Red Dragon Fruit Skin (*Hylocereus costaricensis*) As A Source Of Antioxidants In Natural Dyes And Jelly Making). Jurnal Teknologi Pangan. Vol. 2 (1): 68-83.

Wahyuni R. dan M. Nugroho. 2014. Addition Effect of Super Red Dragon Fruit Peel Extract on Dry Noodle Product Jurnal Teknologi Pertanian Vol. 15 No. (2) 93-102

Wu. L, Ja-an Annie Ho, Ming-Chen Shieh.2005. Antioxidant and Antiproliferative Activities of Spirulina and Chorella Water Extract. Departement of Applied Chemisrty, National Chi-Nan University, Puli, Taiwan.

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THE EFFECT OF SEASON ON THE BOTANICAL COMPOSITION AND QUALITY OF FEEDSTUFF USED IN BALI CATTLE FATTENING AT SMALLHOLDER FARMS

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ABSTRACT

The research was aimed to determine the effect of season on botanical composition and quality of feedstuffs used at Bali cattle fattening. The research was done during at two season, from January to March for the rainy season and June to August for the dry season. The variables observed include the type and amount of feedstuff used by farmers, nutrient content, and dry matter and organic matter digestibility (DMD and OMD). Nutrient composition and digestibility were determined proximate and *in vitro* analysis. Data collected were analyzed by quantitative descriptiption. The results showed that the dominant type of feedstuff used during rainy season were *L.leucocephala* (56.00%), *Zea mays* fresh straw (18.44%), natural grass (13.00%), *P.purpuroides* (3.68%) and *G.sepium* (1.36%). Conversely, dry season were *L.leucocephala* (33.14%), *G.sepium* (29.69%), *natural grass* (22.25%), *S.grandiflora* (4.53%) and *P.purpuroides* (3.99%). The crude protein (CP) during rainy and dry season of *L.leucocephala* (19,4525,65%), *G.sepium* (21.7524.19%), *S.grandiflora* (24.0921.77%), natural grass (11.136.08%), and *P.purpuroides* 12.4711.98%), respectively. The crude fiber (CF) during rainy and dry season of *L.leucocephala* (15,6618,98%), *G.sepium* (12.3514.51%), *S.grandiflora* (9.8113.18%), natural grass (28.9932.80%), and *P.purpuroides* 25.5831.07%), respectively. The DMD (%) during rainy and dry season of *L.leucocephala* (65.6065.39%), *G.sepium* (67.8774.25%), *S.grandiflora* (87.2782.70%), natural grass (65.6448,91%), and *P.purpuroides* 69.7561.62%), respectively. The OMD (%) during rainy and dry season of *L.leucocephala* (58.6260.21%), *G.sepium* (62.7569.77%), *S.grandiflora* (79.0077.39%), natural grass (63.1547.82%), and *P.purpuroides* 65.2765.35%), respectively. It can be concluded the botanical composition of the feedstuffs for Bali cattle in smallholder farms during the rainy season were more varied than during the dry season. Similarly, the forage feedstuffs quality during the rainy season is better than during the dry season.

INTRODUCTION

Climate factor had become a universal phenomenon that determines the quality and quantity of feed given to livestock farmers in West Timor, East Nusa Tenggara. As the rainy season is relatively short (about 3 - 4 months), the availability of forage is abundant with a high enough quality. On the other hand, in dry season that is relatively long (8-9), the productivity of forage is decrease. Aoetpah (2002) reported, the carrying capacity (CC) during the rainy season in the savanna Timor reached 4.8 UT/ha, crude protein (CP) 10-12%, cell wall/neutral detergent fiber (NDF) 34-48% and dry matter production (DMP) from 2.23 to 3.39 ton/ha. In contrast to the peak of the dry season in October - November, the production and forage quality was at its lowest. Dry matter production (DMP) only reached 0.46 to 0.71 ton/ha, the CC was 0.54 UT/ha, CP of 2.67% and 80% CF. As the result, cattle's performance was higher during the rainy season because there was enough forage available, compared to the dry season when forage was less available.

Inventory of the botanical composition and nutritive value as well as digestibility were an important aspects that need to be done in order to give a picture of the real condition in Bali cattle fattening on the smallholder farms, especially regarding to the feed management in both rainy and the dry season. This study aims to determine the composition of botanical and nutritional value as well as evaluating the dry matter and organic matter digestibility through *in vitro* analysis of feedstuffs used in Bali cattle fattening on the smallholder farms in different seasons.

MATERIALS AND METHODS

The study was divided into two stages. First stage was field observation to determine the botanical composition, particularly the type of feedstuffs material used in the fattening farmers. Observation was conducted on January to March to represent rainy season and July to August to represent the dry season. Second stage was testing the feed quality by using proximate analysis (AOAC, 2005) and *in vitro* analysis (Tilley and Terry, 1963) in Laboratory of Animal Feed Technology, Departement of Animal Feed and Nutrition, Faculty of Animal Science, Universitas

Gadjah Mada, Indonesia.

The selection of farmer groups was a purposive sampling with a condition of those who was conducting cattle fattening. Members of the group at least owned one male Bali cattle being fattened. Prior to feed the cattle, the feed was weighed to determine the amount of the provision on cattle, as well as to identify kinds of feedstuffs. For the laboratory analysis, the feedstuffs samples were taken in sufficiently amount, sun dried, grinded, weighed, then put into oven at a temperature 105 °C, and analyzed the nutrient content and digestibility. The observed variables included botanical composition nutrient content DMD and OMD. The data collected were analyzed by quantitave descriptively.

RESULTS AND DISCUSSION

Botanical Composition

Botanical composition feedstuffs used by farmers in Bali cattle fattening did not vary significantly both in rainy and dry season. Forage feed ingredients used by farmers in the rainy season reach 17 types of feed materials, whereas in the dry season reached 16 kinds of feed ingredients where the percentage of over 1% of the 5 feed stuffs in rainy and dry season (Table 1). *Zea mays* straw fresh in the rainy season was used only limited to the harvest season, especially in March.

The results showed that *Leucaena leucocephala* was potential feedstuffs since it was dominantly used by the farmer, but its availability was slowly declining during the dry season. This was caused by the aspect of availability that was decreasing. According to Mathius (1993), as a source of forage feedstuffs, this plant had not been used optimally and had not been widely commercialized as green feedstuffs. Nullik *et al*. (2004) reported that the plant of performance of *Leucaena leucocephala* hybrid KX2 and *L. leucocephala cv. Tarramba* provided a better view than the other types and had the potential to be developed in the province. Production of hybrid *Leucaena* BK KX2 reached 18.1 tons DM/ha/year and *Leucaena Tarramba* reached 10.9 tons BK/ha/year, while the local *Leucaena leucocephala* production was only 8.1 tons BK/ha/year. The usage of *Gliricidia sepium* as feed during the rainy season was quite low at only 1.36%, but in the dry season it increased until 29.69%. The increased of *Gliricidia sepium* utilization as a feed was associated with the availability of other feedstuffs began to decrease its use as *Leucaena leucocephala*. Utilization of Gliricidia sepium by farmers as feed was still low due to its low palatability value since it took time for the adjustment of the cattle to consume.

Utilization of *Sesbania grandiflora* by farmers as feed was still lacking (4.53%). It was due to the development of legumes by farmers still limited.

The utilization of natural grass during the rainy season was relatively low (13.04%), but in the dry season it increased to 22.25%. *Pennisetum purpuroides* as the superior grass was still limited in use, both in the rainy season (3.68%) and dry season (3.99%). This was related to the cultivation carried out by farmers that was still limited. Hasan (2012) stated that the lack of water will slowly result in slower growth, lack of stalk range, leaves widening, and do not even showing up shoot. The results of this study illustrated that there were fluctuations in the availability of feed to ensure the productivity of livestock throughout the year in West Timor, East Nusa Tenggara. Variations in using feed material was caused by several factors, such as the availability of feed material itself that was influenced by season, farmers' access to sources of feed materials as well as the availability of labors.

Nutrient Composition

The crude protein (CP) of *Gliricidia sepium* and *Leucaena leucocephala* in rainy season was lower than the crude protein content in dry season. *Sesbania glandiflora*, otherwise, had high crude protein content in the rainy season, but during the dry season, it decreased (Table 1). The results of this study illustrated that although the forage legume trees were more resistant to environmental stress of extreme climate, but the difference in dry and rainy season turned out to be sufficient to affect the content of the nutritional value of the forage legume. This research report was not much different from the report of Tahuk *et al.* (2013) who obtained the crude protein and energy content of *Leucaena leucocephala* were respectively 25.00% and 4903.41 Kcal/g, *Sesbania glandiflora* were respectively 27.37% and 4378.260 Kcal/g. Jamal and Semiadi (1997) reported that crude protein content of *Sesbania glandiflora*, *Gliricidia sepium* and *Leucaena leucocephala* on Timor Island in the rainy season, respectively 30.85%, 26.80% and 28 48%. This report was higher than the results of this study, both in the rainy season and the dry season. The content of CF, EE, the third of this legume plants had increased during the dry

season when it was compared with the rainy season. Nevertheless, the NFE content of these three legumes were higher in rainy season than the dry season. If it was viewed from the ash content, then *Leucaena leucocephala* showed higher ash content in the rainy season, otherwise *Gliricidia sepium* and *Sesbania grandiflora* indicated higher the ash content in dry season. Mathius (1993) stated that the difference in the nutrient content of *Leucaena leucocephala* mainly due to differences in varieties, the location of the plant is harvested, harvesting age, soil type, climate and comparison of plants section observed.

The natural grass on rainy season had a quite high nutritional value. The CP was 11.43%, in contrast to the dry season (July to August), a decline in nutritional value was significantly up to 6.08%. The content of CF, EE, NFE, and ash increased in dry season when compared to the rainy season and vice versa. This condition affects the availability of nutrients, particularly protein for cattle that was insufficient to increase its productivity.

Dry Matter and Organic Matter Digestibility

The dry matter digestibility (DMD (%) of *Leucaena leucocephala,* natural grass and *Pennisetum purpuroides* were higher during rainy season. Conversely, *Gliricidia sepium* and *Sesbania grandiflora* were higher during the dry season. The organic matter digestibility (OMD) of *Leucaena leucocephala* and *Gliricidia sepium* was higher during the dry season, while the *Sesbania grandiflora* had higher OMD during the rainy season. The natural grass and *Pennisetum purpuroides* had higher DMD and OMD during the rainy season than dry season This illustrates that the season affected the dry matter and organic matter digestibility of feedstuffs (Table 1).

The natural grass had a highly fluctuating digestibility due to the influence of season. The digestibility was higher during the rainy season, but during the dry season the digestibility decreased dramatically. The *Pennisetum purpuroides* showed quite higher digestibility in the rainy season and the dry season. However, the development of this kind of grass by the farmer was still limited so that the availability was insufficient. Climatic factors became a limiting factor in the development of this superior grass, especially with very limited rainfall and a relatively long dry season.

CONCLUSION

The conclusions of this study are:

The botanical feed composition used by the fattening male Bali Cattle Bali farmers during te rainy season were more varied than during the dry season. The farmers use both legumes and grasses, except *Zea mays* fresh straw that is used only during the rainy season. The nutrients content, dry matter and organic matter digestibility of feedstuffs during the rainy season is the better than during the dry season.

KEYWORD : Botanical composition, digestibility, Bali cattle fattening, smallholder farms, rainy and dry seasons

Table 1. Composition of botanical, nutrients, and *in vitro* digestibility of feedstuffs (%) in male Bali cattle fattening in Small farm, North Central Timor, East Nusa Tenggara in rainy and dry seasons

Kinds of Feed	Season	Botanical Composition	Nutrien Composition							Digestibility	
			DM	OM[2]	CP[2]	EE[2]	CF[2]	Ash[2]	NFE[3]	DM	OM
Leucaena leucocephala	Rainy	56.00	20.88	89.32	19.43	2.56	15.66	10.67	41.75	65.60	58.62
	Dry	33.14	25.45	90.19	25.65	8.41	18.98	9.81	29.52	65.39	60.21
Gliricidia sepium	Rainy	1.36	19.01	88.82	21.75	2.93	12.35	11.19	38.12	67.87	62.75
	Dry	29.69	24.27	87.56	24.19	12.55	14.51	12.44	36.31	74.25	69.77
Sesbania grandiflora	Rainy	0.77	16.75	89.49	24.09	3.31	9.81	10.51	39.62	81.27	79.00
	Dry	4.53	20.33	85.29	21.77	8.90	13.18	14.71	32.94	82.70	77.39
Natural grass	Rainy	13.04	18.09	89.44	11.13	2.44	28.99	14.68	33.28	65.64	63.15
	Dry	22.25	36.54	84.71	6.08	4.11	32.80	15.29	41.72	48.91	47.82
Pennisetum purpuroides	Rainy	3.68	14.20	81.37	12.47	0.71	25.58	18.63	32.47	69.75	61.62
	Dry	3.99	25.24	86.62	11.98	4.68	31.07	13.38	32.37	65.27	63.35
Zea mays fresh straw	Rainy	18,44	-	-	-	-	-	-	-	-	-
	Dry	-	-	-	-	-	-	-	-	-	-
Others feedstuffs	Rainy	6,61	-	-	-	-	-	-	-	-	-
	Dry	6,39	-	-	-	-	-	-	-	-	-

[1]Based on proximate analysis; [2] % of DM; [3]:NFE = 100 – (% CP + % EE + % CF + %ASH); DM=dry matter, OM=organic matter, CP=crude protein, EE=Ether extract, CF= crude fiber, NFE = nitrogen free extact

REFERENCES

AOAC, 2005. Official Method of Analysis of the Association of Official Analitycal Chemists. 12th Ed. Benjamin Franklin Station. Washington. Pp. 129-137.

Aoetpah, A., 2002. *Fluctuations in the availability and nutritional quality of natural pastures on the Timor Island*. Journal of Dryland Agriculture Information. 11: 32-43.

Hasan, S. 2012. *Tropical Forage Feed*. First Edition. Publisher: IPB Press. 89 – 94

Jamal, Y dan G. Semiadi.1997. *Kandungan senyawa alkaloida, tanin serta nilai nutrisi beberapa jenis hijauan yang diberikan pada ternak di Pulau Timor (The Contents of Alkaloid, Tannin and its Nutritional Values from Several Browse Fed to Livestock in Timor Island)*. Biology News, 4 (I) : 9 - 14.

Mathius, I. W. 1993. *Leucaena leucocephala as a Qualified Forage Feed Bank For Goat and Sheep*. Wartazoa. 3 (1) : 24 - 29

Nulik, J., D. kana Hau, P.TH. Fernadez dan S. Ratnawati.2004. *Adaptasi beberapLeucaena species di Pulau Timor dan Sumba, Nusa Tenggara Timur (Adaptation of Several Leucaena species in Timor and Sumba, Nusa Tenggara Timur)*. National Seminar on Livestock and Veterinary. 825 - 831.

Tahuk, P.K., A.A. Dethan dan S. Sio. 2013. Performance of Male Bali Cattle on Feed Fattening Using Single Forage and Concentrate With Combination of Forage and Feed with Different Protein Levels in Timor Island. Reports of Higher Education Competitive Research Grant, The Directorate General of Higher Education.

Tilley, J.A.M. and R.A. Terry, 1963. Two-stag tecnique for the in vitro digestion of forage crops. J.British Grassland Soc. 18: 104.

O-38-9

Participation of local Sumbawanese and Balinese migrant women in cattle production system in Sumbawa eastern Indonesia

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Introduction

It is commonly believe that woman play an important role in livestock sector to support household livelihood. In many places the extent of roles and contribution of women have been proved to make beef cattle development program successful at the farmer level (FAO, 2011, 2012 Sajogyo,1983). It is well known locally that Balinese women involve heavily in beef cattle production system in vice versa to Sumbawanese women. However the information on the degree of involvement of Sumbawanese women in comparison to Balinese women in cattle rearing is limited.

Therefore it is necessary to conduct research about the extent of women contribution in beef cattle activities in both Sumbawa and west Sumbawa districts. Research is needed to find out factors that lead to less involvement of women as working partner in cattle farming activities and then to propose alternative solutions.

Methodology

Research to understand the involvement of women in beef cattle activities was conducted in Sumbawa and West Sumbawa districts between November and December 2015 by using Focus Group Discussions (FGD) approached. The FGDs were carried out in Rhee and Penyaring villages for Sumbawa district and in Senayan village for West Sumbawa district. Twelve participants who owned cattle were selected each site. Key questions were prepared accordingly to get answers which were in line with the objectives of the research. Parameters recorded were participation of women in collecting fodder, feeding, and providing drinking water and housing, cattle mating, vaccination and medication and decision making process. Data were tabulated and analyses descriptively.

Result and Discussion

Source of Household Income

The main sources of income in the three study locations are from cattle farming followed by grain crops such as rice and maize. The crops farming are dominated by rainfed upland thus the activities only occur during wet season and mostly no crops activities following crop harvesting time. In addition to rice and maize, farmers in the area also grow beans such as peanut, mungbean and other beans and tuber crops such as cassaava and sweet potatoes.

Women involve in agricultures to sell farm labors ranging from planting, weeding up to harvesting with an average cost of labor is IDR of 50.000/day. The women also diversify their sources of income from other activities beyond agriculture sector such as selling foods mainly by a Balinese respondent in Rhee village, home industries such as making buffalo skin cracker, milk candy and trading of domestic need such as clothes, tablecloth, linen, blanket and other clothing material.

This indicated that women in the three study sites has multitude role on household activities ranging from taking care family life as a domestic duties and to some extend on economic activities.

Involment of Women in cattle farming In general there are no specification or separation activities between man and women in cattle farming. Women participation in cattle farming involve in various activities such as collecting fodder, feeding, provide drinking water and housing, mating and vaccination.

Collecting fodder

In the Balinese society, women involvement not only in collecting fodder to satisfy the cattle need but also growing and managing forage and when forage supply insufficient they also collecting forage from other places nearby such as from road side, creek side and other unoccupied land. There were no difference between Balinese women and Sumbawanese women in Sumbawa district on cattle farming activties of which women in this area

help their husband in collecting fodder and often travel to a distance of 7-8 Km from their place to collect fodder.

Table 1. Women involvement in cattle farming activities based on ethnic

Type of Activity	Sumbawa District		West Sumbawa District
	Balinese (%)	Sumbawanese (%)	Sumbawanese (%)
a. Provision of fodder	100	41.7	8.3
b. Feeding, drinking and housing	100	16.7	8.3
c. Cattle mating	58.3	0	0
d. Vaccination and medication	0	0	0

However, Sumbawanese women involve in growing fodder is very uncommont for both districts and planting forages is man responsibility by culture although some women that participating in FGD willing to help their husband.

Feeding, providing drinking water and housing

Balinese women commonly involve in feeding, providing drinking water and housing. This is contrast to most Sumbawanese women who rarely involve in feeding, providing drinking water to their cattle. Some reason less involvement of Sumbawanese women is association with the distance of cattle housing and their house. In addition, some man prefer to manage their cattle by man perse. However, either Balinese or Sumbawanese women commonly involve in managing small ruminant such as goat, and poultry.

This practice follow common phenomenon that handling and managing big animal such as buffalo, cattle and horse is dominaed by man. However, there always an exception such as the involvement of Balinese women on cattle farming and this is open possibility of man and women collaboration in raising large animal. This finding is in agreement with Sari et al.,2009, who stated that cattle farming demand more physical work so it more suitable to man although it there possibility of women become a cattle raiser.

Cattle mating

Balinese women are heavily involved in cattle mating, they know exactly the sign of oestrus to indicate mating time and most of them also involve in selecting a good bull to mate the cow. In addition Balinese women also monitore the pregnancy upto labor time. This is extremly contrast to Sumbawanese women, they never involve in any reproduction activities eventhough they understand the signs of a cow that is in oestrus.

Vaccination and

Vaccination is part of goverment responsibility to maintain healthiness and weel being of animal including cattle. Goverment provide vaccination free of charge one a year and other health issues service provide by paramedic and farmer responsible to pay the transport cost of the paramedic. Some farmer prefer to cure the sick animal with traditional remedy this is associated with cost and paramedic often come not on time and first aid to handle sick animal need to be done immediately.

Women rarely involve in vaccination activities and they also have a limited knowledge on cattle health. They keen to improve their knowledge on animal health to increase their involvement in cattle rearing however it is difficult for them to get first hand information as they did not receive regular visit either from veterinarian or paramedic.

Gender issues in cattle farming

Balinese women in Sumbawa were really realised that helping family to have a better life is compulsary. This maybe associated as they are migrant in addition they need sufficient cash for their culture activities and ritual. This is may be one of the factor motivate Balinese woman working very hard. This is in agreement to Sadli (1988) who said that women have various reason and motivation to have activties in public sector, some due to individual decision and other may related to culture environtment where the community exist.

Sumbawanese women on the other hand realise they need to be more active in economic sector to have a better

family life. Cattle farming is one of activties that they can participate to improve their family livelihood. However, most of goverment policies and program in increasing farmer welfare through increased cattle production put heavily on man. This is indicated that goverment should be gender responsive to accelerate farmer welfare and village production.

Women involvement in decision making Decision to sell cattle

Reason to sell cattle for both Balinese and Sumbawanese in Sumbawa district were associated with family need of living, cattle already reach mature age and farmer happy to the price offered by the trader. Decision to sell is based on husband and wife agreement for the Balinese but not for Sumbawanese. Often Sumbawanese women did not know numbers and cattle price have been saled. They only received the cattle sold money to any amount handed by their husband. Balinese women generally manage the money from cattle sale and normally use to buy feeder stock and fodder and others domestic need.

The practice by balinese women in Sumbawa is in agreement to Suratiyah (1983) who mentioned that women in farmer family generally dominan on decision making of money utilisation for daily need as women in charge mainly in domestic duties. However, this is not in agreement to Sumbawanese where man dominate the money allocation, women only received some money for daily need.

Table 2. Women involvement in decision making

Type of decision	Balinese (women:men)	Sumbawanese in Sumbawa District (women:men)	Sumbawanese in West Sumbawa District(women:men)
Decision to sell of cattle	50:50	30:70	10:90
Decision making in Production and Marketing	45:55	25:75	20:80
Management of Income from Cattle	60:40	60:40	10:90

Both Balinese and Sumbawanese place women as additional worker in household without payment as they are family members, not as professional business partner. Further, both ethnic have no earn fund allocated for saving or reserve for health and unexpected events.

Conclusions:

Balinese women involve in cattle husbandry including collecting feed, cattle feeding, housing and have significant role in decision making including cattle purchase, selling and cash arrangement but not for Sumbawanese women thought they are willing to be involved.

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KEYWORD：Women, Cattle production, Culture norms, Decision making

REFERENCES

FAO 2011, ESA Working paper No. 11-02: The role of women in agriculture.

FAO 2012, Invisible Guardians; Women manage livestock diversity. FAO Animal Production and Health, 174.

Sadli, Saparinah, 1988. Perempuan, Dimensi Manusia dalam Proses Perubahan Sosial.Pidato Ilmiah diucapkan pada Dies Natalis UI XXXIX pada tanggal 2 Februai 1998 di Depok.

Sari, AI., S.H. Purnomo., dan E.T. Rahayu. 2009. Sistem pembagian kerja, akses dan kontrol terhadap sumber daya ekonomi dalam keluarga peternak rakyat sapi potong di kabupaten grobogan. Sains Peternakan, 7 (1). pp. 18-26. Universitas Sebelas Maret, Surakarta.

Sajogyo, P. 1983. Partisipasi masyarakat desa dalam pembangunan pertanian khususnys partisipasi wanita dalam proses pembangunan pertanian. Makalah pada seminar IKIP Jakarta.

Suratiyah, 1993 dalam Dina Lesmana, Roles of Women in Decision Taking to Adoption on Farming Technology of Salak Pondoh Nglumut.

O-38-10

Changes in bali cattle production systems in sumbawa, eastern indonesia and the implications on feed supply systems

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Introduction

About 70% of beef in Indonesia is produced domestically. One of the production centers is Sumbawa Island of West Nusa Tenggara province. Sumbawa Island was declared a center for Bali cattle purification for Indonesia in 1976 and a Livestock District in 2005 to strengthen this region as a major producer of beef. However, the development of beef cattle in Sumbawa has faced many constraints due to limited feed resources (Dilaga, 2014).

Traditionally, cattle in Sumbawa have been raised by an extensive system in communal land during the wet season while cropping lands have been used to plant food crops. In the dry season, after crop harvesting, the cropping lands, including rice fields, are open for free grazing of livestock. This system has been a very efficient way of producing beef that enables the region to supply beef to other areas of Indonesia at competitive prices.

Recently, the communal areas have been declining in size and carrying capacity due to overgrazing, which has stimulated invasions by weeds (Sutaryono et al., 2012). Rice fields cannot be utilized optimally for livestock grazing due to crop intensification and diversification. These changes have been stimulating farmers to allocate their private lands for cattle production, especially in the wet season. This has implications on the cattle feed supply and feeding system. This paper discusses factors contributing to the change in the production system and its implication on systems of feed supply and cattle rearing.

Materials and methods

To obtain information on changes due to declining availability of communal grazing area, in-depth interviews were conducted with key farmers in the Empang and Tarano subdistricts, who migrate their cattle to a nearby small island during crop planting season. In-depth interviews were also conducted with key farmers in the Moyo Hulu subdistrict, who experienced changes in the production system due to the development of large dam named Batu Bulan Dam. To identify drivers for the shift into more intensive cattle production, a survey was conducted involving 35 farmers in the Sumbawa and West Sumbawa districts who have been raising cattle by extensive system (free grazing on communal lands), but have recently changed their cattle raising system to a more controlled system by planting leucaena on their own land, or utilizing wild leucaena and other tree legumes.

Results and discussion

Declining availability of communal grazing land

There are 60 sites (27,783 ha) of communal grazing areas in Sumbawa Districts, but only 5 of them have clear legitimation (with government decree) for livestock grazing (Dinas Peternakan Sumbawa, 2012). Many of these areas are under land conflict between crop farmers and cattle farmers. Some have been converted into cropping land, fish ponds and used for transmigration. Sutaryono et al (2012) reported that communal grazing areas in Sumbawa are heavily invaded by weeds especially *Chromoline odorata*, *Lantana camara, Ziziphus jujuba* and *Jatropha sp*. As a result, the carrying capacity has significantly declined.

Based on in-depth interview with key farmers, due to declined availability and carrying capacity of communal grazing area in the Empang and Tarano subdistricts, local farmers migrate their cattle to a nearby island when cropping lands are cultivated (December - May). However, this does not completely solve the problem because this island is now also overgrazed and livestock are susceptible to theft.

Intensification of cropping land

The best example of change in land use in Sumbawa Island is the development of a large dam in Batu Bulan village (Moyo Hulu sub district of Sumbawa). According to key farmers in Batu Bulan, this Batu Bulan dam was

established in 2002, providing irrigation for about 5,000 ha of rice field in the region. As described in Table 1, establishment of this dam has dramatically altered cattle production system in the surrounding area.

Table 1. Change in land use due to establishment of large dam (case study of Moyo Hulu sub district of Sumbawa)

Variable	Before the dam	After the dam
Land use	1 rice season (Dec-May) then fallow (for grazing)	Crops all year round
Type of livestock	Mainly buffalo	Mainly cattle
Buffalo/cattle ownership	Many farmers have more than 50 per household	max 15 heads/household
Buffalo/cattle rearing system	In the forest during wet season (December – May), in the rice field in dry season (May – December)	Cows and calves on private land, small-scale fattening of bulls in pens

Drivers of change in the cattle production system

Based on interviews with farmers who have changed their cattle raising system to a cut- and-carry system, farmers confirm that this change is mainly due to declining availability of grazing areas driven by changes in land use. Other factors that contributed to these changes include improved farmer knowledge on cattle fattening, availability of suitable land to plant forages and increased cattle pricse.

Improved knowledge on cattle fattening system

All respondents interviewed have been involved in an Australian Centre for International Agricultural Research (ACIAR) - funded project on cattle fattening based on tree legumes since 2011, so they have improved knowledge on cattle fattening, especially regarding the value of using leucaena to increase cattle growth rate.

There were 31 of 33 respondents interviewed (94%) stated that they experience lack of feeds during September to December each year. To overcome this feed scarcity, farmers are willing to travel up to 5 km away from home using a motor bike to collect tree legumes (mostly wild leucaena), a practice they never did before.

Table 2. Number of respondents feeding forages as 1st, 2nd and 3rd preference for cattle fattening in Sumbawa and West Sumbawa districts

Forages	1st preference	2nd preference	3rd preference	Total
Leucaena	28	2	2	32
Grass	5	9	10	24
Gliricidia		15	8	23
King grass		1	1	2
Sesbania grandiflora			1	1
Rice straw		1	1	2
Corn stover		1	3	4
Rice bran		1	1	2

Limited availability of wild leucaena stimulated farmers to plant leucaena on their own land. 32 of 33 (97%) respondents interviewed planted leucaena on their own land. 69% of respondents plant leucaena on non-irrigated low land, 28% respondents plant leucaena on irrigated low land and 3% respondents planted leucaena on other lands.

Availability of private lands for planting forages

Table 3 shows that respondents who adopt cattle fattening and plant forages have land that is more suitable for

planting tree legumes. These lands include unirrigated low land and highlands

Table 3. Land ownership and land use of farmers with intensive cattle production system

Type of land	No of respondents	Land area (ha)	Land use
Rice field, irrigated and rainfed	12	1.1	2 x rice, 1 x cash crop
Low land, no irrigation	29	3.2	cash crop (mainly corn) one crop a year, leucaena all year round
Up land	3	1.4	Leucaena all year round

Land ownership of farmers in Sumbawa is much larger than that of farmers in Lombok. Dahlanuddin *et al*. (2016) reported that only 60% of cattle farmers in Central Lombok have access to land, with average land ownership at 0.4 ha per household. Therefore, farmers in Sumbawa have much higher potential to feed a large number of cattle compared with their counterparts in densely populated islands like Lombok.

Increase in cattle price

Trends in cattle prices in Sumbawa and West Sumbawa districts are presented in Table 4 price of cattle (all class) increased in the last 2 years. The biggest increase occurred for mature males and females mainly due to increase in demand from other provinces, especially Kalimantan.

Table 4. Average increase of cattle price in Sumbawa in the last 2 years.

Cattle type	Two years ago (IDR)		2016 (IDR)	
	Male	Female	Male	Female
Calves	2,833,000	2,180,000	3,833,000	3,030,000
Young	4,145,000	3,368,000	5,495,000	4,405,000
Mature	6,163,000	4,943,000	9,117,000	7,566,000

Increase in cattle prices occurred throughout eastern Indonesia due to an increase in beef prices. Waldron et al. (2013) reported that beef prices in eastern Indonesia have been increasing steadily in the last 10 years. Male cattle are always more expensive than females, and that price increase is highest during festive periods such as *Idul Fitri* and *Idul Adha*.

Implications of the change on feed supply and feeding system

A consequence of the change in Sumbawa's cattle rearing system from semi-extensive to a more controlled system such as fattening cattle in pens is that farmers should produce forages on their own land. It has been identified that the most suitable high protein forage type to be developed for cattle feed in Sumbawa is *Leucaena leucocephala* (leucaena). Demonstration farms and farmer-to-farmer training activities were facilitated by an ACIAR-funded project. Subsquently, a project funded by the Applied Research and Innovation Systems in Agriculture project is now supporting the scale-out the use of leucaena for cattle fattening involving key market actors such as local government and a traders association.

The scale-out of this leucaena feeding system has been constrained by a) farmers' perception that they still have enough grazing area so they do not have to plant forages, b) lack of labor to do cut-and-carry, c) the mistaken notion of some farmers that it is safer to let cattle free grazing that putting them in pens and d) farmers find it difficult to securely fence planted leucaena from free-grazing animals (Kana Hau et al., 2013).

Support from local government and private sector to overcome these barriers are required to scale out the leucaena feeding system in the dry areas of Sumbawa. Failure to improve farmer capacity to produce forages on

their own land will change cattle production system into unprofitable small-scale systems, resulting in high costs of production and making the production system less competitive.

Concluding remarks

Farmers in Sumbawa have started to change their cattle rearing system from semi-intensive system to cut-and-carry. This change has been driven by declining carrying capacity of communal grazing areas and availability of irrigation that drives intensive crop production. Other drivers of this shift include improved knowledge of farmers on good cattle fattening practices, availability of dry land suitable for planting leucaena, and increases in cattle price. These factors suggest that in order to remain competitive, farmers in Sumbawa should have the capacity to produce high-quality forages on their own land.

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KEYWORD：Bali cattle, intensive production systems, feed supply

REFERENCES

Dahlanuddin, Zaenuri L A, Sutaryono Y A, Hermansyah, Puspadi K, McDonald C, Williams L J, Corfield J P and van Wensveen M 2016. Scaling out integrated village management systems to improve Bali cattle productivity under small scale production systems in Lombok, Indonesia. *Livestock Research for Rural Development. Volume 28, Article #79*. Retrieved June 29, 2016, from http://www.lrrd.org/lrrd28/5/dahl28079.htm

Dilaga, S.H. 2014. Sapi Sumbawa, Sumber Daya Genetik Ternak Indonesia. Penerbit Pustaka Reka Cipta, Bandung.

Dinas Peternakan Kabupaten Sumbawa 2012. Laporan Tahunan Dinas Peternakan Kabupaten Sumbawa.

Kana Hau D, Panjaitan T, Jacob Nulik, Dahlanuddin and Elske van de Fliert 2013. Barriers to and opportunities for the use of forage tree legumes in smallholder cattle fattening systems in Eastern Indonesia. *Tropical Grasslands – Forrajes Tropicales*. Vol. 2; 79 — 81.

Sutaryono Y A, Dahlanuddin, S D Hasan and Mastur 2012. Climate change adaptation of smallholder livestock farmers in West Nusa Tenggara Province Indonesia. Proc. 15th AAAP Animal Science Congress Bangkok, Thailand pp. 968-972.

Waldron S, Dianne Mayberry, Dahlanuddin, Marthen Mulik, Simon Quigley, Dennis Poppi 2013. Eastern Indonesia agribusiness development opportunities - analysis of beef value chains. Final Report Australian Centre for International Agricultural Research.

O-39-5 EFFECT OF Aerva lanata AND Cassia auriculata EXTRACTS AND SYNTHETIC ANTIOXIDANT: BUTYLATED HYDROXYTOLUENE ON THE RANCIDITY OF VEGETABLE OIL

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INTRODUCTION

Vegetable oil is an important animal feed ingredient. Major problem of vegetable oil storage is rancidity which adversely affect on feed quality and animal health. Antioxidants are compounds that reduce rancidity. Specifically approved antioxidants are able to be added to lard, tallow, and other foods susceptible to rancidity (Chow, 1980). Butylated Hydroxytoluene (BHT) is a synthetic antioxidant used in food industry (De Koning, 2002 Thorisson *et al*., 1992). Natural antioxidants are food additives, they are free radical scavengers, and also healthier and safer than synthetic antioxidants (Chu *et al*., 1999).*Aerva lanata Linn*. (Sinhala: Polpala) and *Cassia auriculata* (Sinhala : Ranawara) are medicinal plants which is used in Sri Lanka. The chemical components such as flavonoids, alkaloids, steroids, polysaccharides, tannins, saponins can be found in *A.lanata* (Muthukumaran, 2011 Shirwaikar, 2004). The phytochemical screening of ethanol extract of *C.auriculata* flowers revealed the presence of flavonoids, phenolic acids, sterods / triterpenoids, alkaloids, tannins and anthocyanins (Lukmanul, 2007).

The flavonoid and phenols amount in ethanolic extraction of *A.lanata* is 11.83mg/g and 24.23mg/g (Ragavendran, 2012).The flavonoid and phenolic content of ethanol extract of *C.auriculata* is 13.21mg/100 g and 16.32 mg/100 g of dry weight of the flower powder (Lukmanul, 2007).

The main objective of this study to evaluate effect of *A.lanata* and *C.auriculata* plant extracts and Butylated Hydroxy Toluene (BHT) on rancidity of vegetable oil. The second objective was to compare the efficacy of natural extracts of *A. lanata* and *C.auriculata* with the recommended level of commercial antioxidant, Butylated hydroxytoluene (BHT).

MATERIALS AND METHODS

Location and duration

The study was carried out at the Gold Coin Feed Mills (Lanka) limited, Colombo 15, and Sabaragamuwa University Sri Lanka during June to August 2015.

Materials

Vegetable oil was obtained from the Gold Coin Feed Mills (Lanka) limited. Butylated hydroxyl toluene (BHT) was purchased from Glorchem chemical company at Colombo 11. *A.lanata* and *C.auriculata* were obtained from Homagama, Sri Lanka.

Methodology Method of sampling

Eight liters (8L) composite sample of vegetable oil were prepared by mixing 2L from four different oil containers on receiving day at receiving point.

Plant extract preparation

The extracts of *A.lanata* and *C.auriculata* were used as natural antioxidants. Plant materials were oven dried at 30 ^{0}C with a good air draft for three days and were powdered through a mechanical grinder.Ethanol extracts were obtained by mixing dried powder and ethanol at 1:5 ratio and were kept for 24 h. Extracts were collected by filtering through a cotton. The extraction process was repeated twice. The collected extracts were pooled and solvent was evaporated under reduced pressure using Rotary - evaporator at water bath of 40 ^{0}C. The extracts were stored in freezer for subsequent analysis (Mali *et al*, 2013).

Incorporation of natural antioxidants (A.lanata and C.auriculata) and synthetic antioxidant (BHT)

Plant extracts were incorporated to the vegetable oil at 200ppm and 400ppm concentrations. Food and drug

administration permits 0.02% (200 ppm) total antioxidant based on fat weight. Thus the amount of BHT antioxidant used for the treatments was 200 ppm. Three replicates of each sample were maintained and analyzed. All the replicates were stored at room temperature (27 ^{0}C) for five weeks of experimental period.

Determination of Free Fatty Acid value (FFA)

Free fatty acid values of samples were analyzed by AOCS official method (1998). Fifty milliliters of ethanol was boiled to 70 ^{0}C. Ethanol was neutralized with 0.1N sodium hydroxide (NaOH) using 0.5 ml of phenolphthalen in as an indicator. Neutralized ethanol was poured on the 0.5g fat in the extraction flask and the contents of the flask were mixed. Sample was titrated immediately with a 0.1N sodium hydroxide (NaOH) solution shaking vigorously during titration (color change was from colorless to pink). The end point of the titration was reached when the addition of a single drop produced a slight but definite pink color which persisted for at least 1 minute.

Calculation

FFA (Vegetable oil as palmitate) = V × 0.1 × (25./M)
0.1 = Strength of sodium hydroxide
20 = Factor for vegetable oil
M = Weight of sample (g)
V = Volume of sodium hydroxide (ml)

Determination of Peroxide value (PV)

Peroxide values of samples were determined by iodometric titration method (ISO 39, 2007). Five grams of oil was weighted (with precision of 0.001g) in to an erlenmeyer flask and 50 ml of glacial acetic acid and isooctane solution (4:6) was poured into the flask. After adding 0.5 ml of KI solution to flask it was swirled for 60sec. Immediately after 100 ml distilled water and 1 ml starch indicator were poured in to the flask. Solution was titrated with 0.01N sodium thiosulfate until blue color disappears. Blank determination was carried out without oil.

Calculation

PV (meq/kg) = $[(V_1-V_0) \times T \times F \times 1000]/M$
V_1 = Volume of thiosulfate solution required to titrate the sample (ml)
V_0 = Volume thiosulfate solution required to titrate the blank determination (ml)
T = Concentration of the sodium thiosulfate solution (normality)
F = Factor for 0.01N thiosulfate solution (1.0081)
M = Mass of the sample (g)

Determination of Iodine value (IV)

Iodine values of samples were analyzed according to the method described by ISO 3961, (2013). 2.5 g of oil was weighted accurately into a 250 ml of glass stoppered flask. Twenty milliliters of solvent (cyclohexane (50ml) and glacial acid (50ml)) was added and dissolve the oil. Then 25 ml of wij's solution (ICl in acetic acid) was spoured into the flask. Flask was stoppered and mix well and then the mixture was kept in dark place for 60 minutes at 25 ^{0}C. Fifty milliliters of 10% KI solution and 100 ml distilled water were poured into the flask. The sample was titrated with 0.1M sodium thiosulfate solution using 1% starch solution as indicator. The end point was when the blue color just disappeared after vigorous shaking. Blank determination was carried out, omitting the fat, at the same time and under the same condition.

Calculation

IV (I_2/100g) = $V_0 - V_1 \times 12.7 \times (100/1000) \times M$
V_0 = Volume of 0.1 sodium thiosulfate required to titrate the blank solution (ml)
V_1 = Volume of 0.1 sodium thiosulfate required to titrate the test sample (ml)
12.7 = Amount (g) of iodine contained in one liter of 0.1 N iodine
M =Amount of fat taken (g)

Data analysis

Data were analyzed in by two way ANOVA and means were separated by DUNCAN's multiple range test by SAS

system 9.0 version.

RESULTS

Effect of plant extracts and BHT on free fatty acid formation in vegetable oil

Free fatty acids (FFAs) are formed due to hydrolysis of triglycerides by the actions of enzymes and reaction of oil with heat and moisture. Initial FFA value of vegetable oil was 13.5%. The FFA content of vegetable oil has been slightly increased during the five weeks of storage period at room temperature (27 ^{0}C) in all the treatments (Figure 1). It indicates that hydrolysis of triglycerides has been occurred in all tested samples. But FFA formation was reduced in both synthetic antioxidants and plant extract treated vegetable oils compare to the control.

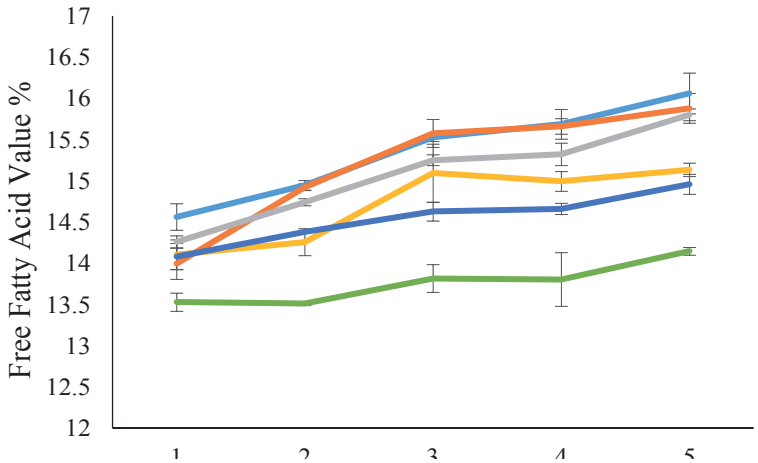

Figure 1: Free fatty acid formation in storaged vegetable oil

BHT = Butylated hydroxytoluene, P200 = 200ppm *A.lanata* leaves extract, P400 = 400ppm *A.lanata* leaves extract, R200 = 200ppm *C.auriculata* flowers extract and R400 = 400ppm *C.auriculata* flowers extract

In first week, there was not significant ($p \leq 0.05$) effect of BHT, *A.lanata* and 200ppm *C.auriculata* on FFA formation. In second week, there was not a significant ($p \leq 0.05$) effect of treatments on FFA formation except 400 ppm of both plant extracts treated samples. BHT and control shows same trend of FFA formation from second week onwards. But in fifth week FFA formation was slightly reduced in BHT treated sample than control. The effect of *A.lanata* (200ppm, 400ppm) and 200ppm *C.auriculata* for FFA formation was not significantly different ($p \leq 0.05$) in third week of storage but 400 ppm *C.auriculata* treated samples shows significantly ($p \leq 0.05$) higher effect for reduction of free fatty acid formation.

In fourth week both natural extracts were affected on FFA formation, but *C.auriculata* treated samples were recorded with higher effect on reduction of FFA formation over *A.lanata* treatments. The *C.auriculata* flowers extract at 400 ppm concentration prevents hydrolytic rancidity of vegetable oil significantly which stored for five weeks period.

Effect of plant extracts and BHT on peroxide value of vegetable oil

The oxidative changes of vegetable oils can be measured by the peroxide value where it indicates primary compounds of autoxidation. Results of peroxide values of vegetable oil during five weeks of storage period are shown in figure 2. The initial peroxide value of vegetable oil was 7.5 meq Kg^{-1}.

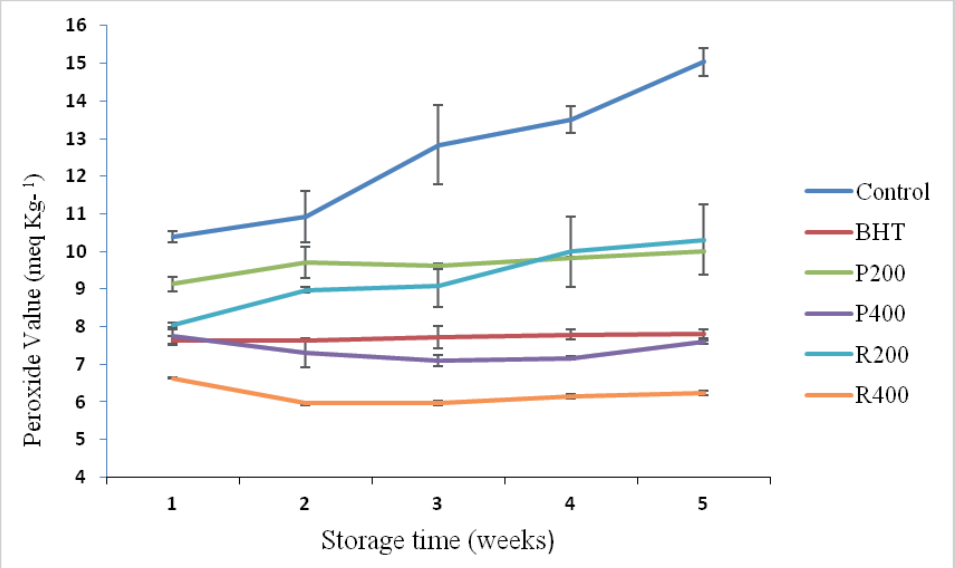

Figure 02: Effect of plant extracts and BHT on peroxide values (meqKg^{-1}) of vegetable oil

BHT = Butylated hydroxytoluene, P200 = 200ppm *A.lanata* leaves extract, P400 = 400ppm *A.lanata* leaves extract, R200 = 200ppm *C.auriculata* flowers extract and R400 = 400ppm *C.auriculata* flowers extract

The control sample PV has increased rapidly (7.5 to 10.92 meqKg^{-1}) fallowed by slight increase of PV. The rapid increase of PV during the first few weeks could be attributed to surge propagation phase which forms the largest amount of hydro peroxides. Then the observed slight increase of the PV could be due to the division of peroxides into secondary oxidation compounds (Cornel, 2010).

During the initial phase, oxidation proceeds at a relatively slow and uniform rate. Peroxides are formed at a faster rate than they are destroyed. So, that their content increases in conjunction with the oxygen absorption. After a certain oxidation has occurred, the reaction enters a second phase, so rapidly accelerating rate of oxidation (Cornel, 2010).

As oil oxidation continues with time, the peroxides that are formed decompose to generate volatile and nonvolatile compounds. They contribute to flavor and odor deterioration of oils and fats. The extreme stages of oxidation, polymerization, and degradation are accompanied by rapid increase in the viscosity of the oil.

Peroxide values were increased than the initial value in all the oil samples except 400ppm concentration of both *C.auriculata* and *A.lanata* under five weeks storage period.

Table 1: Means of peroxide value of each treatment at five weeks storage

Treatment	Mean value of PV (meqKg^{-1})
Control	11.29^{a}
P200	9.57^{b}
R200	9.29^{b}
BHT	7.69^{c}
P400	7.01^{d}
R400	6.43^{e}

BHT = Butylated hydroxytoluene, P200 = 200ppm *A.lanata* leaves extract, P400 = 400ppm *A.lanata* leaves extract, R200 = 200ppm *C.auriculata* flowers extract and R400 = 400ppm *C.auriculata* flowers extract

Means with same letters are not significantly different ($p<0.05$).

A significant difference ($p \leq 0.05$) was observed between the control and all other treatments. The control had the highest peroxide value followed by *A.lanata* leaves extract 200ppm, *C.auriculata* flowers extract 200ppm and BHT. The lowest peroxide value was observed in *C.auriculata* flowers extract 400ppm (6.43 $meqKg^{-1}$) fallowed by *A.lanata* leaves extract 400ppm (7.01 $meqKg^{-1}$) having a high stability of the oil in during storage.

In synthetic antioxidant BHT show slight increase of PV during five weeks period from 7.72 $meqKg^{-1}$ to 7.91 meq Kg^{-1}.The BHT treated oil samples were stabilized due to its antioxidant property.In *C.auriculata* flowers extract 400ppm and *A.lanata* leaves extract 400ppm, peroxide values were increased in first week and began to decline than initial PV. The PV was recorded as 7.16 $meqKg^{-1}$ and 6.15 $meqKg^{-1}$ at the last week of storage for 400ppm $meqKg^{-1}$ and 400ppm *C.auriculata* respectively. This might be due to high concentration of phytochemical compounds in extracts.

Extract of *A.lanata* has antioxidant activity due to high phenolic content. Phenolic compounds are powerful chain breaking antioxidants, they possess scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). Many flavonoids are strong antioxidants (Dziedzic *et al*., 1983). And also these are superoxide, hydroxyl and hydrogen peroxide scavengers (Somashekaraiah, 2012) and presence of these along with other phytoconstituents suggests synergistic/antagonistic actions. The activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition, metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction. The above mentioned reasons may be the cause for decreased PV in 400 ppm concentration of both *C.auriculata* and *A.lanata* plant extracts.

Effect of plant extracts and BHT on the Iodine values of vegetable oil

The Iodine value (IV) shows the degree of unsaturation of oil sample. Higher iodine value reflect that the higher degree of unsaturation. The initial IV of vegetable oil was 50.37 I_2/100g of oil and was reduced during the storage. Figure 3 show the IV of vegetable oil at the end of the fifth week of storage.

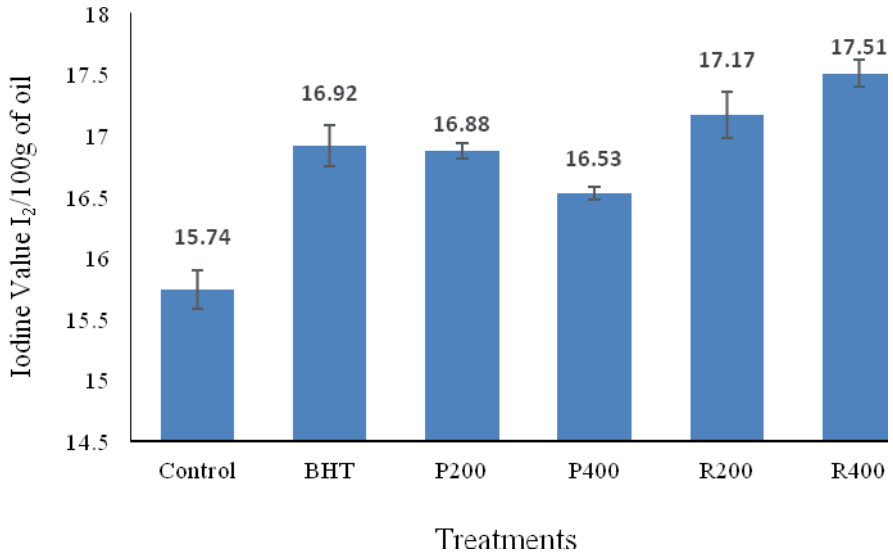

Figure 03: Effect of treatments on iodine value of vegetable oil

BHT = Butylated hydroxytoluene, P200 = *A.lanata* leaves extract 200ppm, P400 = *A.lanata* leaves extract 400ppm, R200 = *C.auriculata* flowers extract 200ppm and R400 = *C.auriculata* flowers extract 400ppm

The IV of control was lower than treated samples. All the treatments show significantly affect ($p \leq 0.05$) on Iodine Value of vegetable oil. Oxidation caused a increase in the relative percentages of the saturated fatty acids. So high iodine value means lower saturation and low iodine value means high saturation of fatty acids.

Considering natural plant extracts, *C.auriculata* flowers extract give better effect than *A.lanata* leaves extract on unsaturation of fatty acids in vegetable oil. That means the saturation of fatty acids during the storage is less in samples treated with *C.auriculata* flowers extracts. But IV of samples were affected differently according to the

treated concentrations of plant extracts.
The synthetic antioxidant 200ppm BHT was performed better than *A.lanata* extraction and less effective than *C.auriculata* flowers extraction on degree of unsaturation in stored vegetable oil.
Finally it can be conclude that *C.auriculata* flowers extraction at 400ppm is significantly ($p \leq 0.05$) reduce level of saturation in vegetable oil with the storage.

CONCLUSIONS

All tested concentrations of plant extracts and BHT at 200ppm were significantly retarded the lipid autoxidation of vegetable oil. The effectiveness of plant extracts of *A.lanata* and *C.auriculata* as natural antioxidants at 400 ppm concentration were significantly ($p \leq 0.05$) higher than BHT 200ppm in preventing vegetable oil autoxidation. The extracts of *A.lanata* and *C.auriculata* were suppressed the hydrolytic rancidity significantly ($p \leq 0.05$) over the synthetic antioxidant BHT. *C.auriculata* at 400ppm concentration can be used to safeguard vegetable oil against both oxidative and hydrolytic rancidity during the storage for five weeks period which stabilize the keeping quality.

KEYWORD : Aerva lanata, Butylated Hydroxytoluene, Cassia auriculata, Rancidity, Vegetable oil

REFERENCES

Akpulu, I.N., Dada, J.D., Odama, L.E. and Galadima, M. 1994. Antibacterial activity of aqueous extracts of some Nigerian medicinal plants, Nigerian J Botany 7: 45-48.

Chu, Y.H., Hsu, H.F.1999. Effects of antioxidants on peanut oil stability. *Food Chem* 66:29-34.

Cornel, 2010. Research consenting sensory and physiochemical changers of animal fat during storage: 5-8.

Dziedzic, S.Z. and Hudson, B.J.F. 1983. Hydroxyl isoflavones as antioxidants for edible oils. *Food chemistry* 11:161-166.

Hatano, T., Edamatzu, R., Mori, A., Fujita, Y., Yasuhara, T., Yoshida, T. and Okuda, T. 1989. Effect of interaction of tannins with co-existing substances. *Chem. Pharm. Bull* 37: 2016-21

Lukmanul Hakkim F., Girija, S., Senthil Kumar, R. and Jalaludeen, M.D. 2007. Effect of aqueous and ethanol extracts of Cassia auriculata L. flowers on diabetes using alloxan induced diabetic rats. *Int J Diabetes & Metabolism* 15:100-106

Mail, A.A., Bandawane, D.D. and Hivrale, M.G. 2013. Evaluation of Anti-inflamatory and Analgesic Activity of Methanolic Extract of Cassia Auriculata Leaves, Pharmacologia 117:125-118.

Muthukumaran, P., Shanmuganathan, P. and Malathi, C. 2011. In vitro antioxidant evaluation of *Mimosa pudica*, Asian J Pharm Res. 1(2):44-46.

Ragavendran. P., Sophia, D., Raj, C. A., Starlin, T. And Gopalakrishnan, V. K. 2012. Phytochemical screening, antioxidant activity of *Aervalanata* (L) – an Invitro study. *Asian Journal of Pharmaceutical and Clinical Research*. 5(2):0974-2441.

Shirwaikar, A., Issac, D., Malini, S. 2004. Effect of *Aerva lanataon* cisplatin and gentamicin models of acute renal failure. *J Ethnopharmacol*. 90:81-86.

Somashekaraiah, B.V. 2012. In vitro and In vivo Antioxidant and Antidiabetic Efficacy of *Cassia auriculata L*. Flowers. 55 *Global Journal of Pharmacology* 6 (1): 33-40.

Thorisson, S., F. Gunstone, and R. Hardy 1992. The antioxidant properties of ethoxyquin and of some of its oxidation products in fish oil and meal. *Amer. Oil Chem. Soc* 69, pp: 806-809.

O-39-6

Effect of different long-term storage with rice bran-CaCO3 of aerobic thermolignocellulolytic inoculums on fiber compound content of fermented rice straw

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INTRODUCTION

One of the obstacles in the development of livestock business is the problem in feed availability either for its quality, quantity, continuity, and also its competitiveness to food needs for human. One of alternatives to solve the problem is by utilizing the agricultural waste that is available throughout the year.

The use of rice straw as feed alternative by adding microbe to improve the nutrition value has been widely performed. The addition of microbe to the rice bran-$CaCO_3$ as carrier that is further known as inoculum aims to change the physical structure of rice straw with delignification enzyme by degrading the lignocellulose, a fiber component that interfere the digestion.

The microbial growth in the fermentation process is influenced by the environmental factors such as temperature, pH, air, and osmotic pressure, while the nutritional factors are including carbon sources, nitrogen sources, minerals (inorganic ion), essential metabolites (vitamin and amino acids), and water (Nester *et al*., 1983). The activity of cellulase enzyme is influenced by the temperature, pH, substrate type and concentration, and also concentration of inhibitor from the final product (Enari, 1983).

Thermophiles have some advantages than mesophiles and psychrophiles such as able to produce thermostabel enzyme so that the enzymatic reaction can run faster. These microbes can grow at temperature 45 - 65 ℃ and not be able to proliferate at the temperature below 40℃ (Alexander, 1985). At an appropriate temperature, the microbial growth and enzyme activity produced by those microbes are expected to be optimum, so the increase of temperature up to optimum will cause the activity of aerobic thermolignocellulolytic microbe degradation will be much better.

The objectives of the research were to investigate the effect of rice straw fermentation by using aerobic thermolignocellulolytic inoculums in the rice bran-$CaCO_3$ carrier and the effect of using aerobic thermolignocellulolytic inoculums in the rice bran-$CaCO_3$ carrier at the different long-term storage to the concentration of cellulose, hemicellulose, lignin, organic matter, dry matter, and pH value of fermented rice straws.

MATERIALS AND METHODS

This research used aerobic thermolignocellulolytic inoculums in the rice bran-$CaCO_3$ carrier 5% at the long-term storage 0, 2, 4, 6, 8, 10 and 12 weeks, rice straws, and water so it will result in fermented rice straw with concentration 50%. Inoculums used was the compound of mold 2 (M2), mold 5 (M5), bacteria 3 (B3), bacteria 4 (B4), and *Termonospora fusca* (Tf) that were the isolate collection of Laboratory of Nutritional Biochemistry, Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada. Fermentation was performed every two weeks in 12 weeks. To identify the result of fermentation, on the 21st day the sample was taken to be analyzed its concentration of cellulose, hemicellulose, lignin, organic matter, dry matter, and pH value of the fermented rice straws. As comparison for each treatment, the sample of fermented rice straws at incubation 0 (before fermentation) was taken.

The data obtained were analyzed by using CRD variance, analysis of Split-plot design. The significant difference of each treatment was tested with Duncan's new Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Means of concentration of cellulose, hemicellulose, lignin, dry matter, and organic matter of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier with different age are presented in the Table 1 - 5.

The research result showed that utilization of aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier could decrease the concentration of hemicellulose, lignin, and pH value of fermented rice straws, while the concentration of cellulose, organic matter, and dry matter a showed a non-significant difference. The decrease of concentration

of cellulose was not significant because the effect of cellulose enzyme activity resulted in by inoculums occurred at the stage loosening the lignocellulose bonds and had not reached the stage of final cellulose degradation. The concentration of hemicellulose and lignin decreased because there were hemicellulose and ligninase enzyme activities (Yusiati *et al*., 1995), while the decrease of pH value was assumed caused by accumulation of lactate that was produced by the natural microbes in the rice straws, which is lactic acid microbes.

The long-term storage of inoculum 0 to 12 weeks gave significant effect to the concentration of cellulose, hemicellulose, and pH value of fermented rice straws, while the concentration of lignin, organic matter, and dry matter were not different. The decrease of cellulose, hemicellulose, and pH value was assumed caused by the existence of other enzyme beside β-glucosidase that have role in the degradation because the environment of carrier was kept optimum so the microbes persisted in the lag phase.

This research can be concluded that the fermentation of rice straws by using aerobic thermolignocellulolytic inoculums in the rice bran-$CaCO_3$ carrier could decrease the concentration of hemicellulose, lignin, and pH value of fermented rice straws, while the concentration of cellulose, organic matter, and dry matter did not decrease. Aerobic thermolignocellulolytic inoculums in the rice bran-$CaCO_3$ carrier have ability to saccharify and fermented rice straws that were constant until the long-term storage time 12 weeks so that the concentration of lignin, organic matter, and dry matter did not change, while the concentration of cellulose, hemicellulose, and pH value changed.

KEYWORD : aerobic, carrier, fermented rice straw, inoculums, storage

Table 1. Concentration of cellulose of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier under different length of storage (%DM)

Fermentation treatment	Length of storage time (weeks)							**Mean^{ns}**
	0	2	4	6	8	10	12	
Before	36.33	33.76	33.76	33.47	34.72	34.16	35.5	**34.53**
After	36.1	33.72	32.17	33.09	34.47	35.51	32.53	**33.94**
Mean	**36.22^{b}**	**33.74^{ab}**	**32.97^{a}**	**33.28^{ab}**	**34.60^{ab}**	**34.84^{ab}**	**34.02^{ab}**	

^{ab} different superscript at the same row shows a significant difference (P<0.05)
^{ns} non-significant different

Table 2. Concentration of hemicellulose of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier under different length of storage (%DM)

Fermentation treatment	Length of storage time (weeks)							**Mean**
	0	2	4	6	8	10	12	
Before	27.97	28.05	28.39	28.39	27.45	29.00	26.72	**28.00^{x}**
After	27.77	27.66	27.62	27.40	28.72	27.19	25.30	**27.38^{y}**
Mean	**27.87^{b}**	**27.86^{b}**	**28.01^{b}**	**27.90^{b}**	**28.09^{b}**	**28.10^{b}**	**26.01^{a}**	

^{ab} different superscript at the same row shows a significant difference (P<0.05)
^{xy} different superscript at the same column shows a significant difference (P<0.01)

Table 3. Concentration of lignin of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier under different length of storage (%DM)

Fermentation treatment	Length of storage time (weeks)							Mean
	0	2	4	6	8	10	12	
Before	3.24	3.21	3.13	4.41	3.48	3.50	3.16	**3.45^{x}**
After	3.21	3.06	3.4	3.06	3.40	2.66	3.40	**3.17^{y}**
Mean^{ns}	**3.23**	**3.14**	**3.27**	**3.74**	**3.44**	**27.19**	**3.28**	

^{xy} different Superscript at the same column shows a significant difference (P<0.01)
^{ns} non-significant difference

Table 4. Concentration of organic matter of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier under different length of storage (%DM)

Fermentation treatment	Length of storage time (weeks)							Mean^{ns}
	0	2	4	6	8	10	12	
Before	75.75	74.28	74.47	75.18	75.66	77.86	75.69	**75.56**
After	75.09	75.14	68.85	75.33	70.38	76.48	75.36	**73.80**
Mean^{ns}	**75.42**	**74.71**	**71.66**	**75.26**	**73.02**	**77.17**	**75.53**	

^{ns} non-significant difference

Table 5. Concentration of dry matter of fermented rice straws using aerobic thermolignocellulolytic in the rice bran-$CaCO_3$ carrier under different length of storage (%DM)

Fermentation treatment	Length of storage time (weeks)							Mean^{ns}
	0	2	4	6	8	10	12	
Before	49.28	55.09	49.28	50.04	51.92	50.23	51.39	**51.03**
After	51.96	51.64	51.27	48.34	48.32	48.36	46.33	**49.46**
Mean^{ns}	**50.62**	**53.37**	**50.28**	**49.19**	**50.12**	**49.30**	**48.86**	

^{ns} non-significant difference

REFERENCES

Alexander, M. 1985. Introduction to Soil microbiology. 2nd edition. Wiley Eastern Ltd. New Delhi.

Enari, TM. 1983. Microbial cellulose. In: Microbial Enzime and Biotechnology. Ed: WM. Fogarty. Applied Science Publ. London.

Nester, EW., CE. Robert, ME. Lidstorm, N. Pearsall, and MT. Nester. 1983. Microbiology. 3rd ed. Saunders Coolege Publishing.

Yusiati, LM, Z. Bachruddin, S. Margino, Widyantoro, Sasmito, C. Anwar. 1998. Production of Ligninase and Carbohydratase of Termophilic Microbes and Their Application on Enrichment of Material Lignocellulose Quality. Report of HB III/4 of Higher Education Research Program. Faculty of Animal Science. UGM.

O-39-7

THE EFFECTS OF COCONUT MEAT WASTE AS FEED ALTERNATIVE IN SHEEP RATION ON CHOLESTEROL CONTENT AND MEAT QUALITY

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OBJECTIVE

Indonesia, a tropical country, has two seasons, those are dry and rainy seasons. The seasons resulted in differences in the availability supply of feed, especially forage as a source of fiber for ruminants. However, recently the population of small ruminants animal in Indonesia is increasing and it forced the increase of feed requirements, especially forage. The problem of forage supply in dry seasons requires the use of alternative materials which is cheap, good in ingredients, as well as continuous availability throughout the year.

Virgin Coconut Oil (VCO) is known as coconut oil has many benefits, for human health and its demand is increasing in recent years. This encourages the increased of VCO production. The VCO production, generated the by-product, which is coconut meat waste. The coconut meat waste contained high percentage of crude fiber (36.13%). According to crude fiber contents, coconut meat waste, the byproduct of the manufacture of VCO is possible to be used as fiber-source-feed alternative, to solve the problem of limited supply of forage during dry season in Indonesia. This study aimed to determine the effect of coconut meat waste, by-product of the manufacture of VCO as an alternative of fiber source on the meat and blood cholesterol content, as well as the chemical composition of lamb meat.

METHODOLOGY

Animals and feeding trial. Sixteen male thin tail sheep (±8-12 months old±15 kg) were reared for 12 weeks (July-September) on a separate cage in Experimental farm of Faculty of Animal Science, Universitas Gadjah Mada, Indonesia. The sheep were separated into 4 groups of fiber-source substitution treatments. The control group was fed with Concentrate:King grass:Coconut Meat Waste (C:K:CW) with composition 60:40:0, and other groups were 10%CW (C:K:CW=60:30:10), 20%CW (C:K:CW=60:20:20), and 30%CW (C:K:CW=60:10:30). The concentrate composition is presented in table 1. Coconut meat waste was obtained from VCO home-factory in Kulon Progo District, Yogyakarta Province. Coconut meat waste was sun-dried for 3 days. The dried coconut meat was ground on a grinder and were shieved through a 3 mm diameter shiever. The diet was fed on daily basis as much as 4% from animal body weight, twice a day at 6 AM and 4 PM.

Blood sampling. The blood was obtained three time during feeding trial, at 0, 6 and 12 weeks. Blood were obtained from all animals. Blood sampling was performed approximately 1 hr before feeding in the morning. Five ml blood was collected using a vacutainer tube from the jugular vein (neck). The blood sample will be used for analysis of cholesterol content according to Leiberman Burchard method (Plummer, 1977).

Animal slaughter. Slaughtering was done at the end of feeding trial (2 weeks). Three sheep from each treatment group were chosen as a sample for slaughter and longissimus dorsi and bicep femoris were obtained. The sample were prepared for analysis for cholesterol content according to Leiberman Burchard method (Plummer, 1977) and proximate analysis (AOAC, 2002).

Data analysis. Meat cholesterol and chemical composition data were analyzed using factorial Completely Randomized Design (4x2) to determine the effect coconut meat waste followed by Duncan's Multiple Range Test (DMRT). Blood cholesterol content data were analyzed using one-way ANOVA.

RESULTS

Crude protein (CP) content of meat from all groups ranged between 17.77% - 18.50% (Table 2) and is in normal range according to Judge et al. (1989), which is 16-22%. It shows that the substitution of king grass to coconut meat waste did not show a negative effect on lamb meat protein content. Our results also similar with previous studies, such as the Paradise (1999) CP 18.69% Kusuma (1990) and Sugiyono (1997) CP 19.9% and Wiradarya (1989) CP 19.93%. Soeparno (1985) and Triatmojo (1991) reported that crude protein content of sheep meat is not affected by dietary treatment with different level of feed energy. Craddock et al. (1974) and Theriez et al. (1982) stated that the increase in energy feed has no effect on the carcass quality. Protein content of lamb meat

are relatively constant with relatively small difference which may be caused by the fact in ruminant, protein is not deposite into the carcass or animal body, the excess of protein in ruminant only be removed through the urine (Triatmojo, 1991). Crude protein content also were not different among muscle (BF and LD). Our results was different from those obtained by Triatmojo (1991) in which the crude protein content in BF are higher than those of LD, but similar with results reported by Heru-Mulyanto (1990) in which muscle composition of BF and LD were not different. Crude protein content of BF and LD were not different may be due to the sheep were placed in individual cages during feeding trials, so that the animal is not much activity. The BF (active muscle) and LD (passive muscle) has an activity which is almost the same, that may affect the protein deposition and muscle metabolisme.

Coconut meat waste contain high percentage crude fat, which is 40.78%. Coconut meat waste fed to sheep did not affect cholesterol content in meat and the muscle type also were not affected. Our results was similar with Solomon et al. (1992) who reported that the addition of palm oil in sheep feed has no effect on fat and cholesterol of meat. Average cholesterol content of meat LD obtained in the study was 30.95mg/100g meat and BF was 34.08mg/100g meat (Table 1). This result is lower than Solomon et al. (1992) who noted that the addition of palm oil resulted cholesterol content in LD of 66.9 mg/100g, whereas cholesterol content of control sheep was 66.4mg/100g. Our data were lower compared to Lough et al. (1991) who reported that cholesterol content of lamb fed canola seed was 62.4 mg/100 g and control sheep was 61.2mg/100 g. The content of unsaturated fatty acids in coconut meat waste as high as 50-72 mg/g (Wahlqist, 1982), but when unsaturated fatty acid are fed to ruminants, it will be hydrogenated to saturated fatty acids. Hydrogenation in unprotected fat can happened up to 85-90% (Bauchart et al., 1990). Unsaturated fatty acids in coconut meat waste were not protected, so that the unsaturated fatty acids turn into saturated fatty acids and ultimately did not affect cholesterol meat. The cholesterol content of the meat is associated with fat intake (Solomon et al., 1991). At the time of the study, the average daily consumption of crude lipid (g / head / day) were 23.92, 28.87, 32.84 and 39.45 (g / kg W0,75 / hr) for each treatment respectively From these data, it showed that the daily fat consumption of 30%CW group was highest among other groups. Daily fat consumption was significantly different between treatments. Differences rough daily fat consumption did not lead to differences in the cholesterol content of the meat. Nelson and Cox (2000) states that cholesterol is synthesized from Acetil Co-A through four stages, namely: Synthesis mavolenate of the acetate, conversion of mevalonate into two isoprene active, condensation of six isoprene active as squalene and changes of squalene into cholesterol. Acetyl Co-A in can be derived from glucose (carbohydrates), free fatty acid (fat) and from acetate (Riis, 1983). Fat consumption during feeding trial was to increase as the levels of coconut meat waste increased, whereas according to Natalia (2007) TDN consumption and PK decreased with increasing levels of addition of coconut pulp. This led to the formation of Acetil Co-A only come from fat alone, resulting he formation Acetil Co-A was not optimal. Under normal circumstances, Acetil Co-A can be derived from protein, fat and carbohydrates (Riis, 1983). This causes the number Acetil Co-A is formed only be used for maintenance only and will not be used for the synthesis of cholesterol. Therefore, the cholesterol contained was not affected. Fat is transported in blood plasma in the form of plasma lipoprotein (Nelson and Cox, 2000). That is, the protein is needed in the transport of fats in the blood. Protein consumption during the study decreased with increasing levels of addition of coconut pulp (Natalia, 2007). This leads to the transport of fats in the blood was not optimal, so that the fat metabolism is also not optimal.

Blood cholesterol also was not affected by coconut meat waste dietary supplementaton. It may be due to unsaturated fatty acids in coconut meat waste experienced hydrogenation to saturated fatty acids in rumen (Garton, 1965). The hydrogenation of unsaturated fatty acids into saturated fatty acids have resulted in the fatty acid composition of feed materials are not a lot of influence on the fatty acid deposition. Anggorodi (1980) noted that the human or animal body maintains its normal concentration of cholesterol in plasma by regulating the synthesis and excretion of cholesterol. Guyton (1981) adds that the feedback mechanism participated in maintaining the balance of plasma cholesterol. There is hemostatic mechanism of plasma cholesterol in some mammals (Taylor et al., 1965), thus giving coconut pulp no effect on cholesterol.

Percentage of organic matter in LD and BF was not affected by coconut meat waste dietary substituion. Organic matter of meat of all groups were 98.76 to 98.90%. which is in normal range. According to Judge et al. (1989), the range of ash content of lamb meat is a constant of about 1% (organic matter, 99%). The results also was not much different from the ash obtained by Kusuma (1990) and Sugiyono (1997), which is 1% (99% organic matter) and 1.09% (98.9% organic material).

The organic material consists of protein, fat and carbohydrates (Kamal, 1999). Judge et al. (1989) states that, the ash content is closely linked to its water content, protein and fat. The results showed that levels of protein and

cholesterol meat as a constituent of organic material meat was not different, therefore it did not affect organic matterial content in meat. In addition, the consumption of minerals of animals in each treatment was not different, so that the levels of minerals in the meat that is indicated by the ash, is also relatively the same. Soeparno (1994) noted that feed is the environmental factors that give most influence to the chemical composition of meat. Organic matter content muscle BF and LD are no different. These results differ from the results Heru-Mulyanto (1990) but equal to the result obtained Triatmojo (1991) and Soeparno (1985). Heru-Mulyanto (1990) states there is a difference between the organic matter content of BF and LD muscle mutton, while Triatmojo (1991) states there is no difference between the organic matter content of BF and LD muscle.

Dry matter content of meat of all groups are in normal range compared to other previous research. Dry matter content of several studies are 23.2% (Eden, 1999), 26.3% (Kusuma, 1990), 22.5% (Sugiyono, 1997) and 26.23% (Wiradarya, 1989). The normal range of dry matter content of lamb on the longisimus dorsi is 20-35% (Judge et al., 1989). This showed that coconut meat waste did not give negative effect in dry matter content.

CONCLUSION

It can be concluded that the king grass substitution with coconut meat waste did not cholesterol content of the blood and meat cholesterol, and meat chemical composition. Also, there was no different in meat composition between different muscle type (biceps femoris and longissimus dorsi). Overall, King grass substitution with coconut meat waste did not give negative effect on meat chemical composition.

KEYWORD : Coconut meat waste, Blood cholesterol, Sheep, Meat Quality

Table 1. Feed Composition

No.	Bahan Pakan	C	10% CW	20% CW	30% CW
1.	King grass	40	30	20	10
2	Coconut meat waste	0	10	20	30
3	Rice Bran	30,86	30,86	30,86	30,86
4	Onggok	11,14	11,14	11,14	11,4
5	Corn	4,28	4,28	4,28	4,28
6	Coconut oilcake	8,57	8,57	8,57	8,57
7	Soybean oilcake	4,28	4,28	4,28	4,28
8	Salt	0,86	0,86	0,86	0,86
	Total %	100	100	100	100
	Total CP	11,26	11,03	10,8	10,58
	Total TDN	64,25	65,25	66,25	67,25

Table 2. The effects of coconut meat waste on meat chemical composition and blood cholesterol

Parameter	Type of muscle	CW treatments				Average[ns]
		0	10%	20%	30%	
Crude protein	BF	17,77±1,31	18,21±1,71	17,59±1,91	17,51±2,03	17,77
	LD	18,65±1,32	18,44±1,27	18,41±4,01	18,50±2,90	18,50
	Average[ns]	18,21	18,12	18,00	18,01	
Blood cholesterol	R1	36,26±4,36	34,89±6,12	30,50±13,03	28,57±11,08	
	R2	23,76±2,16	28,48±2,13	24,91±8,08	34,77±6,18	
	R3	15,49±3,30	20,29±3,04	22,15±2,35	23,15±2,21	
	Average[ns]	25,17	27,89	25,85	28,83	
Meat cholesterol	BF	29,04±12,28	35,93±17,64	30,33±13,46	41,03±13,73	34,08
	LD	39,92±22,01	25,72±9,70	29,69±11,63	28,48±7,77	30,95
	Average[ns]	34,48	30,82	30,01	34,76	
Organic matter	BF	98,92±0,19	98,80±0,03	98,86±0,05	98,64±0,05	98,81
	LD	98,87±0,07	98,84±0,05	98,83±0,05	98,87±0,07	98,85
	Average[ns]	98,90	98,82	98,85	98,76	
Dry matter	BF	23,71±2,30	21,20±1,05	20,58±1,48	22,07±3,34	21,89
	LD	23,40±1,04	21,11±1,25	20,84±0,82	20,51±0,68	21,46
	Average[ns]	23,55[a]	21,16[b]	20,71[b]	21,29[b]	

[ab] different suppercripts in the same column showed significantly different average ($P<0,05$)

[ns] non-significant

REFERRENCES

Anggorodi, R. 1994. Ilmu Makanan Ternak Umum. PT. Gramedia, Jakarta.

AOAC. 1985. Official methodes of Analysis of the Association of Official Agriculture Chemist. Washington D.C. Pp. 129-146.

Bauchart, D., F. Legay Carmier, M. Doreau, and B. Gailard. 1990. Lipid metabolism of liquid-associated and solid-adherent bacteria in rumen contents of dairy cows offered lipid-suplemented diets. Br. J. Nutr. 63:563-578.

Bauman, D. E., J. W. Perfield, M. J. De Veth, and A. L. Lock. 2003. New perpective on lipid digestion and metabolisme in ruminant. Proc. Cornell Nutr. Comf. Pp. 175-189.

Borgman, R. F. and F. Wardlow. 1973. Serum cholesterol concentration and cholithiasis in rabbit influenced by dietary fat. Am. J. Res. 36: 92-96.

Czerkawski, J. W. 1986. An Introduction to Rumen Studies. Pergamon Press, Oxford.

Davis, C. L. 1980. The Microbiology and Physiology of Ruminant Nutrition. Departement of Dairy Science, University of Illinois USA.

Harper, H.A., V.M. Ridwell, and P.A. Mayes. 1979. Biochemistry. 17th ed. Los Altos, California.

Heru-Mulyanto. 1990. Pengaruh Jenis Kelamin dan Berat Potong terhadap Komposisi Kimia Daging Domba Lokal. Skripsi Sarjana Peternakan. Fakultas Peternakan Universitas Gadjah Mada, Yogyakarta.

Judge, M.D. Aberle, J.C. Forrest, H.B. Hedrick, and R.A. Merkel. 1989. Principles of Meat Science. Kendall/Hunt Publishing Co. Iowa.

Laugh, D. S., M. B. Solomon, T. S. Rumsey, T. H. Elsasser, L. L. Slyter, S. Kahl, and G. P. Lynch. 1991. Effects of dietary canola seed and soy lechitin in high-forages diets on cholesterol content and fatty acid composition of carcass tissues of growing ram lambs. J. Anim. Sci. (70):1153-1158.

Lawrie, R.A. 1985. Meat Science. 3rd ed. Pergamon Press, Oxford-New York-Toronto-Sidney-Paris-Frankfurt.

Natalia, C. 2007. Pengaruh Penggunaan Ampas VCO dalam Pakan terhadap Performans Domba. Skripsi Sarjana Peternakan, Universitas Gadjah Mada. Yogyakarta.

Noble, R. C. 1981. Digestion, transport and absorption of lipids. In: W. W. Cristie (Ed.) Lipid Metabolism in Ruminant Animals. Pergamon Press Ltd., Oxford, UK, Pp. 57-93.

NRC. 1976. Nutrient Requirement of Beef Cattle 5th ed. National Academy of Science., Washington D.C.

Plummer, D.T. 1977. An Introduction to Practical Biochemistry. Mc Graw Hill Publ. Co. Ltd. Bombay.

Riis, P.M. 1989. Dynamic Biochemistry of Animal Production. Elsevier Science Publishers B.V., Amsterdam.

Soeparno. 1985. Meat and Carcass Composition of Lambs Fed Diets Varying in Protein and Energy Consentration. Proc. ASAHIL Seminar in Food Tech. and Nutr. pp 217-224. GMU. Yogyakarta.

Soeparno. 1994. Ilmu dan Teknologi Daging. Cetakan ke-2, GMU. Yogyakarta.

Solomon. M.B., G.P. Lynch, E. Paroczay, and S. Norton. 1991. Influence of rapeseed meal, whole rapeseed and soybean meal on fatty acid composition and cholesterol content of muscle and adipose tissue from ram lamb. J. Of. Anim. Sci. 69(10): 4055-4061.

Theriez, M., Y. Villete and Cestrillo. 1982. Influence of Metabolizable Energy Content of the Diet and of Feeding Level on Lamb Performances and Groth of Body Composition. Lives. Prod. Sci. 9: 471-484.

Triatmojo, S. 1991. Analisis Proksimat Otot Longissimus Dorsi dan Bices Femoris pada Aras Energi Pakan yang Berbeda. Laporan Penelitia. Fakultas Peternakan Universitas Gadjah Mada, Yogyakarta.

Tillman, A.D, H. Hartadi, Soedomo-Reksohadiprojo, S. Prawirokusumo, dan S. Lebdosukotjo. 1998. Ilmu Makanan Ternak Dasar. Gadjah Mada University Press, Yogyakarta.

Van Nevel, C. J., and D. I. Demeyer. 1995. Lipolysis and biohydrogenation of soybean oil in the rumen in vitro: Inhibition by antimicrobials. J. Dairy Sci. 78:2797-2806.

Van Soest, P. J. 1994. Nutritional Ecology of The Ruminant. Cornele University. Ithaca and London.

Weismer-Pederson, J. 1971. Chemistry of Animal Tissue: Water. In: The Science of Meat and Meat Products. 2nd Ed. W.H. Freeman and Co. San Fransisco.

Wheller, T.L., G.W. Davis, B.J. Stocker, and C.J. Hammond. 1987. Cholesterol concentration of longisimus muscle, subcutaneous fat and serum of two beef cattle breed types. J. Of. Anim. Sci. 65(6): 1531-1537.

O-39-9

SILAGE QUALITY OF BMR SORGHUM AND SWEET SORGHUM GROWTH ON SEDIMENTATION ULTISOL SOIL

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Introduction

Sorghum (*Sorghum bicolor L.*) was one of important cereal plant with high biomass potential to support forage production and has ability to easy growing on Indonesian soil types. Sorghum plants tolerant to drought and water logging condition, still productive on marginal soil, and relatively resistant to pests or diseases (Sirappa, 2003). Whole sorghum biomass (herbage and grain) can be used as ruminant feed silage based industry. Nowadays utilization of forage-based sorghum used sorghum conventional varieties which are not designed as livestock feed and have potential conflict with food and energy sources. The use of conventional sorghum varieties are constrained due to high lignin content. Mutation breeding techniques produced promising sorghum mutant line for forage with low lignin content and high nutrition forage quality. Initially brown midrib (bmr) lines was produced in some grasses species containing low lignin. Bmr sorghum was obtained by gamma ray irradiation at 250 Gy for producing sorghum mutant lines containing low lignin, which is possible to be used for forage sources in animal

Materials and Methods

Sorghum cultivation and silage production was conducted on September 2013 until March 2014 started with sorghum cultivation in Lalowiu, Southern Konawe Residence, Southeast Sulawesi Province. The location latitude was 4°07'366" SL and 122°48'104" WL with altitude ± 51.6 ASL. The geographic position of research site located on tropic area with annual rainfall recorded as 1816.13 ml year^{-1}, relative humidity 84% (minimum 35% and maximum 98%) and daily temperature 20°C - 38°C. Cultivation location was former swamp area which has drained and categorized as ultisol soil and analyzed result show high acidity soil. Four organic fertilizers (OF) levels (0 ton ha^{-1}, 10 ton ha^{-1}, 20 ton ha^{-1}, and 40 ton ha^{-1}) became one of two research treatment beside sorghum varieties/ lines (Numbu (NB), CTY-33 (CT), PATIR3.2 (P2), and PATIR3.5 (P5)) as second factors. NB and CT varieties were conventional sweet sorghum and it were National Released, while P2 and P5 lines were bmr sorghum. Basic fertilization with NPK applied on 15 and 30 days after sowing (DAS) during research period with comparison N: P: K equal with 4:3:2 (g/g) dosage on 270 kg ha^{-1}. The harvesting held on 80% population blooming with assumed best nutrition and production phase of sorghum as forage.

The silage was produced by chopping 2 - 5 cm long of whole sorghum plant which was harvested on blooming condition requirement and wilting at 24 hours to decrease water content for ensilage process proper level. Ensiling process started by compacting chopped sorghum into three layers plastic bag to minimize oxygen infiltration and sun light inside. The last plastic bag was tightened using plastic rope, as balled silage and stored at warehouse for 21 days before silage harvesting, in which generally lowest silage pH was reached/ 5th phase (Schroeder, 2004). All parameters were analyzed *i.e*: (1) Water Soluble Carbohydrate (WSC) content obtained by composite each replications of forage before and after ensilage process, and prepared using phenol preparation according Jiang and Huang (2001) and measured using Calorimetric method (Dubois *et al*. 1956) on spectrophotometer (LW-200 Series, λ 200 - 1000 µm model), (2) silage pH value was measured on silage harvesting by juicing and screening 10 grams of silage sample and 100 ml distillate water with double replications. Another parameters were obtain on laboratory test include: (3) Dry Matter (DM) contain, was tested by proximate procedure, (4) *Fleigh* values were calculated using Otzurk *et al*. (2006) formula, based on DM content and silage pH: *Fleigh* = 220 + [(2 × DM(%)) - 15] - (40 × pH).

Discussion

Silage is one of feed and forage preservation techniques on certain level of water content through microbial fermentation by lactic acid bacteria called as ensilage and occur on place which named as silo (McDonald *et*

al. 2002). The suitable plant species as silage material should be have high field dry matter production, high digestibility, low buffer capacity, and higher water soluble content (Demirel 2011). Sorghum as rich carbohydrate plant was suitable for fermented based forage. Whole sorghum plant and its bioprocess several side products can be optimized as ruminant feed (Whitfield *et al.* 2012).

Microbes' utilization on fermentation technology, especially lactic acid bacteria (LAB), needed energy source for them to produce fermented product as forage preservation. Forage's WSC was microbes' available energy to produce lactic acid during ensilage process. Lack of WSC would inhibit fermentation, but the excess caused its utilization as harmful organism substrate during silage storage and feed out periods (Cherney, 2000).

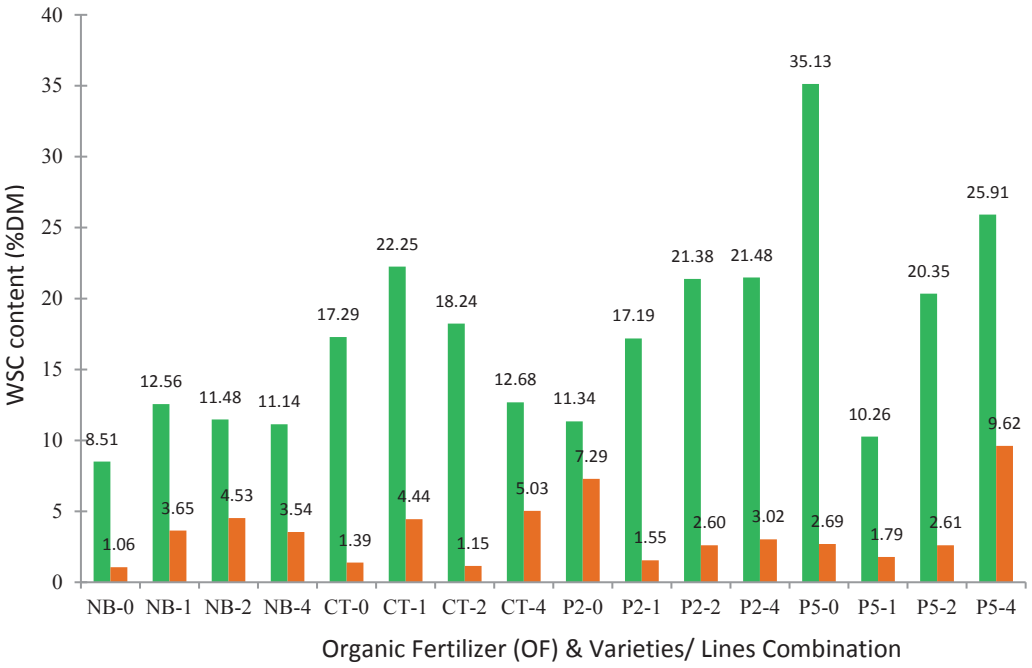

Figure 1. WSC content of forage and silage (%DM), NB: Numbu, CT: CTY-33, P2: PATIR3.2, P5: PATIR3.5, 0: control, 1: OF 10 ton ha^{-1}, 2: OF 20 ton ha^{-1}, 4: OF 40 ton ha^{-1}

Lactic acid was main preserve agent on ensilage process which produced by WSC and organic acid bacteria catabolism. For this reason, WSC content of forages which suitable for silage material were 6 to 8% of DM (Whittenbury *et al.* 1967). Research result (Figure 1) showed the average WSC forage content of NB, CT, P2 and P5 varieties/ lines respectively were 10.92 17.62 17.85 and 22.91%. Based on WSC content on this research, all sorghum varieties/ lines were proper to be used as silage material. WSC loses during ensilage process were calculate by comparing its content between forage and silage. Silage WSC content and it's losing during ensilage on this research respectively (Figure 1.) was: NB 3.19-(71.82), CT-33 3.00-(81.50), P2 3.61-(75.12) and P5 4.18-(81.25)%. WSC decrease rapidly on early two days silage fermentation process (Yang *et al*. 2006), and it was needed by lactic acid bacteria for pH decreasing up to 3.5 (Muck 2011).

The research showed sweet sorghum varieties have higher DM content if compared with bmr lines (Table 1). Highest DM content was NB varieties (20.60%), while the lowest was two bmr lines (P5 and P2 respectively: 18.89 and 18.54%). This DM result was nearby other result by Podkowka and Podkowka (2011) which found sorghum silage DM content around 20.88%.

Whole sorghum silage can be obtained by early harvesting, while its grain still on soft dough maturity stage. The maturity stage will affect on ensilage product. Fermentation acid increase comes from higher moisture content, and it's generally founding on younger sorghum plant. Some case explaining that acid lactic production level was connected with increasing of product quality, but it was affected on decreasing of DM quantity (Wall and Ross, 1970). The proper mature sorghum will produce higher quality and better characterise silage. Silage from immature sorghum plant usually has high acidity on ensilage process (Ahlgren 1956).

Table 1. Sorghum Silage Dry Matter (DM), pH, Fleigh Value, and Crude Protein (CP)

Variety/ line	Variables	Organic fertilizer levels (ton ha^{-1})				Averages
		0	10	20	40	
Numbu	DM (%)	20.64±0.72	20.72±0.51	20.51±2.89	20.55±1.24	20.60±1.40a
	pH	3.55±0.20	3.70±0.17	3.72±0.08	3.80±0.05	3.69±0.15a
	Fleigh	104.27±09.26	98.44±06.31	97.35±02.86	94.09±03.86	98.54±06.51a
CTY33	DM (%)	21.17±0.79	19.41±1.33	19.65±1.27	19.64±1.44	19.97±1.28ab
	pH	3.83±0.13	3.68±0.18	3.73±0.10	3.50±0.17	3.69±0.18a
	Fleigh	94.00±03.74	96.49±08.38	94.98±06.08	101.61±09.44	97.44±07.49a
PATIR3.2	DM (%)	19.03±3.65	17.03±0.53	19.41±0.72	18.69±1.40	18.54±1.96 b
	pH	4.12±0.67	4.28±0.08	3.93±0.28	4.07±0.33	4.10±0.37b
	Fleigh	78.40±33.31	67.73±02.04	86.48±11.18	79.72±16.03	78.08±17.92b
PATIR3.5	DM (%)	18.96±1.87	18.56±0.85	19.43±3.03	18.60±2.57	18.89±1.94b
	pH	4.30±0.28	4.08±0.13	4.12±0.33	3.77±0.49	4.07±0.35b
	Fleigh	70.91±12.93	78.78±04.44	79.19±14.40	91.53±23.82	80.10±15.30b
Averages	DM (%)	19.95±2.07	18.93±1.58	19.75±1.95	19.37±1.70	
	pH	3.95±0.44	3.94±0.29	3.88±0.26	3.78±0.34	
	Fleigh	86.89±20.88	86.66±14.19	89.37±11.18	92.40±15.90	

Number followed by different alphabetic in same variable's row indicate significant different ($p<.05$)

pH was one of general main criteria to evaluate silage fermentation process. Generally, lower pH reflected better and stable silage preservation (Seglar 2003) and lactic acid content (Amer *et al*. 2012). There was no interaction ($p>.05$) between OF levels and sorghum varieties/ lines on silage pH (Table 1). Silage pH was influence ($p<.05$) by sorghum varieties/ lines rather than FO levels. Sweet sorghum silage pH was lower (3.69) compare with bmr lines silage pH (4.10 and 4.07). Similar on Di Marco *et al*. (2009) that compare silage pH on three types of sorghum seed producer, sweet, and bmr sorghum sweet sorghum silage pH was the lowest one (3.86), seed producer (3.95) and bmr sorghum (4.08).

Fleigh value determined by DM content and pH of silage. Better *Fleigh* value will be obtained on higher DM and lower pH. The high DM content reflected ensilage process able to preserve/ keep the material, while low pH reflected ensilage process was occur properly. The minimal material lost, low pH, silage structure and flavour indicated ensilage process occur properly and it's should be have high silage recovery (Yosef *et al*. 2009). *Fleigh* value as silage quality evaluation in this research (Table 1) has been influenced by sorghum varieties/ lines ($p<.05$), and there was no interaction ($p>.05$) between OF level and varieties/lines. Sweet sorghum varieties have higher *Fleigh* value compare with bmr lines. Silage was categorized as very good quality if they have Fleigh value for 85 - 100, good 60 - 80, average 50 - 60, fair 25 - 40, and poor if et al. 2006). Based on this criteria, sweet sorghum varieties silage quality was categorized as very good (NB 98.54 CT 97.44), while bmr lines silage quality was good (P2 78.08 P5 80.10).

Conclusions

The result generally showed silage quality made of Numbu and CTY-33 varieties were classified as very good silage quality (*Fleigh* Value: 98.54 - 97.44), while PATIR3.2 and PATIR3.5 mutant lines were classified as good silage quality (*Fleigh* Value: 78.08 - 80.10). Another good sign was found that there was little difference between forage nutrient compare with sorghum silage nutrient. It was mean favourable fermentation occurred during ensilage process.

KEYWORD : Silage, Bmr, Ultisol, Fleigh Value

REFERRENCES

Ahlgren GH. 1956. Forage corp 2nd edition. New York (US):McGraw-Hill Book Company INC.

Amer S, Hassanat F, Berthiaume R, Seguin P, Mustafa AF. 2012.Effects of water soluble carbohydrate content on ensiling characteristics, chemical composition and in vitro gas production of forage millet and forage sorghum silages. *Animal Feed Science and Technology* 177 (2012) 23– 29 29.

Cherney DJR. 2000. Forage Evaluation in Ruminant Nutrition. Givens DI, Owen E, Axford RFE, Omed HM, editor. Oxon (UK): CABI Publishing.

Demirel R, Akdemir F, Saruhan V, Demirel DS, Akinci C, Aydin F. 2011. The determination of qualities in different whole-plant silages among hybrid maize cultivars. *Afr. J. Agri. Res*. Vol. 6(24), pp. 5469-5474, 26 October 2011.

Di Marco ON, Ressia MA, Arias S, Aello MS, Arzadun M. 2009. Digestibility of forage silages from grain, sweet and bmr sorghum types: comparison of i*n vivo, in situ* and *in vitro* data. *Animal Feed Science and Technology* 153 (2009) 161–168.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analy. Chem*. 28:350–356.

Jiang Y, Huang B. 2001. Osmotic adjustment and root growth associated with drought preconditioning-enhanced heat tolerance in kentucky bluegrass. *Crop Science* 41:1168-1173.

Kjeldahl J. 1883. A new method for the determination of nitrogen in organic matter. *Zeitschreft fur Analytische Chemie*. 22: 366.

McDonald P, Edwards R, Greenhalgh J. 2002. Animal Nutrition. 6th. New York (US): Scientific and Tech John Willey & Sons. Inc.

Muck RE. 2011. The art and science of making silage. Proceedings Western Alfalfa and Forage Conference Las Vegas, 11-13 December 2011.

Ozturk D, Kizilsimsek M, Kamalak A, Canbolat O, Ozkan CO, 2006. Effects of ensiling alfalfa with whole-crop maize on the chemical composition and nutritive value of silage mixtures. *Asian-Australasian Journal of Animal Sciences* 2006;19(4): 526-532.

Podkowka Z, Podkowka L. 2012. Chemical composition and quality of sweet sorghum and maize silages. *Journal of Central European Agriculture*, 2011, 12(2), p.294-303.

Seglar B. 2003. Fermentation analysis and silage quality testing. *Proceedings of the Minnesota Dairy Health Conference*, College of Veterinary Medicine, University of Minnesota. May 2003

Schroeder JW. 2004. Silage fermentation and preservation.Quality Forage NDSU Extension Service. [Internet]. [Juni 2004]. North Dacota (US): North Dacota State University. hlm 1-7;[accessed 2014 Jul 12]. Available on : http://www.ag.ndsu.edu/pubs/ansci/range /as1254.pdf.

Sirappa MP. 2003. Prospek pengembangan sorgum di Indonesia sebagai komoditas alternatif untuk pangan, pakan dan industri. *Jurnal Litbang Pertanian* 22(4).

Wall JS, Ross WR. 1970. Sorgum production and utilization: major feed and food crops in agriculture and food series. Westport Connecticut (US):The Avi Publishing Company, Inc.

Whitfield MB, Chinn MS, Veal MW.2012. Review: processing of materials derived from sweet sorghum for biobased products. *Industrial Crops and Products* 37 (2012): 362– 375.

Whittenbury R, McDonald P, Bryan-Jones DG. 1967. A short review of some biochemical and microbiological aspects of ensilage. *J. Sci. Fd Agric*. 18, 441-444.

Yang HY, Wang XF, Liu JB, Gao LJ, Ishii M, Igarashi Y, Cui ZJ. 2006. Effect of water-soluble carbohydrate content on silage fermentation of wheat straw. Journal of Bioscience and Bioengineering.Vol.101, No. 3, 232-237.

Yosef E, Carmi A, Nikbachat M, Zenou A, Umiel N, Miron J. 2009. Characteristics of tall versus short-type varieties of forage sorghum grown under two irrigation levels, for summer and subsequent fall harvests, and digestibility by sheep of their silages. *Anim. Feed Sci. Technol*. 152, 1–11.

O-40-1 ESTIMATION OF MICROBIAL PROTEIN SYNTHESIS BASED ON EXCRETION OF PURINE DERIVATES USING SPOT SAMPLING METHODE IN FAT-TAIL AND THIN-TAIL SHEEP

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INTRODUCTION

The fluctuation of feed nutrient supply was one of the major limiting factor in livestock production in developing countries (Makkar, 2004). Microbial protein was one of the most important protein sources for ruminant. It provides variety nutrients including the majority of the amino acid available for the animals which depend on diet based on roughage (Nolan and Khan, 2004 FAO and IAEA, 1997). Therefore, the study of the microbial contribution to the nutrition of the host animal is imperative for developing feed supplementation strategies to improve ruminant production.

Various methods were used to determine microbial protein production based on internal and external markers. These methode was less applicable, need cannulated animal and contrast to animal welfare. Therefore, it was required a better method that relatively simple and invasive such as a method based on purine derivatives (PD) total excretion method. The PD total excretion method needs a total collection of 24 hours urine, it was less applicable in the field and need to be modified by using spot sampling technique. Spot sampling technique requires the concentration of urinary creatinine that combined with concentration of PD, in the form of PD:Creatinine ratio (PDC index) (Chen and Ørskov, 2003).

Purine derivatives was known as the result of purine bases metabolism in the animal body (Ørskov, 2003). Each species and breeds have different metabolic tendencies. This is caused by the different genetic factors (Yusiati, 2005). Genetic and environmental differences between two sheep breeds could raise potential difference in the digestive process, including rumen microbial protein synthesis. Thin-Tailed Sheep (TTS) and Fat-Tailed Sheep (FTS) were considerably breed in the small farms in Indonesia. It was necessary to have a study on the rumen microbial contribution to the host, by using PD excretion method with spot sampling technique in (TTS) and (FTS).

METHODOLOGY

This research was conducted in 10 days of adaptation period and followed by 8 days of collection period. Six male sheep from each breeds (TTS and FTS), 18 months old, and 20.5 kg average body weight, were placed in individual cage. Peanut crop straw as a single diet and water were provided ad libitum. Feed, residual feed, and feces samples were collected to determine the DM by drying the sample in 100^{o}C and OM by ashing the sample in 550^{o}C (AOAC, 2007), whereas urine samples were collected every three hours for four days in spot sampling method and daily for eight days in total collection method to determine PD and creatinine concentration (Chen dan Gomez, 1995). The data of each breed (TTS and FTS) were analyzed using t-test. The data concentration of PD and creatinine of spot sampling urine were used to calculate the ratio of PD:Creatinine (PDC index). PDC index then correlated with total excretion of PD and multiplied by metabolic body weight ($W^{0.75}$) to estimated daily total excretion. This total excretion can be used to estimated microbial protein synthesis with linear equation for TTS and FTS respectively $Y=0.84X+(0.143\ W^{0.75}e^{-0.25X})$ and $Y=0.84X+(0.091\ W^{0.75}e^{-0.25X})$ (Yusiati et al., 2015). The results of the correlation test between urinary PD:C concentration and total PD excretion, show the equation and apropriate time of spot sampling to be applied to the PD test on FTS and TTS.

RESULTS AND CONCLUSION

Concentration of PD and Creatinine

Concentration of allantoin, uric acid, xanthin-hypoxanthine, PD and ceatinine from TTS and FTS urine was showed in Table 1.

Table 1. Concentration of allantoin, uric acid, purin derivate and creatinine of FTS and TTS (μmol/L)

Concentration[ns] (μmol/L)	Sheep breed	
	TTS	FTS
Allantoin	3507.47± 996.27	2475.15± 1321.03
Uric Acid	329.45± 178.47	205.91± 72.99
Xanthine-Hypoxanthine	316.59± 55.22	345.47± 95.53
Purine Derivate	4168.31± 1148.91	3026.53± 1444.29
Creatinine	3364.49± 1097.55	2928.89± 1177.76

[ns]) not significant

In this research, peanut crop straw was given as single diet for both breeds. This is intend that there is no potential difference which came from feed factor. If there is any difference, it is due to the difference of breeds. This recent study, revealed that urinary allantoin, uric acid, PD and creatinine concentration (μmol/L) in TTS were not differ compared to FTS. Factors affecting the concentration of PD which are a source of energy, protein feed and availablility in the rumen (Andrade-Montemayor et al., 2009) and the volume of urine excreted (Yusiati, 2005). Creatinin concentration in urine is not depend on feed and physiology status of animals, but it is depend on body weight (George et al., 2006). Concentration of PD compounds and creatinine could be used to measure the total daily excretion of those compounds, both in units of μmol/day or μmol/$W^{0.75}$.

Excretion of PD and creatinine

Urinary excretion of allantoin, uric acid, xanthin-hypoxanthin, PD and creatinine in TTS and FTS both in units of μmol/day or μmol/$W^{0.75}$ was showed in Table 2.

Table 2. Total daily excretion of Allantoin, Uric Acid, xanthin-hypoxanthin, PD and Creatinine in TTS and FTS.

Excretion [ns] μmol/day	Sheep breed	
	TTS	FTS
Allantoin	4430.70± 900.91	3328.69± 1282.98
Uric Acid	436.75± 26.73	442.84± 11.57
Xanthine+Hypoxanthine	435.08± 68.93	549.23± 126.67
Purin Derivate	5302.53± 1104.08	4189.67± 1371.83
Creatinine	4242.27± 846.92	4236.97± 1213.69
Excretion μmol/$W^{0.75}$	**Sheep breed**	
	TTS	**FTS**
Allantoin*	491.04± 52.36[b]	330.73± 97.45[a]
Uric Acid [ns]	48.18± 3.84	31.26± 1.38
Xanthine+Hypoxanthine [ns]	45.91± 4.89	51.32± 10.50
Purin Derivate**	585.86± 72.64[b]	413.67± 100.75[a]
Creatinine[ns]	471.34± 56.28	414.16± 86.27

[ns]) not significant
*[a,b]) different superscript at the same row indicate significant differences (P<0.05).
** [a,b]) different superscript at the same row indicate significant differences (P<0.01).

Total excretion of allantoin, uric acid, xanthine+hypoxanthine and PD in unit (μmol/day) was not significantly different between TTS and FTS. Based on metabolic weight unit (μmol/$W^{0.75}$), TTS was significanly higher than FTS in allantoin and PD excretion. The difference of PD excretion which was corrected by metabolic weight and same diet showed the effect of the difference of breed. Total PD excretion in TTS reached the value 585,86

μmol/$W^{0.75}$/day, while in FTS was 362.36 μmol/$W^{0.75}$/day. The relative proportions (%) of the different derivates (allantoin: uric acid: xanthin+hypoxanthine) was 83.74: 7,95: 8,30 in TTS and 78,30: 7,88: 13,82 in FTS. In both of breeds allantoin concentration was the highest propotion. Xanthin+hypoxanthine was higher than uric acid, it was in accordance with (Poshiwa et al., 2005). It was suggested that high roughage diets may promote xanthin+hypoxanthine higher than uric acid.

Total excretion of PD could be used to Estimated Microbial Nitrogen Synthesis (EMNS) with linear equation for TTS and FTS respectively $Y=0.84X+(0.143\ W^{0.75}e^{-0.25X})$ and $Y=0.84X+(0.091W^{0.75}e^{-0.25X})$ (Yusiati et al., 2015). Based on this study also known the value of EMNS was 4.33 g N/day for TTS and 3.38 g N/day for FTS. This result was in acordance with Mupangwa et al. (2000) which reported that EMNS of sheep given by tropical legume was 2.70-4.30 g N/day. Microbial Nitrogen Synthesis Efficiency (MNSE) which were calculated by devided (EMNS) with DOMR. In the TTS was significanly higher than FTS (13.13 vs 9.47 g N/kg DOMR), this result was lower than result have been reported by Chen and Gomes (1995) was 23.4 g N/kg DOMR in the european sheep breed.

Spot Sampling time

Table 3 shows Correlation test between PDC index in each time of the spot sampling with total PD excretion in the TTS. The result of correlation test between PDC index in each time of the spot sampling with total PD excretion revealed that, there are four spot sampling time of TTS that had a significant correlation with the total PD excretion. On the other hand, there is no spot sampling time of FTS that had significant correlation with the total PD excretion.

Table 3. Correlation test between PDC index in each time of the spot sampling with total PD excretion in the TTS

spot sampling time	N treatment	P	R^2	linear regression equation
07.00 - 10.00*	6	<0.05	0.7362	y= 287.07+28.57x
10.00 - 13.00	6	>0.05	0.1371	y= 420.78+15.07x
13.00 - 16.00	6	>0.05	0.0558	y= 423.92+11.71x
16.00 - 19.00	6	>0.05	0.6248	y= 328.27+19.70x
19.00 - 22.00	6	>0.05	0.3005	y= 253.89+27.04x
22.00 - 01.00*	6	<0.05	0.7607	y= 260.23+24.49x
01.00 - 04.00**	6	<0.01	0.8540	y= 83.082+33.52x
04.00 - 07.00*	6	<0.05	0.6858	y= 290.24+29.73x

*) significant differences (P<0.05)
**) significant differences (P<0.01).

This research also revealed the most applicable time with highest correlation between urinary PD:C concentration and total PD excretion of TTS was 7:00-10:00 am with linear regression equation y= 287.07+28.57x (0-3 h after feeding), which was similar range of time reported by Salman et al. (2013) that, spot sample time were collected between 10:00 am and 12:00 am (2 h after grazing) in grazing karayaka sheep.

It can be concluded that TTS was more efficient to convert DOMR (kg) to be microbial protein (g N). PD:C concentration in spot sampling urin in TTS can be used to estimate total PD's excretion which can be aplicated on field to estimate microbial protein synthesis. The most applicable time with highest correlation between urinary PD:C concentration and total PD excretion of TTS was 7.00-10.00 am with linear regression equation y=287.07+28.57x.

KEYWORD : Purine derivates excretion, Spot sampling method, Fat-tail sheep, Thin-tail sheep

REFERENCES

Andrade-Montemayor, H., T.G. Gasca, and J. Kawas. 2009. Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. R. Bras. Zootec. 38: 277-

291.
Association of Analytical Communities. 2007. *Official Method of Analysis 18th ed*. Gaithersburg (US): AOAC International.
Chen, X.B., Gomes Jm. 1995. Estimation of Microbial protein supply to sheep and cattle based on urinary excretion of purine derivates and overview of technical detail. International feed resources unit. Rowett research institute, Bucsburn Aberdeen, UK. Occasional Publication, pp. 1-21.
Chen, X.B. and Orskov. 2003. Research on urinary excretion of purine derivates in ruminants: past, present and future. Review. International feed resourcesunit. Macaulay land use research institute, Craigiebuckler Aberdeen, UK.
FAO and IAEA. 1997. Estimation of rumen microbial protein production from purine derivates in urin. A laboratory manual. Viena, Austria
George, S.K., M.T. Dipu, U.R. Mehra, A.K. Verma, and P. Singh. 2006. Influence of level feed intake on concentration of purine derivatives in urinary *spot samplings* and microbial nitrogen supply in crossbred bulls. Asian-Aust. J. Anim. Sci. 19: 1291-1297.
Makkar, H. P. S. 2004. Development, standardization and validation of nuclear based technologies for estimatinh microbial protein supply in ruminant livesctock for improving productivity. In: Makkar, H. P. S., Chen, X. B. Estimation of microbial protein supply in ruminants using urinary purine derivates. IAEA. Kluwer academic pub, Vienna.
Mupangwa JF,. Ngongoni N.T, Topps J.H, Acamovic T., Hamudikuwanda H., Ndlovu L.R. 2000. Dry matter intake, apparent digestibility and excretion of purine derivatives in sheep fed tropical legume hay. Small Rumin Res. 2000 Jun 1;36(3):261-268.
Nolan, J.V., Khan, L.P. 2004. The use of urinary excretion of purine metabolites as an index of microbial protein supply in ruminanat, Estimation of microbial protein supply in ruminants using urinary purine derivates. IAEA. Vienna, Austria.
Poshiwa, X., Ngongoni, N. T., Manyuchi, B., Chakoma, C., Tigere, A. 2004. The effect of plane nutrition on the urinary purine derivative excretion in sheep and goats. In: Makkar, H. P. S., Chen, X. B. Estimation of microbial protein supply in ruminants using urinary purine derivates. IAEA. Kluwer academic pub, Vienna.
Yusiati, L. M. 2005. Development of Microial protein synthesis method using purine derivates excretion of some Indonesian ruminant species. Disertasi. Faculty of animal science. UGM, Yogyakarta.
Yusiati, L.M., Widyobroto, B.P., Bachruddin, Z., Hanim, C., Aulyani, T.L., Dughita, P.A. 2015. Microbial rumen protein synthesis estimaation based on purine derivates excretion with spot sampling method in Thin Tailed sheep and fat tailed sheep. Final research report of postgraduate research grant programme faculty of Animal Science universitas Gadjah Mada.

O-40-2

Effect of Fungal Treated Oil Palm Fronds in the Diet of Goats

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INTRODUCTION

Thailand is a leading country in palm oil production, which is associated with production of a variety of agricultural co-products which are leaves and petioles from oil palm trees which also called oil palm fronds (OPF). OPF in particular has been given emphasis lately with great potential to be utilized as a roughage source or as a component in complete feed for ruminants. However, the use of oil palm fronds in livestock production is limited for their complex biological structure, low protein content (Ishida and Abu Hassan, 1997), and as up to 20% of their dry biomass is lignin. This is consistent with low OPF digestibility in ruminant. As a result, OPF have a low energy value, varying between 4.9 and 5.6 MJ metabolisable energy (ME)/kg DM (Zahari and Alimon, 2005). In order to break down the lignocellulosic bonds and to increase bioavailability of nutrients, various physical and chemical delignification methods have been examined in agricultural co-products such as rice and wheat straw (Hamed and Elimam, 2010). Although these methods have advantages, those are costly, low in effectiveness, and not environmentally friendly. And also, application of technology is required. Additionally, colonization with white rot fungi (WRF) is considered to be a promising technique because of its preferential degradation of lignin (Okano et al., 2005). This biological treatment has already been used to improve the nutritive value and ruminal digestibility of poor quality forages (Zadrazil et al., 1997 Okano et al., 2005). However, few experiments have been investigated on the effects of feeding biological treated OPF for ruminants including in the utilization of fungal (*Lentinus sajor-caju*) treated oil palm fronds (FTOPF) to *in vitro* and *in vivo* digestibility by goats. Therefore, this study was conducted to investigate the utilization of FTOPF in the diet of goats and compared it with untreated OPF (UOPF).

MATERALS AND METHODS

Treatment of oil palm fronds: A *Lentinus sajor-caju* strain was selected and used in this study. The study was divided into 3 treatments as follow: i) Initial oil palm frond without inoculation of *Lentinus sajor-caju* mycelia (control), ii) Oil palm frond and inoculation with *Lentinus sajor-caju* mycelia, and iii) Oil palm frond + 1% urea and inoculation with *Lentinus sajor-caju* mycelia. Before inoculating with the white rot fungi (WRF), the study adjusted the moisture content of OPF at 600-650 g/kg by adding 45 ml of distilled water to 40-45 g OPF directly in each inoculation recipient.

For the preparation of spent oil palm frond (OPF), fresh OPF were chopped into 1-2 cm length and air dried at ambient temperature (30-35°C). Then prepared OPF was packed in plastic bags (30x45 cm), and autoclaved twice at 100-102°C for 2 h with cooling between cycles. The autoclaved OPF were removed from plastic bags, inoculated with sorghum grain spawns of *Lentinus sajor-caju*, LSc) cultures at the rate of 3-5% w/w (fresh weight basis), and re-packed into plastic bags. The culture bags were transferred into the fermentation room and incubated at room temperature (28-32°C) or full colonization of the mushroom mycelia on the substrate for 21 days. After 21 days of incubation, all bags were removed from the fermentation room. Then the plastic bags were removed from the oil palm frond substrates and sun dried by spreading on plastic sheet for 3-5 days. The air-dried spent oil palm fronds were packed in plastic bags (45×90 cm) and stored at room temperature until feeding for the goats.

Animals, design and experimental diets: Six male crossbred (Thai native x Anglo Nubian) goats (average BW 33.5+1.70 kg) were randomly assigned according to a 3x3 replicated Latin square design to investigate the effect of fungal treated oil palm frond on feed intake and blood metabolites. There were three treatments of i) untreated oil palm frond (UOPF), ii) fungal treated oil palm frond (FTOPF), and iii) fungal treated oil palm frond with urea 1% (FTOPFU). Three experimental diets were formulated with roughage: concentrate ratio of 30:70. The roughage component of the diets included 30% of oil palm frond either untreated (UOPF) or treated with *Lentinus sajor-caju* (FTOPF) with or without urea (FTOPFU) (Table 1). The diets were formulated to provide the nutrient allowances to meet or exceed the NRC (1981) requirements of growing goats.

All goats were kept individually in pens (0.50x1.20m) under well-ventilated sheds where water and mineral salt were available at all time. The experiment was conducted for 3 periods, and each period lasted for 21 days. During

the first 14 d of each period, all animals were fed by respective diets for *ad libitum* intake, whereas during the last 7 d, the animals were moved to metabolism crates for total collection during the time goats with restriction to 90% of the previous voluntary feed intake to ensure total feed intake. Feeds were provided as total mixed ration (TMR) twice times in two equal portions daily at 0800 and 1600 h. For determination of daily DMI, refusals were collected and weighed daily before feeding. Feed samples obtained each time were oven dried at 60℃ for 72 h, grounded to pass through a 1-mm sieve, and composited by period on an equal weight basis, and analyzed for DM, ether extract, ash, and CP content (AOAC, 1995). Digestion coefficients were calculated using the formula given by Schneider and Flatt (1975). Goats were individually weighed before the morning feeding at the beginning and ending of each experimental period. At the end of each period, rumen fluid was collected from all goats by using a stomach tube at 0 and 4 h-post feeding during the digestibility trial. This was strained through 4 layers of cheese cloth and pH measured immediately using a pH meter (HANNA instruminalts HI 98153 microcomputer pH meter, Singapore) fitted with a combined electrode. The ruminal fluid was then acidified with 3 mL of 1 M H_2SO_4 added to 30 mL of ruminal fluid. The mixture was centrifuged at 16,000 ´ *g* for 15 min, and the supernatant was stored at -20℃ before NH_3-N analysis by using the micro-Kjeldahl methods (AOAC, 1995). Blood samples (about 10 mL) were collected from a jugular vein into tubes containing of 12 mg of EDTA. Plasma was separated by centrifugation at 2500 × *g* for 15 min at 5℃ and stored at -20℃ until analysis. Plasma glucose, insulin, BHBA, and packed cell volume (PCV) were measured by using commercial kits (No. 640, Sigma Chemical Co., St. Louis, USA). All data were subjected to the analysis of variance by using Proc. GLM and treatment means were performed and compared by using Tukey's significant test. Contrasts were considered significant when the P-value was ≤0.05, with a p-value of ≤ 0.10 considered as a tendency approaching significance.

RESULTS AND DISCUSSION

The results showed that (Table 2) No significant differences attributable to dietary treatments were observed in DM intakes (total DMI, %BW, and g/kgW0.75), although the average DM intake was numerically higher in FT groups. Total tract digestibility of DM, OM, CP, NDF, ADF, and ADL were significantly ($p<0.05$) higher in both FTOPF and FTOPFU than UOPF. However, there is no information about the intake of fungal treated oil palm frond by goats. Our results were in agreement with a study by Fazaeli et al. (2004) who fed diets containing fungal treated wheat straw to native bulls, and reported that total tract digestibility of DM, OM, and gross energy were significantly ($p<0.05$) higher in fugal treated wheat straw and spent wheat straw (treated straw after harvesting of mushroom) than untreated wheat straw. Such improvements could be as a result of the changes in non structural carbohydrate to structural carbohydrate ratio of the straw (Tan et al., 2002). These results were supported by the findings of the *in vitro* digestibilities of this study and other reports (Zadrazil et al., 1997). Ruminal pH was unchanged by dietary treatments in this study (Table 3), was within the optimum range for cellulolytic bacteria activity (Russell and Wilson, 1996), and also digestion of protein (6.0-7.0). Likewise, NH_3-N and BUN concentration were similar among treatments. It was close to the optimal level in normal goats which had been reported in the ranges of 11.2 to 27.7 mg/dL (Lloyd, 1982). No significance ($p>0.05$) of FTOPF inclusion was detected for blood glucose, insulin, BHBA and PCV and all were within the normal ranges 50-75 mg/dL and 22-38 mg/dl, respectively (Lloyd, 1982). In the present experiment, these data indicate that goats consuming the diets with FTOPF were in a normal energy status. This may be the possible reason for the lack of differences among treatments, and there were no deleterious effects on feed intake or the metabolism of the goats. Based on the experimental data, FTOPF and FTOPFU can be used as substitution for UOPF in total mixed ration, Thus, FTOPF or FTOPFU could be a beneficial alternative source of roughage for ruminants.

KEYWORD : Fungal Treatment, Oil Palm Fronds, Goats

Table 1. Ingredients and chemical composition of the experimental diets (% DM basis)

Items	Dietary treatments		
	UOPF	FTOPF	FTOPFU
UOPF[1]	30.0	-	-
FTOPF	-	30.0	-
FTOPFU	-	-	30.0
Ground corn, GC	45.0	45.0	45.0
Soybean meal, SBM	7.3	7.3	7.3
Fish meal	0.4	0.4	0.4
Leucaena leave meal, LLM	7.0	7.0	7.0
Palm kernel cake, PKC	7.0	7.0	7.0
Molasses	2.1	2.1	2.1
Dicalcium phosphate	0.4	0.4	0.4
Salt	0.2	0.2	0.2
Mineral and vitamin mix[2]	0.7	0.7	0.7
Estimated nutrients (%)			
CP	15.0	15.0	15.0
TDN	76.0	76.0	76.0

[1] Treatments: UOPF = untreated oil palm frond, FTOPF = Fungal treated oil palm frond, FTOPFU = Fungal treated oil palm frond with 1% urea.
[2]Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

Table 2. Effects of diet on feed intake and nutrient digestibility of goats

Item	Dietary treatments[1]			SEM[2]	Contrast, *p*-value	
	UOPF	FTOPF	FTOPFU		UOPF vs. FT	FTOPF vs. FTOPFU
Total DMI, kg/d	0.992	1.051	1.095	0.10	0.38	0.66
DMI, %BW	2.98	3.14	3.33	0.18	0.18	0.37
DMI, g/kg $W^{0.75}$	71.69	75.64	79.79	5.28	0.22	0.45
Nutrient digestibility, %						
DM	69.33[b]	73.06[a]	72.22[a]	0.39	0.03	0.58
OM	70.67[b]	74.57[a]	73.69[a]	0.39	0.04	0.58
CP	73.50[b]	77.50[a]	78.18[a]	0.80	0.01	0.64
NDF	56.61[b]	64.66[a]	62.49[a]	1.31	0.02	0.43
ADF	35.77[b]	43.47[a]	40.81[a]	1.05	0.05	0.41
ADL	23.50[b]	27.71[a]	29.33[a]	0.94	0.38	0.80

[1] Treatments: UOPF = untreated oil palm frond, FTOPF = Fungal treated oil palm frond, FTOPFU = Fungal treated oil palm frond with 1% urea, FT = Fungal treated, [2] SEM = Standard error of the mean (n = 6).

Table 3. Effects of diet on rumen fermentation of goats

Item	Dietary treatments[1]			SEM[2]	Contrast, *p*-value	
	UOPF	FTOPF	FTOPFU		UOPF vs. FT	FTOPF vs. FTOPFU
Temperature, oC	39.16	39.33	39.16	0.25	0.78	0.63
Ruminal pH	6.53	6.51	6.48	0.06	0.72	0.70
NH3-N, mg/dL	19.52	19.76	21.43	0.36	0.49	0.81
BUN, mg/dL	17.69	20.31	20.65	1.63	0.58	0.95
Glucose, mg/dL	84.66	83.25	82.80	2.27	0.47	0.91
BHBA, mg/dL	3.96	4.03	3.93	0.05	0.95	0.77
PCV, %	31.66	30.66	31.83	2.00	0.79	0.54

[1] Treatments: UOPF = untreated oil palm frond, FTOPF = Fungal treated oil palm frond, FTOPFU = Fungal treated oil palm frond with 1% urea, FT = Fungal treated, [2] SEM = Standard error of the mean (n = 6).

REFFERENCES

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Fazaeli, H., H. Mahmodzadeh, A. Azizi, Z. A. Jelan, J. B. Liang, Y. Rouzbehan and A. Osman. 2004. Nutritive value of wheat straw treated with *pleurotus* fungi. Asian-Aust. J. Anim. Sci. 17:1681-1688.

Hamed, A. H. M. and M. E. Elimam. 2010. Performance and digestibility in Nubian goats fed stream treated sorghum stover. Pak. J. Nutr. 9:298–301.

Ishida, M. and O. Abu Hassan. 1997. Utilization of oil palm frond as cattle feed. Japan Agric. Res. Quart. 31:41–47.

Lloyd, S. 1982. Blood characteristics and the nutrition of ruminants. Br. Vet. J. 138:70-85.

NRC. 1981. Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperate and Tropical Countries. National Academy Press, Washington, DC., USA.

Okano, K., M. Kitagawa, Y. Sasaki and T. Watanabe. 2005. Conversion of Japanese red cedar (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. Anim. Feed Sci. Technol. 120:235–243.

Russell, J. B. and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79:1503–1509.

Tan, Z. L., D.-X. Lu, W.-Y. Niu, C.-Y. Han, X.-P. Ren, R. Na and S.-L. Lin. 2002. Effects of dietary structural to nonstructural carbohydrate ratio on rumen degradability and digestibility of fiber fractions of wheat straw in sheep. Asian-Aust. J. Anim. Sci. 15:1591-1598.

Zadrazil, F. 1997. Changes in In vitro digestibility of wheat straw during fungal growth and after harvest of oyster mushrooms (*Pleurotus* spp.) on laboratory and industrial scale. J. Appl. Anim. Res. 11:37-48.

O-40-3

Nutritional Status of Kacang Goats Fed Ruminally Undegradable Protein to Improve their Productivity

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ABSTRACT

Kacang goat is one of the local goats in Indonesia that is usually reared by farmers. They are grazed during the day and housed at night fed only roughage without concentrate, therefore, their productivity is low. The aim of this study was to improve local goats productivity using ruminally undegradable protein (fish meal and soybean meal) in the ration. Twelve Kacang goats, 1-1.5-year-old, with the initial body weight of 17.84 + 1.57 kg (CV =8.80%) were used in this study and arranged with a Completely Randomized Design. The goats were fed by 3 different rations: T0 (control)= natural grass, T1 = *Pennisetum purpureum* (30%) + *Gliricidia* leaves (30%) + concentrate with fish meal and T2 = *Pennisetum purpureum* (30%) + *Gliricidia* leaves (30%) + concentrate with soybean meal. The ration was made total mixed ration containing 14% crude protein and 1% of the mineral mix, except for T0. The goats were fed about 4.5% dry matter ration of the body weight. Water was provided *ad libitum*. Parameters observed were an average daily gain (ADG), nutrients intake, digestible nutrients, and feed conversion ratio (FCR). Data were analyzed by analysis of variance and Duncan's Multiple Range Test. The ADG of T0 (41.57 g) was lower ($P<0.01$) than that of T1 (72.75 kg) and T2 (91.71 kg), because the DMI/$BW^{0.75}$, CP, ether extract and NFE intake of T0 was lower ($P<0.05$) than those of T1 and T2. The ADG of goats were higher in fish meal ration (T1) by 75.01% and in soybean meal ration (T2) by 120.62% over control (T0). The digestible energy conversion and FCR of T1 and T2 were better ($P<0.01$) than that of T0. It can be concluded that the use of ruminally undegradable protein (fish meal and soybean meal) in the ration can improve the productivity of Kacang goats.

INTRODUCTION

Kacang goat is one of the local goats in Java, Indonesia that is usually reared traditionally by farmers. They are grazed during the day and housed at night fed only roughage without concentrate, therefore, their productivity is low. Nowadays, the population of Kacang goats decreases gradually because their size is smaller and cheaper than other breeds so that farmers get less benefit. Mahmilia and Tarigan (2004) stated that Kacang goats are relatively small in size, about 55 cm of height at withers. Actually, although having small in size, their carcass percentage of Kacang goats were reported the same as Etawah goat and Kejobong goat that was about 40.86% (Sumardianto et al., 2013). Hutama (2014) stated that dressing percentage of Kacang goat reared intensively with slaughter weight of 18.52-21.33 kg were 46.67% (Hutama, 2014). In addition, Kacang goats are well adapted to their condition (Sianipar et al., 2005). Some efforts have to be done to increase their productivity so that farmers will get more benefit, and in the long run it will increase the population of Kacang goats.

Fish meal and soybean meal are protein source feedstuffs that are potentially not degraded in the rumen. Stern et al. (2006) reported that fish meal contained 65+4% ruminally undegradable protein of total crude protein, and was digested about 80+5% of them in the small intestine. Soybean meal contains 44-48% of crude protein (Cromwell, 1999) and about 73.4% of dry matter protein is undegraded in the rumen (Widyobroto et al., 2010). Therefore, the aim of this study was to improve Kacang goats productivity using ruminally undegradable protein (fish meal and soybean meal) in the ration.

MATERIALS AND METHODS

Twelve Kacang goats, 1-1.5-year-old (the incisors have erupted 1), with the initial body weight of 17.84 + 1.57 kg (CV =8.80%) were used in this study and arranged with a Completely Randomized Design. The goats were fed by 3 different rations: T0 (control)= natural grass, T1 = *Pennisetum purpureum* (30%) + *Gliricidia* leaves (30%) + concentrate with fish meal and T2 = *Pennisetum purpureum* (30%) + *Gliricidia* leaves (30%) + concentrate with soybean meal. The rations were set up to be made a total mixed ration containing 14 of crude protein and 1% of the mineral mix, except for T0. However, the composite sampling of the ration showed differently (Table 1). The goats were fed three times a day at 08.00, 12.00, and 16.00 about 4.5% dry matter ration of the body weight. Water was provided *ad libitum*. This experiment consisted of 3 periods: adaptation period (5 weeks), preliminary

period (2 weeks), and treatment period (12 weeks). Parameters observed were an average daily gain (ADG), nutrients intake, digestible nutrients, and feed conversion ratio (FCR). Data were analyzed by analysis of variance and Duncan's Multiple Range Test.

Table 1. Feed Ingredients and Nutrients Content in the Ration

Feedstuffs / Nutrients	T0	T1	T2
Feed ingredients:	---------- % ----------		
Natural grass	100	0	0
Pennisetum purpureum	0	30	30
Gliricidia leaves	0	30	30
Cassava waste product	0	20.10	19.20
Wheat bran	0	13.75	13.80
Fish meal	0	6.15	0
Soybean meal	0	0	7.00
Nutrients content in the ration:			
Dry matter (%)	18.58	91.26	91.53
Ash (100% dry matter)	12.06	10.41	10.11
Ether extract (100% dry matter)	2.37	2.48	2.56
Crude Fiber (100% dry matter)	34.62	29.68	29.18
Crude Protein (100% dry matter)	10.92	13.64	15.59
Nitrogen free extract (100% dry matter)	40.04	43.80	42.56
TDN (%)	63.23	56.21	57.95

RESULTS AND DISCUSSION

The average daily gain (ADG) of T0 (41.57 g) was lower ($P<0.01$) than that of T1 (72.75 kg) and T2 (91.71 kg), because the dry matter intake (DMI)/$BW^{0.75}$, Crude Protein (CP), ether extract, and Nitrogen-free extract (NFE) intake of T0 was lower ($P<0.05$) than those of T1 and T2 (Table 2). This indicated that Kacang goats prefer ration containing concentrate rather than mainly natural grass. This is because the protein content of T1 and T2 ration were higher than protein content of natural grass (T0). In this case, the higher ADG in T1 and T2 were because of higher nutrients intake. Grovum (1993) stated that palatability is one of the factors that influence ruminant intake. In addition, Riaz et al. (2014) stated that increasing crude protein content in the ration increased feed intake. The ADG of goats were higher in fish meal ration (T1) by 75.01% and in soybean meal ration (T2) by 120.62% over control (T0). The ADG of T0 was higher than those of Restitrisnani et al. (2013) found that Kacang goats fed *Pennisetum purpureum* and concentrate (9.20% of CP) had ADG of 23.46 gram. Gafar et al. (2013) also reported lower ADG of Kacang goats fed *Pennisetum purpureum* and concentrate (16% of CP) that was 37.8 gram. However, by improving CP content in the ration of T1 and T2, the ADG increased. This was in agreement with Restitrisnani et al. (2013) that higher CP content in the ration (11.67-18.33%) increased ADG of Kacang goats (61.86-69.41 gram).

Table 2. The Average Daily Gain, Nutrients Intake, Digestible Nutrients, and Feed Conversion Ratio of Kacang Goats fed Ruminally Undegradable Protein

Parameters	T0	T1	T2
Average daily gain (g)	$41.57^{A} \pm 5.61$	$72.75^{B} \pm 4.93$	$91.71^{C} \pm 6.97$
Dry matter intake (g)	$502.17^{Aa} \pm 39.11$	$597.86^{a} \pm 51.10$	$707.48^{Bb} \pm 92.81$
Dry matter intake (g/kg $BW^{0,75}$)	$53.88^{Aa} \pm 3.48$	$65.11^{b} \pm 6.60$	$70.75^{Cb} \pm 9.24$
Dry matter intake (% BW)	$2.56^{a} \pm 0.19$	$3.12^{ab} \pm 0.36$	$3.29^{b} \pm 0.44$
Digestible dry matter (g)	$326.06^{a} \pm 51.80$	$363.28^{a} \pm 43.18$	$447.51^{a} \pm 123.62$
Dry matter digestibility (%)	$67.62^{a} \pm 4.35$	$58.40^{b} \pm 2.68$	$59.68^{b} \pm 4.38$
Dry matter feed conversion	$12.20^{A} \pm 1.49$	$8.22^{B} \pm 0.38$	$7.72^{B} \pm 0.88$
Crude Protein (CP) intake (g)	$54.85^{A} \pm 4.27$	$81.54^{B} \pm 6.97$	$110.32^{C} \pm 14.47$
Digestible CP (g)	$35.20^{Aa} \pm 6.26$	$51.91^{a} \pm 7.20$	$79.07^{Bb} \pm 19.27$
CP digestibility (%)	$66.71^{a} \pm 4.51$	$61.14^{a} \pm 4.24$	$67.93^{a} \pm 3.05$
Intake protein conversion	$1.33^{a} \pm 0.16$	$1.12^{a} \pm 0.05$	$1.20^{a} \pm 0.14$
Ether extract intake (g)	$11.89^{Aa} \pm 0.93$	$14.82^{b} \pm 1.27$	$18.09^{Cc} \pm 2.37$
Crude fiber intake (g)	$173.83^{a} \pm 13.54$	$177.45^{a} \pm 15.17$	$206.44^{a} \pm 27.08$
Nitrogen free extract (g)	$201.05^{Aa} \pm 15.66$	$261.84^{b} \pm 22.38$	$301.08^{Bb} \pm 39.49$
TDN intake (g)	$317.78^{a} \pm 34.74$	$336.76^{a} \pm 41.75$	$412.35^{a} \pm 80.07$
TDN (%)	$63.23^{a} \pm 3.99$	$56.21^{b} \pm 2.42$	$57.95^{ab} \pm 3.63$
Gross energy /GE intake (MJ)	$42.92^{A} \pm 3.34$	$10.81^{B} \pm 0.92$	$13.08^{B} \pm 1.72$
Digestible energy (MJ)	$40.23^{A} \pm 3.09$	$6.04^{B} \pm 0.87$	$7.76^{B} \pm 1.26$
Energy digestibility (% GE intake)	$93.73^{A} \pm 0.69$	$55.65^{B} \pm 3.49$	$59.13^{B} \pm 2.23$
Digestible energy conversion	$1.04^{A} \pm 0.13$	$0.15^{B} \pm 0.01$	$0.14^{B} \pm 0.02$

Note: different capital letters superscripts in the same row indicate the highly significant difference ($p<0.01$) while different lower case superscripts indicate significant difference ($p<0.05$).

The ADG of goats fed soybean meal (T2) was higher than those fed fish meal (T1) because of higher nutrients intake. The DMI, ether extract, and CP intake of T2 goats were higher than those of T1 goats. This indicated that soybean meal was more palatable than fish meal for Kacang goats. In addition, the CP content of T2 ration (15.59%) a little bit higher than that of T1 ration (13.64%).

The CP digestibility (%) and the intake protein conversion of all treatments were relatively the same. It means that the difference of digestible CP was due to DMI difference (T0 was similar to T1 but lower ($P<0.01$) than T2 and T1 was lower ($P<0.05$) than T2).

The DMI, CP, and TDN intake of T0 did not meet the standard requirements for a goat to achieve ADG of 50 gram (Kearl, 1982) that were lower than 600 gram of DMI, 56 gram of CP, and 360 gram of TDN, therefore the ADG of T0 were below 50 gram. On the other hand, the ADG of T2 goats were higher than 50 gram, because the nutrients intakes of T2 were higher than Kearl (1982) recommendation.

Gross energy intake, digestible energy, and energy digestibility (% GE intake) of T0 were higher ($P<0.01$) than those of T1 and T2 (Table 2). However, the digestible energy conversion of T0 was higher than that of T1 and T2. It indicated that energy from T0 feed did not effectively converted to weight gain. It also happened to the feed conversion ratio of T0 that was higher ($P<0.01$) than that of T1 and T2. In other words, the FCR of T1 and T2 were better ($P<0.01$) than that of T0.

CONCLUSION

It can be concluded that the use of ruminally undegradable protein (fish meal and soybean meal) in the ration can improve the productivity of Kacang goats.

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KEYWORD : FCR, Fishmeal, Gliricidia leaves, Goat, Soybean meal

REFERENCES

Cromwell, G.L. 1999. Soybean Meal - The "Gold Standard". The Farmer's Pride, KPPA News. Vol. 11 No. 20, Nov. 10, 1999.

Gafar, A.A., A.R. Alimon, A.Q. Sazili, Y.C. Man, dan A.R. Abubakr. 2013. Effect of varying levels of palm oil decanter cake on feed intake, growth performance and carcass characteristics of Kacang goats. IOSR J. Agric. and Vet. Sci. 3 (4): 24 - 29.

Grovum, W.L. 1993. Appetite, palatability and control of feed intake. In The Ruminant Animal. Digestive, Physiology, and Nutrition. Church, D.C. (Ed). Waveland Press, Inc. Prospect Heights. 202 - 216.

Hutama, Y.G. 2014. Persentase Karkas dan Komponen Non Karkas Kambing Kacang Jantan Akibat Pemberian Pakan dengan Kadar Protein dan Energi yang Berbeda. Fakultas Peternakan dan Pertanian, Universitas Diponegoro, Semarang, Indonesia. Retrieved Sept. 23, 2014 from http://eprints.undip.ac.id/42789/

Kearl L. C. 1982. Nutrient Requirements of Ruminant in Developing Countries. International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University, Logan.

Mahmilia, F. and A. Tarigan. 2004. Karakteristik morfologi dan performans kambing Kacang, kambing Boer, dan persilangannya. Prosiding Lokakarya Nasional Kambing Potong. Bogor, Indonesia, Agust. 6, 2004. Puslitbang Peternakan, Bogor. 209-212.

Restitrisnani, V., A. Purnomoadi dan E. Rianto. 2013. The production and body composition of Kacang goat fed different quality of diets. J. Indonesian Trop. Anim. Agric. 38 (3): 163 - 170.

Riaz, M.Q., K.H. Sudekum, M. Clauss, dan A. Jayanegara. 2014. Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: a meta-analysis. Livest. Sci. 162: 76 - 85.

Sianipar,J., A. Batubara, S. Karokaro, and S.P. Ginting. 2005. Efisiensi nutrisi pada kambing Kosta, Gembrong, dan Kacang. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, 2005. 630-636.

Stern, M.D., A. Bach, and S. Calsamiglia. 2006. New Concepts in Protein Nutrition of Ruminants. Proceedings of the 21st Annual Southwest Nutrition & Management Conference, February 23-24, 2006, Tempe, Arizona. 45-66.

Sumardianto, T.A.P., E. Purbowati, and Masykuri. 2013. Karakteristik karkas kambing Kacang, kambing Peranakan Ettawa, dan kambing Kejobong jantan pada umur satu tahun. Anim. Agric. Journal. 2 (1): 175-182. Retrieved Sep. 19, 2013 from http://ejournal-S1.undip.ac.id/index.php/aaj.

Widyobroto, B.P., S.P.S. Budhi, and A. Agus. 2010. Effect of protein undegraded supplementation on production and composition of milk in dairy cows. J. Indonesian Trop. Anim. Agric. 35 (1): 27-33.

O-40-4

Effects of replacing concentrate with wet soya milk residue on intake, digestibility and growth performance of goats

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Abstract

This study was aimed to determine the effects of replacing concentrate with soybean milk residue on feed intake, digestibility and growth rates of post weaning goats. Sixteen female crossbred (Boer x Black bengal) goats, (3-5 month of age and average live weight of 14.4±1.4 kg) were divided into four isonitrogenous diet groups under a Randomized Complete Block Design (RCBD), with four goats in each group. Four dietary treatments were ratios of pelleted compound diet and soybean milk residue a rate of 4.1:0, 3.4:0.5, 2.3:1 and 1.9:1.2 % of BW per day on a dry matter basis. All animals were kept individually pen with free access water and mineral block and offered Napier grass *ad libitum*. The results indicated that nutrients intake and digestibility of goats were not significant (P>0.05) different among the diets, except for fiber fraction. No differences were observed between groups for average daily gain, feed efficiency and feed conversion ratio of goats. However, the goats fed soybean milk residue at 1 and 1.2 %BW have a low feed cost per kilogram of body weight gain than those other diets. In conclusion, goats could be fed wet soybean milk residue at high level (1.2 % DM of BW) with pelleted compound feed during growing period without detrimental effects on intake, digestibility and their growth performance.

Introduction

Soybean is made into various foods such as tofu, soymilk, soymilk powder, soy sauce, soy flour, dried tofu. Soymilk residue (waste), waste from soybean milk industry is high in moisture and spoil very quickly in room temperature. Every kilogram of dry beans made into soymilk generates about 1.1 kg of soymilk residue, which contain 80-90% moisture. Soymilk residue is often considered as waste, which is mostly dumped and burned, and is a potential environmental problem because it is highly susceptible to putrefaction (Rahman et al., 2015 Li et al., 2013). Li at al. (2013) reported that soya milk waste is rich in protein, fiber, fat and trace elements, and is alternative sources of energy and protein for ruminants (Rahman et al., 2014a). Farmers are also interested in using soymilk residue as goat feed for long-term feeding, and fed ad libitum. The use of soybean milk residue as ruminant feed on the growth performance of goats has been limited. Although the goat is considered superior to other ruminant species in its utilization of poor quality, high fiber forages and household waste for its body maintenance and production. Therefore, this study investigated the effects of supplementing soymilk residue on intake, digestibility and growth performance of Bore crossbred goats.

Materials and Methods

Sixteen female Boer crossbred (Boer x Black bengal) goats, weighing 14.4 ± 1.4 kg initial body weight (BW), and approximately 3 to 5 months old were used for 90 days. The goats were assigned at random to four isonitrogenous dietary treatments groups, with four goats in each group under a Randomized Complete Block Design (RCBD). Four dietary treatments were ratios of pelleted compound feed and soya milk residue a rate of 4.1:0, 3.4:0.5, 2.3:1 and 1.9:1.2 % of BW/d (DM basis), respectively. All animals were kept individually pens (1x1.2 m) with free access water and mineral block. The goats fed ad *libitum* with Napier Pakchong grass (*Pennisetum purpureum* x *P. americanum*) as a basal diet. Soymilk residue was supplied by a market women from a local soybean milk processing (home made) in the evening every day. Soymilk residue was contained 315 g CP/kg DM and 12 % DM. The pelleted compound feed and Napier Pakchong grass were contained 14 and 8 % CP, respectively. Feeds were offered twice a day at 0800 and 1600 h. Amounts of feed offered and refused were recorded daily to estimated feed intake. Subsamples of faeces, feed offered and residues were taken weekly for dry matter determination and dried samples were ground through 1 mm screen and then analyzed for DM, ash, CP and EE (AOAC, 1990) and fiber fraction as described by Van Soest et al. (1991). After the completion of the 90-d feeding trial, a digestibility trial was carried out for 7 d using the total faeces collection method. The body weight of the goat was recorded every 15 days for feeding adaption. The data were analyzed using the General Linear Model procedure of Program R (version 3.2.5).

Results and Discussion

Nutrients intake and digestibility of goats are given in Table 1, showing CP and GE intake were not significant different in among dietary treatments. The goats on the soymilk residue at 1 and 1.2 % BW had lower intake of dry matter in term of %BW and g/kg $^{0.75}$ than those fed low level of soymilk residue (0 and 0.5 %BW). It was similarly to those suggested by Rahman et al. (2015 2014a), who found that supplementation of soya waste in the diet was decreased dry matter intake of goats. This might be due to the higher content of soymilk residue compared with the concentrate pellet diet (12 and 89% DM, respectively). High moisture content of feeds increases the bulkiness of diets, and is negatively related to the capacity of the rumen (Rahman et al., 2014b), which was reduced fiber fraction intake. Nutrients digestibility of goats were not differ (P>0.05) between dietary treatments (Table 1). Rahman et al. (2015) reported that intake and digestibility of nutrients increased with the increased dietary level of soya waste (0.5 to 2.0 %BW), it was due to isonitrogenous diet in this study.

Average daily gain and feed conversion ratio of goats fed the treatment diet did not differ (P>0.05) in each treatment group (Table 2), it can be reduced compound feed at 53 % in goats fed 1.2 % BW of soymilk residue. This might be attributed to the high nutritional characteristics of soymilk residue, and is positively related to the activity of rumen microbe, and lead to similar body weight change of goats (Rahman et al., 2014b). The feed cost per kilogram gain was significantly (P<0.05) lower in the high level of soymilk residue (1 and 1.2 %BW) compared with low level of soymilk residue (0 and 0.5%BW). The conclusion, supplementation of locally wet soybean milk residue was not affected on nutrient digestibility, BW gain and feed efficiency of goats. It can be reduced feed cost and is thus recommended for use by farmer.

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KEYWORD : soya milk residue, goat, growth performance, feed intake, boer crossbred

Table 1. Nutrients intake and digestibility by goats fed soybean milk residue and pelleted compound feed

Parameters	Dietary level of soymilk residue				SEM	*P-value*
	0	0.5	1	1.2		
Dry matter intake (DMI)						
Total DMI (g/d)	654.42	656.79	540.29	557.121	38.32	0.114
Total DMI (% BW)	3.77 [a]	3.72 [a]	3.09 [b]	3.13 [b]	0.107	0.002
Total DMI (g/kgBW$^{0.75}$)	76.80 [a]	76.00 [a]	63.26 [b]	64.32 [b]	2.609	0.007
Organic matter (OM)	592.46	597.98	496.63	513.01	34.05	0.136
Crude protein (CP)	80.29	88.84	80.43	87.85	5.62	0.587
Ether extract (EE)	29.89 [c]	38.78 [b]	41.87 [ab]	47.42 [a]	2.43	0.004
Neutral detergent fiber	369.75 [a]	355.98 [a]	277.05 [b]	281.82 [b]	22.22	0.030
Acid detergent fiber	207.41 [a]	196.36 [a]	147.53 [b]	145.70 [b]	12.27	0.010
Gross energy (J/kg DM)	117.24	121.23	103.18	108.08	6.79	0.284
Apparent digestibility (%)						
Dry matter (DM)	70.86	69.22	69.64	68.37	1.61	0.750
Organic matter (OM)	73.62	75.66	71.34	72.30	0.65	0.730
Crude protein (CP)	70.68	69.79	72.21	72.31	2.51	0.867
Ether extract (EE)	84.20	86.57	86.95	86.80	1.13	0.326
Neutral detergent fiber	62.99	60.09	61.08	59.21	2.58	0.634
Acid detergent fiber	52.23	49.50	48.23	44.48	2.52	0.251
Digestible energy ()	73.05	71.68	71.28	70.90	1.62	0.803

The means within rows with different letters (a, b, c) differ significantly ($p<0.05$).

Table 2. Average daily gain and feed conversion ratio (FCR) in goat fed soybean milk residue and pelleted compound feed

Parameters	Dietary level of soymilk residue (%BW)				SEM	*P-value*
	0	0.5	1	1.2		
Initial weight (kg)	14.50	14.62	14.75	14.62	0.39	0.977
Final weight (kg)	20.37	20.87	20.37	21.0	0.85	0.927
Average dairy gain (g/day)	65.28	69.44	62.50	70.83	7.14	0.83
Feed efficiency	0.100	0.101	0.115	0.127	0.0	0.279
Feed conversion ratio	9.99	9.48	9.25	8.10	0.68	0.314
Feed cost (bath/d)	8.66 [a]	8.16 [a]	6.96 [b]	6.54 [b]	0.27	0.001

The means within rows with different letters (a, b) differ significantly ($p<0.05$).

REFERENCES

AOAC. 1990. Official methods of analysis of the association of Official analytical chemists (AOAC). 15th ed. AOAC International, Arlington, Virginia, USA.

Li S., D. Zhu, K. Li, Y. Yang, Z. Lei, and Z. Zhang, 2013. Soybean curd residue: Composition, utilization, and related limiting factors. ISRN Industrial Engineering, Hindawi publishing corporation. Available online at : http// dx.doi.org/10.1155/2013/ 423590.

Rahman, M.M, M.R. Rahman, T. Nakagawa, R.B. Abdullah, W.E. Wan Khadijah, and R. Akashi, 2015. Effects of wet soya waste supplementation on the intake, growth and reproduction of goats fed Napier grass. Anim. Feed Sci. Technol. 199: 104-112.

Rahman, M.M, R.B. Abdullah, W.E. Wan Khadijah, T. Nakagawa and R. Akashi, 2014a. Feed intake and growth performance of goats offered Napier grass (*Pennisetum purpureum*) supplemented with concentrate pellet and soya waste. Sains Malaysiana. 43: 967-971.

Rahman, M.M, T. Nakagawa, R.B. Abdullah, W.E. Wan Khadijah and R. Akashi, 2014b. Feed intake and growth performance of goats supplemented with soy waste. Pesq. Agropec. Bras., 49 : 554-558.

Van Soest, P. J., J. B., Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. J. Dairy Sci. 74: 3583-3589.

O-40-6

Digestibility and Nitrogen Balance of Male Bligon and Kejobong Goat Fed Peanuts Straw

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OBJECTIVE

Bligon and Kejobong are Indonesian local goat breeds which are highly potential to be developed due to their good performances. The problem commonly arise in goat fattening is feed supply which is less than the requirement as it is depends on season. Therefore, the study on alternative feed stuffs that can fulfill the needs of the animal, for instance nutrient contents and its quality as well as its availability in area of goat reared is needed. One of the potential feedstuff for goat was agriculture by products such as peanut straw which is easy to find during harvesting time. Usually farmers use agriculture by products such as peanut straw as sole diet for ruminant. The question is that the diet can fulfill N requirement of the ruminants. Ruminant require protein in the diet to supply N for rumen microbes activity and for animal tissue metabolism. Microbial requirement are met at 6-8% crude protein in the diet while animal requirement range from 7-20% in the diet depending upon species, sex, and physiological status (Huston, and Pinchak, 2016)

Measurement of Nitrogen (N) balance is a useful and accurate method to evaluate the nutrient quality of feedstuff, especially the values of nitrogen to ruminants. The method were used to evaluate the multipurpose trees which may suitable for feed supplements (Jetana et al. 2010), to evaluate the effect of physical treatments of feedstuff on N utilization (Mbewe, et al. 2014). The method also used to evaluate the response of different species (Yusiati et al., 2000) and different breed (Elamin, et al., 2012, Yusiati et al., 2015) of ruminants to feedstuff or diets offered in order to increase efficiency of livestock production.

Based on the review, it is necessary to evaluate a single feed from peanuts straw to supply protein requirement of goats, either Bligon or Kejobong, using nitrogen balance experiment.

MATERIALS AND METHODS

Peanut crop straw obtained from the same area in the Yogyakarta province were used in this experiment as sole diet. It was served without the empty root. The hard woody straw also was throw away, and only the edible portion which were used as the feedstuff. The chopped straw was served in fresh condition, one day after harvesting.

Six male Bligon and six male Kejobong goats, age 11 month and average body weight 18 kg were used in this experiment to evaluate the effect of peanut straw as a sole diet on N retained in their body. Animal were put in the individual metabolism cages, to get good separation of the urine and feces. The feeding trial was run for couple month including one week collection period. All animals were weighed before and after the trial. The diets and drinking water were served twice a day at 09.00 am and 16.00 pm.

During the collection period, 200 g of daily feed offered samples were collected. Uneaten feed were also collected daily, early in the morning before feeding time and then were weighed. Individually, 20% of the uneaten feed samples were taken. After drying at 55℃ , the samples were bulked and ground in a hammer mill to pass a 1mm screen diameter and then sub samples were taken out for nutrient analysis. Individual feces samples as much as 10% of total excretion were taken out at the end of each 24 hours period, put in poly bag and kept in fridge through the collection period. The samples were made into individual composite and got sub samples for the same analysis as the feed samples.

Urine was collected daily into plastic bucket placed under the cages and containing 10% sulfuric acid solution to reached the urine pH below 3 to avoid microbial growth. Urine volumes were measured and filtered through the 2 layers of gauze. Daily samples were taken, put into 20 ml plastic vials and brought to the laboratory for analysis.

Laboratory analysis

Representative samples of feed, refusal feed and feces were subjected to proximate analysis including dry matter and, organic matter determination following the AOAC procedure (1984), as well as crude protein by the Kjeldahl method, ether extract by Soxhlet and crude fiber by . Nitrogen content of the urine samples were also measured by Kjeldahl method. All analysis was done in duplicate.

The data obtained were used to calculate the values of Nutrient digestibility. Nitrogen balance was calculated as the amount of average daily nitrogen intake which is not excreted in feces and urine (g/day).

Statistical analysis

All data collected were subjected to statistical analysis of compared means by independent samples T-Test design.

RESULT AND DISCUSSION

Nutrient intake and Digestibility Coefficient

Nutrient contents of peanut straw was dry matter (DM) 20.13%, organic matter (OM) 87.39%, crude protein (CP) 17.62%, ether extract (EE) 3.88%, crude fiber (CF) 30.57%, and nitrogen free extract (NFE) 35.32%.

It showed in Table1.there were no significant differences in the nutrient intake of Bligon goat compared with those of Kejobong goat whether the values presented in g/day or in $g/W^{0.75}$/day. Dry matter intake of Bligon and Kejobong goat were 2.59% and 3.08% of their live weight, which were enough for maintenance need as mentioned it should be 2.4% of animal live weight (NRC,1981). Bligon goats with 20kg life weight, fed by diet consisted of 65% King Grass, 15% soybean meal and 20% rice bran supplemented with 3% protected crude palm oil (DM bases) showed the DM and OM intake were 642 ± 22 gram/d and 554,77 ± 17 gram/d (Yusiati *et al.,* 2015). Lower intake in this recent finding compared with the previous finding due to the different component of the diet.

Nutrient digestibility coefficient of the peanut straw in Bligon and Kejobong goat, were shown in Table 2. Nutrient digestibility, included DM, OM, CP, EE, CF and NFE digestibility were significantly higher in Bligon goat compared with ones in Kejobong goat.

Nutrient rumen digestibility was related to the rumen microbial activity. Digestibility of the diet can be different among the breeds as the digestive processes were directed by enzyme activities of the rumen microbes as well as the host enzyme activities which are species specific. It was not expected that the nutrient digestibility coefficient of the diet in Bligon goat higher than in Kejobong goat. Estu et al. (2015) reported that total excretion of PD in Bligon goat were 114.14 μmol/$W^{0.75}$/hari, with microbial protein synthesis efficiency reach out 4.61 g N/kg degraded of organic matter in rumen (DOMR), while in Kejobong goat were 180.18 μmol/$W^{0.75}$/hari, with microbial protein synthesis efficiency 6.90 g N/kg DOMR when the animal received the peanut straw as sole diet. It is also reported that efficiency of protein microbial synthesis was higher in Kejobong when the goat received diet containing King grass and peanut straw (Yusiati and Hanim, 2013). It seemed that the higher digestibility of peanut straw in Bligon goat was not only as an effect of higher rumen microbial enzyme activities, but also as the effect of the digestive enzyme activities of the host. It needs to be evaluated in the future.

This recent study found that crude protein intake of Bligon and Kejobong goat, less than 100g/day. It was lower than the goat requirement. Lamb with 20kg live weight need crude protein 112g/day to have 100g daily body weight gain (NRC. 1985).

Nitrogen intake, fecal and urinary N Excretion and N balance.

The result of N balance study was presented in Table 3. It was shown there were not significant differences of N consumption, N feces excretion, and N urinary excretion between Bligon and Kejobong goat. When the values were presented in metabolic body weight unit, N intake, fecal N and urinary N of Bligon goat were 1,62±0,11, 0,33 ± 0,05 and 0,11 ± 0,04 $g/W^{0.75}$/d, while Kejobong goat were 1,85 ± 0,20, 0,44 ± 0,06 and 0,20 ± 0,12 $g/W^{0.75}$/d. The N intake and fecal N excretion were not also significantly affected by the breed, but the urinary N excretion in Bligon goat tended lower than that in Kejobong goat when expressed in $g/W^{0.75}$/d unit.

The N absorbed of Bligon goat was not significantly differ from Kejobong goat. When it was expressed in percentage of N intake, N absorbed in Bligon goat was significantly higher compared with that in Kejobong goat (Table3).

The nitrogen content of Bligon feces in this study amounted to 2.04%, while Kejobong of 2.01% (DM basis). The N retained was higher in Bligon than Kejobong goat ($P \leq 0.05$) when expressed in g/day unit as well as in percentage of N intake unit.

Urinary N excretion (g/ head/day) of Kejobong tend to be higher than Bligon goat. This may be due to differences of protein metabolism in the both breeds. Based on study, it was known that the N retained in both breeds were positive. This indicated that nitrogen balance was also positive, means there were N retained in the body which used for basic living and production.

The present study was not in line with El-Meccawi et al. (2009), who reported that feeding wheat straw as a single feed for *Local-cross* goats gave negative gave nitrogen balance in a negative position. It means that wheat straw as a single feed can not supply the protein requirements of livestock. Paengkoum and Paengkoum (2009) reported that *Boer-cross* receiving complete diets from rice straw got 5.6 g/head/day N retention, supported by average daily weight gain of 59.5 g/head/day. This means, complete diets from rice straw can supply protein requirement of the goats.

In this study, positive nitrogen balance supported by the positive daily gain weight. The N retention value of Bligon goat was 11.33 g/head/day which gave 87.30 g/head/day of daily gain weight, while N retention of Kejobong goat was 10.08 g/head/day with 91.27 g/head/day of daily gain weight. The result showed that the both breeds can use peanut straw as a sole diet.

CONCLUSSION

Male Bligon and Kejobong goat received the peanut straw diet, showed the positive N balance, therefore peanut straw can be used as a sole diet in the both goats. Bligon goat more efficient in using Nitrogen compared with Kejobong when they received peanut straw as sole diet.

KEYWORD : Bligon goat, Kejobong goat, Peanut straw, Nitrogen balance

Table 1. Nutrient intake of Bligon and Kejobong goat fed by peanut straw as sole diet (mean ±SE)

Nutrient	Intake (g/day or $g/W^{0.75}$/day)	
	Bligon goat	Kejobong goat
Dry matter ^{ns}	525.36 ± 26.20 (55,01±3,99)	519.67 ± 28.42 (62,31±7,36)
Organic matter ^{ns}	464.34 ± 22.10 (48,61±3,37)	457.91 ± 25.34 (54,91±6,51)
Crude Protein ^{ns}	96.72 ± 3.77 (10,13±0,66)	96.39 ± 3.81 (11,55±1,25)
Ether Extract ^{ns}	20.20 ± 1.17 (2,11±0,17)	20.19 ± 1.30 (2,42±0,26)
Crude Fiber ^{ns}	160.11 ± 8.66 (16,77±1,41)	159.82 ± 7.40 (19,17±2,27)
N Free Extract ^{ns}	187.31 ± 10.24 (19,60±1,25)	181.52 ± 13.98 (21,77±2,85)

ns. not significant

Table 2. Nutrient digestibility coefficient of Bligon and Kejobong goat fed by peanut straw as a sole diet (mean ± SE[1]).

Nutrient digestibility (%)	Bligon goat	Kejobong goat
Dry matter	70.77 ± 1.72^{b}	64.65 ± 1.58^{a}
Organic matter	75.68 ± 1.72^{b}	69.07 ± 1.62^{a}
Crude Protein	79.90 ± 1.20^{b}	76.15 ± 1.22^{a}
Ether Extract	55.71 ± 4.27^{b}	45.43 ± 4.86^{a}
Crude Fiber	72.95 ± 1.59^{b}	65.89 ± 2.25^{a}
N Free Extract	77.94 ± 2.55^{b}	70.71 ± 1.77^{a}

[1] Standard Error of the Mean.

[a,b,c] The means with different superscripts at the same row differ significantly (P<0,05).

Table 3. Nitrogen intake, fecal nitrogen, urinary nitrogen, digested nitrogen, N balance of Bligon and Kejobong goat fed by peanut straw as sole diet .

Parameter	Bligon goat	Kejobong goat
N Intake (g/day) ^{ns}	15,48± 0,61	15,42 ±0,61
Fecal N excretion (g/day) ^{ns}	3,12 ± 0,50	3,69 ±0,56
Urinary N excretion (g/day) ^{ns}	1,04 ± 0,41	1,66 ±0,90
Absorbed N (g/day) ^{ns}	12,36 ± 0,57	11,74 ±0,37
Retained N (g/day)	11,33 ± 0,65^{b}	10,08 ±0,99^{a}
Absorbed N (% N intake)	79.89 ± 1.20^{b}	76.14 ±1.23^{a}
Retained (%N intake)	73.15 ± 0.95^{b}	65.33 ±2.42^{a}
Retained (% absorbed N)	91.62 ± 1.36	85.84 ±3.11

ns: non significant

^{ab} Mean within the same row with different superscript letters differ significantly (P<0,05)

REFFERENCES

AOAC. 2005. Official Method of Analysis of the Association of Official Analytical Chemists. 18th ed. Maryland: AOAC International. William Harwitz ed. United States of America.

Elamin,K.M., A.A. Tameem Eldar, A.E.Amin, F.S. Abdala and H.E.Hassan. 2012. Digestibility and Nitrogen Balance of Suddan Goat Ecotypes Fed Different Energy Levels. Asian J. Anim Sci

Jetana,T, C. Vongpipatana , S.Thongruay, S. Usawang and S. Sophon.2010. Apparent Digestibility, Nitrogen Balance, Ruminal Microbial Nitrogen Production and Blood Metabolites in Thai Brahman Cattle Fed a Basal Diet of Rice Straw and Supplemented with Some Tropical Protein-rich Trees. Asian-Aust. J.Anim. Sci. Vol. 23 (4):465 – 474.

Huston, J. E. and W. E. Pinchak. Range Animal Nutrition in Heitschmidt R.K. and J.W. Stuth. Grazing Management. An ecological perspective. http://cnrit.tamu.edu/rlem/ textbook/Chapter2.htm

Mbewe, M. R., V. R. Hamandishe, V. E. Imbayarwo-Chikosi and B. Masunda. 2014. Nitrogen Balance And Rumen Microbial Protein Synthesis In Goats Fed Diets Containing Soaked And Roasted Mucuna Bean (Mucuna Pruriens). Online J. Anim. Feed Res., 4(1): 06-09.

NRC. 1981. *Nutrien Requirments of Domestic Animal. Nutrient Requirments of Goats: Angora, Dairy, and Meat Goats in Temperate and Tropical Countries. National Academy Press*. Washington, D. C.

Paengkoum, P. and S. Paengkoum. 2009. Effects of supplementing rice straw with Leucaena (Leucaena leucocephala) and Madras thorn (*Pithecellobium dulce*) foliages on digestibility, microbial N supply and nitrogen balance of growing goats. J. Anim. Physio. Anim. Nutr. 9: e59–e65.

Putra,D., L.M. Yusiati and Utomo, R. 2015. Estimation Of Rumen Microbial Protein Synthesis Based on Purine Derivatives In The Urine Using Spot Sampling Technique on Bligon and Kejobong Goat. Thesis. Graduate Programe. Faculty of Animal Science. Universitas Gadjah Mada.

Qingxiang , M. 2001. Composition, Nutritive Value and Upgrading of Crop Residues. In Animal Production Based On Crop Residues. FAO Corporate Document Repository. www. FAO Org/Docrep/005/Y1936e/ Y1936e06. htm

Yusiati, L. M. 2005. Pengembangan metode estimasi sintesis protein mikrobia rumen menggunakan ekskresi derivat purin dalam urin berbagai ternak ruminansia Indonesia. Desertasi. Fakultas Peternakan. Universitas Gadjah Mada. Yogyakarta.

Yusiati, L. M. and Hanim C. 2013. Estimation of rumen microbial nitrogen supply based on purine derivatives excreted in the urine of kejobong and bligon goat fed by king grass and peanut straw. Proceeding 3rd Aini International Seminar ISBN: 978-602-96934-85. Padang.

Yusiati, L.M., M. Soejono, S.P.S. Budhi, B.P. Widyobroto, Z. Bachrudin , 2000. The response of Ongole Crossed Bred Cattle toward different kind of forages as a sole diet. Research report. Faculty of Animal Science. Gadjah Mada University.

O-40-7

The comparison of nutrient digestibility of Bligon and Kejobong goats fed king grass and peanuts straw

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Objective

Bligon goats is one of goat breed which has good fertility and productivity, their population in Yogyakarta is up to 60% from total goats population and their productivity 12,000 tails per year. While Kejobong goats are another local goat breed found in Purbalingga, Central Java which has similar productivity with Bligon goats. Their population is 15,317 tails in Kejobong distric, Purbalingga, Central Java (Sodiq and Haryanto, 2007).

Smallholder goat production systems are mostly based on traditional methods. This situation exists in many regions of the tropics, where goats raised in traditional systems mostly roam freely in fallow land, forest and grassland. The main feed resources of animals are native grasses, legumes that occur naturally in grass lands, tree leaves and crop residues (Osakwe and Udeogu, 2007). In Java, feed resources of goats are king grass as native grasses and peanuts straw as legumes. In wet season, those production are high in several region.

The nutrient requirements of goats are determined by age, sex, breed, production system (dairy or meat), body size, climate and physiological stage. Feeding strategies should be able to meet energy, protein, mineral, and vitamin needs depending on the condition of the goats. Goats do not depend on intensive feeding systems except some supplemental feeding during growth, lactation, pregnancy and winter (Rashid, 2008). This study is designed to evaluate nutrient digestibility between bligon and Kejobong goats fed king grass and peanuts straw.

Methodology

2.1. Animals

Five Bligon and five Kejobong entire male goats aged 6-8 months and initial body weight 15-20 kg were used. All animals were confined in individual, well-ventilated separate metabolism cages and nylon nets were fitted below the cages for faecal collection..

2.2. Diets

King grass aged 49 d was collected and stored in a protected shed, as well as peanuts straw were collected after harvesting the peanuts and stored in a protected shed. Then both of diet were choped through a 5 cm. They were offered as the diet with ration 50:50 (w:w).

2.3. Experimental procedure

This experiment was done with 14 d adaptation period and 7 d collection period. Plenty of clean drinking water were offered *ad libitum* throughout. The diets were offered twice daily at 08:00h and 15:00h *ad libitum* and feed refusals collected and weighed in the morning before fresh diet was offered for calculating feed intake. Daily feed intake was feed offered minus refusal feed.

2.4. Digestibility and faeces collection

Total daily faecal output from individual animal was collected for 7 d during collection period. After recording the weight, 10% proportions of the 24 h individual faeces were collected and stored at 5℃ to composite with another faeces collected during collection period. Then they were dried in a draft oven at 55 ℃ for 3 d, milled trough a 2mm mesh and stored for later chemical analysis.

2.5. Chemical analysis

Both feed and faecal samples were ground using a hammer mill to pass through a sieve of 2mm diameter and analysed for their proximate constituents. The proximate composition of feed and faeces, including dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF), crude protein (CP) and nitrogen free extract (NFE) content was determined following standard methods (AOAC, 2005). Data collected were used to compute the nutrient intake (feed intake times to nutrient in feed), digested nutrient (nutrient intake minus nutrient in faeces) , as well as nutrient digestibility (nutrient in feed minus nutrient in faeces devided by nutrient in feed times to 100%).

2.6. Experimental Design and Statistical Analysis

This experiment was arranged in a T-test, with the factor being goat breed (Bligon and Kejobong). Goat breed were

separately conducted for each treatment with five replicates. King grass and peanuts straw were utilized as the diet. The data were analyzed as T-test (Rosner, 1990).

Results

The animals remained healthy throughout the entire period of the experiment. The nutrient composition including DW, OM, CP, CF, EE, and NFE of the king grass and peanuts straw are presented in Table 1. While Table 2 showed mean and deviation standard of nutrient intake , digested nutrient, and nutrient digestibility of Bligon and Kejobong goats. Nutrient intake and digestibility did not show any significant difference (P>0.05) between Bligon and Kejobong goats, except EE intake and NFE digestibility of Kejobong was higher than Bligon. This indicates that the incorporation of king grass and peanuts straw in the diets of the goats did not have negative effect on digestibility. All the experimental animals had adequate total dry matter intake (DMI) which ranged from 668.03 to 679.37 g/animal/day. These values were comparable to values ranging from 336.39 to 392.46 g/animal/day for West African Dwarf (WAD) goats fed concentrate diets at various levels of inclusions of *Moringa oleifera* leaf meal (Tona et al., 2014), and 294 to 449 g/animal/day for WAD goats fed Maize stover and supplemented with Acacia tortilis or Balanites aegyptiaca leaf browses (Ondiek et al., 2013). This comparable of DMI due to body weight of Bligon and Kejobong (15 to 20 kg) was heavier than WAD (7.5 to 12 kg).

Eventhough DMI of Bligon and Kejobong fed king grass and peanuts was higher than WAD goats, but their nutrient digestibility were lower than WAD goats fed diets containing sweet orange peel meal as reported by Oloche et al. (2013), and higher than West African Dwarf goats fed diets containing maize stover (Ondiek et al, 2013). The goat breed and diets influenced feed intake and digestibility. According Rashid (2008) the nutrient requirements of goats are determined by age, sex, breed, production system (dairy or meat), body size, climate and physiological stage.

Conclusion

There were no differences in nutrient digestibility for Bligon and Kejobong goats, NFE digestibility of Kejobong goats was better than Bligon goats fed king grass and peanuts straw.

KEYWORD : Nutrient digestibility, Bligon goat, Kejobong goat

Table 1. Nutrient composition of king grass and peanuts straw (% DM)

Nutrient composition	Kind of diets	
	King grass	Peanuts straw
Dry matter	23.03	31.08
Organic matter	86.57	87.42
Crude protein	5.82	13.22
Crude fiber	33.29	20.88
Ether extract	0.47	3.72
NFE	46.98	41.06

Table 2. Mean and deviation standard of nutrient intake (g/animal/d), digested nutrient (g/animal/d), and nutrient digestibility (%) of Bligon and Kejobong goats fed king grass and peanuts straw

Variabels measured	Goats breed	
	Bligon	Kejobong
Nutrient intake (g/animal/d)		
Dry matter^{ns}	668.03 ± 34.18	679.37 ± 14.29
Organic matter^{ns}	586.12 ± 27.80	595.81 ± 11.77
Crude protein^{ns}	66.78 ± 4.92	70.74 ± 1.70
Crude fiber ^{ns}	169.85 ± 10.50	173.63 ± 11.43
Ether extract*	15.47^{a} ± 0.58	16.18^{b}± 0.27
NFE ^{ns}	334.04 ± 12.71	335.26 ± 5.93
Digested nutrient (g/ animal/d)		
Dry matter^{ns}	472.97 ± 29.69	505.89 ± 43.47
Organic matter^{ns}	434.26 ± 22.85	461.96 ± 33.90
Crude protein^{ns}	45.89 ± 5.18	48.96 ± 8.14
Crude fiber^{ns}	115.31 ± 10.00	125.80 ± 20.70
Ether extract^{ns}	11.05 ± 1.06	12.58 ± 1.67
NFE*	262.02^{a} ± 7.04	274.62^{b}± 7.91
Nutrient digestibility (%)		
Dry matter^{ns}	70.82 ± 3.16	74.39 ± 4.95
Organic matter^{ns}	74.11 ± 2.70	77.47 ± 4.28
Crude protein^{ns}	68.60 ± 3.94	69.08 ±10.64
Crude fiber^{ns}	67.88 ± 4.10	72.10 ± 7.57
Ether extract^{ns}	71.37 ± 5.45	77.65 ± 9.19
NFE*	78.49^{a} ± 2.23	81.92^{b} ± 2.17

^{ns} not significantly different
* ($P<0.05$)

REFFERENCES

AOAC Intl. 2005. *Official Methods of Analysis of Association of Official Analytical Chemists*. 18th ed. W. Horwitz and G. W. Latimer (eds). AOAC Int. Maryland, USA.

Oloche, J., Ayoade, J. A., and Oluremi, O. I. A. 2013. Effect of replacement of sweet orange (*Citrus Sinensis*) peel meal with maize offal on apparent digestibility and nutrient intake of West African Dwarf (Wad) goats. IOSR J. Agric.Vet. Sci. (IOSR-JAVS). 5 (3), 1-4..

Ondiek, J. O., Ogore, P. B., Shakala, E. K., and Kaburu, G. M. 2013. Feed intake, digestibility and performance of growing small East African goats offered maize (*Zea mays*) stover supplemented with *Balanites aegyptiaca* and *Acacia tortilis* leaf forages. Basic Res. J. Agric. Sci. Rev. 2(1), 21-26.

Osakwe, I. I., and Udeogu, R. N. 2007. Feed intake and nutrient digestibility of West African Dwarf (WAD) goat fed *Pennisetum purpureum supplemented with Gmelina arborea*. Anim. Res. Int. 4(3), 724 – 727.

Rashid, M. 2008. Goats and their nutrition. Manitoba Goat Association. https://www.gov.mb.ca/agriculture/livestock/production/goat/pubs/goats-and-their-nutrition.pdf.

Rosner, B., 1990. *Fundamentals of Biostatistics*. 3rd ed. PWS-KENT Pub. Co., Boston, Massachusetts.

Tona, G. O., D. O. Ogunbosoye, and B. A. Bakare. 2014. Growth performance and nutrient digestibility of West African Dwarf goats fed graded levels of *Moringa oleifera* leaf meal. Int. J. Curr. Microbiol. App. Sci. 3(8), 99-106.

O-40-8

STUDY FOR DOMINANCE AND NUTRITION OF WEEDS AS FEED IN VARIOUS CROP LAND IN YOGYAKARTA

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The study aimed to determine the dominant species and nutrient content of weeds as feed on a variety of agricultural land in Yogyakarta. Observations were done on of paddy fields,corn,beans and vacant land of Pakem upperland area (621 m asl), as well as paddy fields, chili and vacant land Samas lowland area (10 m asl), three area each. The research was conducted in September-October 2015. The observations was done include measurement of dominance, plant identification and quality of weed. Dominance of weed was measured by using line intercept transect technique. Sample weed identification and quality examination (proximate) was done at the Laboratory of Forage and Pasture Science, Faculty of Animal Science UGM. Results showed that dominant of weeds in the lowland for shrub type were *Phyllantus amarus,Commelina benghalensis,Ludwigia octovalvis* and *Portulaca oleracea* while the type of grass were *Leptochlo achinensis, Eleusinei ndica, Cyperus compressus, Paspalumdistichum* and *Brahiariamutica* Dominant of weeds in the upperland for shrub type were *Ageratum conyzoides,Eclipta alba,Ludwigia octovalvis,Cleome rutidosperma* and *Portulaca oleracea* for type of grass were *Digitaria setigera,Eleusine indica,Cyperus iria,Echinochoa oryzides* and *Fimbristylismiliacea*. Thus, found three dominant of weeds with quality of DM and OM respectively *Ludwigia octovalvis* (18,76%90,95%), *Portulaca oleracea* (8,09%76,70%) and *Eulisine indica* (20,67%88,38%).

INTRODUCTION

Forage crops is the main feed for ruminants.However, usually forage production and crops decreases in the dry season.This situation impact to potential feed for livestock. These deficiencies must be fulfilled in order not to lower productivity of livestock.One of alternatif is to find plants that can grow during the dry season.One such plant is a weed.Purnomo (2010) stated that the weed is often defined as a plant that grows in places that are not desired by humans through the competition space of time, and a source of nutrients.

There are several types of plants that are classified as weeds, namely grass, shrubs, and legumes.Barus (2003) states there are also ways that can be used to classify weeds, for example based on morphology, life cycle, habitat, or under the influence on agricultural crops.Types of weeds that grow in an area of course different from other regions.It was influenced by growth factors, namely light, water, temperature, and soil types.

Special Region of Yogyakarta area consists of lowlands to highlands. There are an agricultural land that can not be separated from the weeds.Differences location in Yogyakarta might be affect the types of weeds that grow and also quality of nutrients that exist.Results of research Suwignyo et al.(2012) showed that the corn crop is in the lowlands have the potential of dry material better than on corn from the highlands.

MATERIALS AND METHODS

Time and Place Research

Researchwas conducted at two locations in the lowlands and highlands in Yogyakarta.Land used on the plateau is a land area of Rice, Corn, Beans and vacant land, while the land is lowland rice, chili and vacant land.The research was conducted from September to October 2015, located in Pakem (plateau) and Samas (lowlands).ie by 2 different places and carried out from September to October 2015.

Tools

The tools used in this study include field equipment to test the productivity include scissors, tape measure, sickles, digital scales, camera, plastic samples, pencils, books and also a set of tools for proximate analysis in the laboratory.

The materials used in this study include some farms in Yogyakarta with the height difference, but it is also a set of materials used for proximate analysis in the laboratory.

Method

Research methods

The study was conducted in three phases namely weed identification, measurement dominance of weeds, and the weeds quality test.

weed identification

Various types of weedsderived from field observations identified in the laboratory.Forage Forage and Pasture Faculty of Animal Husbandry Universitas Gadjah Mada.Identification was conducted on the classification of plants, the growth cycle, breeding and habitat.Identified weed species are weeds types of grasses, legumes, and shrubs.

Measurement dominance of weeds

*Weed*productivity test was performed *usingline intercept transect*

Intercept line transect technique. Measurement productivity *weed* with *an intercept line transect technique performed according to the method Moodyet al.(1984).* Made path/transect along 30 m using raffia .The line is divided into intervals.Each interval represents one sample unit. All the plants are off ended by the line transect situated below and above the line was observed and recorded.

Sampling

Weeds that of the land studied is taken as a sample of approximately 500 g/proximate weeds that will be analyzed in the laboratory.Not all weeds are taken for analysis of proximate but only five of the most widely weeds for each type.

Proximate analysis

Analysis of samples that will be done is the Proximate analysis includes dry matter (DM) and organic matter (OM).

Data analysis

The data retrieved is data weeds in the highlands and lowlands in the Yogyakarta region and will be analyzed descriptively.

RESULTS AND DISCUSSION

This research was conducted in the Territory of Yogyakarta.Precisely in the area Samas, Bantul with a height of 10 meters above sea level and in Pakem, Sleman with a height of 621 meters above sea level.Here are the climate data obtained from BMKG at the time of the study from September to October 2015:

Table 1. Climate data Yogyakarta

Month
Air temperature(0C)
Humidity (%)
Wind speed (m / s)
Solar radiation (%)
September
25.6
77
0.2
89
October
26.8
75
0.2
92

Table 2. Data monthly rainfall (mm)

locations
September
October
Lowland
0
0
Plateau

0
0

Based on Table 1 it can be seen that in September 2015 major air temperature, air humidity, wind speed, and solar radiation respectively is 25.6^{0}C, 77%, 0.2 m / s, 89%.Large air temperature, air humidity, wind speed, and solar radiation respectively is 26.8^{0}C, 75%, 0.2 m / s, and 92%.While in table 2 shows rainfall in September and October show the number 0. It shows the sampling carried out during the dry season.

The elements of the existing climate affects the growth, including whole plants and weeds.Darmawijaya (1997) stated the elements - elements of the climate that can affect the quality of plants such as rainfall, temperature, humidity, length of the dry months (rainfall less than 60 mm / month), and altitude above sea level.The main components of the climate is rainfall and temperature.Both components were interrelated.

Domination

Based on research, there are some dominant weed species and can grow in a wide variety of land in the lowlands and the highlands can be seen in the following table:

Table 3. Weeds are dominating in the lowlands and highlands

locations
name of species
Type
High land
Fimbristylis miliacea
grass
Echinochoa oryzides
grass
Cyperus iria
grass
Eleusine indica
grass
Digitaria setigera
grass
Portulaca oleracea
bush
Cleome rutidosperma
bush
Ludwigia octovalvis
bush
Eclipta alba
bush
Ageratum conyzoides
bush
Mimosa pudica
legumes
Lowland
Brachiaria mutica
grass
Paspalumdistichum
grass
Cyperus compressus
grass
Eleusine indica
grass
Leptochloa chinensis
grass
Portulaca oleracea
bush

Ludwigia octovalvis
bush
Commelina benghalensis
bush
Phyllantusamarus
bush
Weeds bush*
bush
* Description: Weed identification is unknown bushes

Based on Table 3. note that the weed species of grasses and shrubs were encountered both in the highlands and lowlands.As for the type of legume weed is only in the highlands.The data in Table 3 show only five dominant weeds every kind from any location.All data of the weed in getting from all the land of each location.Land used in high flatness is the land of rice, beans, corn, and vacant land.While in the low-lying land used was paddy field, chili, and vacant land.

Rukmana and Sugandi (1999), stated category includes grass weed species belonging to the family Gramineae types.Besides being the largest component of the entire population of weeds, the family have a fairly high adaptability, distribution is very broad and can grow well in dry or waterlogged soil.

Levels Dry Matter and Organic Matter

Dry matter (DM) and organic matter (OM) content data and weeds from the lowlands and the highlands can be seen in Table 4 below:

Table 4. Dry matter and organic matter of weeds

locations
name of species
DM (%)
OM (%)
High land
Fimbristylis miliacea
17.3079
80.6918
Echinochoa oryzides
14.1564
87.9473
Cyperus iria
21.3947
90.2614
Eleusine indica
19.5299
88.1041
Digitaria setigera
17.8224
89.7173
Portulaca oleracea
7.5340
75.7711
Cleome rutidosperma
13.1484
86.5825
Ludwigia octovalvis
16.9437
89.9868
Eclipta alba
13.8718
82.1705

ageratum conyzoides
13.3253
86.7715
Mimosa pudica
30.9134
92.6651
Lowland
Brachiaria mutica
33.0626
85.9962
Paspalum distichum
30.0659
85.6284
Cyperus compressus
17.8194
86.6198
Eleusine indica
21.8150
88.6517
Leptochloa chinensis
23.7641
84.0805
Portulaca oleracea
8.6572
77.6093
Ludwigia octovalvis
20.5796
91.9240
Commelina benghalensis
8.5744
76.9111
Phyllantus amarus
21.2369
89.5190
Weeds bush *
13.4665
86.4156
* Description: Weed identification is unknown bushes

Dry matter is a very important parameter to estimate the quality of the feed material and used as a guideline to determine the nutrient content of the feed materials.Based on the data in Table 4, the highest known levels of DM in the highlands of the type of grass, shrubs, and legumes respectively*Cyperus iria*is 21.39%, 16.94%*octovalvis Ludwigia,*and*Mimosa pudica*30, 91%.While DM highest levels in the lowlands of species of grasses and shrubs in a row is*Brachiaria mutica Phyllantus amarus 33.06% and*21.23%.

Looking at the data in Table 4, there are three weeds that grow in the lowlands and highlands.All three of the weed is*Eleusine indica, Portulaca oleracea,*and*Ludwigia octovalvis.*Of the three weeds, levels of BK in lowland larger than the plateau.That is because the conditions in the lowlands are warmer because it is adjacent to the beach.

Based on the data in Table 4, the highest known levels of OM in the highlands of the type of grass, shrubs, and legumes respectively*Cyperus ria*is*90.26%, Ludwigia octovalvis*89.98%, and 92.66%*Mimosa pudica.*While the highest levels of BO in the lowlands of species of grasses and shrubs in a row is*Eleusine IndicaLudwigia octovalvis*88.65% and 91.92%. Of the three weeds that grow in both locations, levels of OM in the lowlands is higher than the weeds in the highlands.

CONCLUSION

Weed species of grasses and shrubs are the dominant weed species both in lowland and highland.Weeds are located in lowland areas have the potential of dry matter and organic matter better than the plateau.

KEYWORD : weeds, domination, feed upperlands, lolands, nutrients

REFERENCES

Barus, Emanuel. 2003. Pengendalian Gulma di Perkebunan. Kanisius. Yogyakarta.

Darmawidjaya, M. I. 1997. Klasifikasi Tanah: dasar teori bagi peneliti tanah dan pelaksana pertanian di Indonesia. Gadjah Mada University Press. Yogyakarta.

Moody, K., C. E. Munroe, R. T. Lubigan, E. C. Paller. 1984. Major weeds of the Philippines. Weed Science Society of the Philippines. University of the Philippines at Los Banos College, Laguna, Philippines.

Purnomo, H., 2010. Pengantar Pengendalian Hayati. Penerbit Andi. Yogyakarta.

Rukmana, Rahmat dan Sugandi Saputra. 1999. Gulma dan Teknik Pengendalian. Kanisius. Yogyakarta.

O-40-9

Effect of whole Krabok seed, krabok oil or krabok residue on in vitro methane production.

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Introduction

Krabok seed is widely available in South-East Asian forests. These seeds contain large amounts of fat (50%) and are therefore of interest to be used as energy source in animal diets. Krabok oil contains large amounts of C12:0 and C14:0 (444 and 437 g/kg, respectievely) and is therefore of interest to reduce methane production (Panykaw et al., 2014a & 2014b) and thus to reduce the energy loss through fermentation (Mitsumori and Sun. 2008). From a practical viewpoint, the use of whole krabok seed (WKS) instead of krabok oil (KO) is preferred. However, the efficacy of WKS *versus* KO to reduce methane production it is currently not known. The aim of the current study was therefore to investigate the potential of WKS to inhibit methane production.

Material and methods

Five dietary treatments were formulated and the iso-lipogenic diets (8.5% crude fat, DM basis) were subsequently subjected to *in-vitro* fermentation. The following diets were used: basal diet (control, C), C + WKS, C + KO, C + krabok seed residue (KSR, WKS - extracted oil) and C + KO + KSR. Approximately 0.5-0.55 g DM of experimental diet was weighed into 250 ml fermentation flasks and 60 ml of buffered rumen fluid was added to each incubation flask. All flasks were incubated at 39°C. Ten microliter of the headspace gas was collected from the bottles at distinct time points (0, 2, 4, 8, 12, 24, 30, 36 and 48 h of incubation) and directly injected into a gas chromatography to determine the CH_4 content as described by Pellikaan et al. (2011), After 48h incubation, the flasks were placed in an ice bath to stop fermentation, then opened, the incubation contents were sampled for volatile fatty acid (VFA) analysis (Cone and Becker. 2012)

Results

The results were shown in Table 1, It appeared that the supplementation of KO reduced the percentage of methane of total gas production (% CH_4) but differences in percent of CH_4 were not found between KSR, KSR+KO and WKS values were 9.93, 11.06, 9.84, respectively. The reproducibility of producing gas production profiles from C, C+WKS, C+KO, C+KO+KSR, C+KSR samples was tested within series incubated in the same rumen fluid/ buffer solution at different time was shown in Fig.1. Furthermore, supplemental KO decreased the concentrations of acetate, iso-butyrate and total fatty acids ($P<0.05$), whereas the propionate- and butyrate concentrations remained unchanged.

Conclusions

The efficacy of WKS versus KO to reduce methane production is similar, thereby implicating that WKS can be used under practical feeding conditions to reduce methane production

KEYWORD : krabok oil, krabok seed, krabok residue, methane

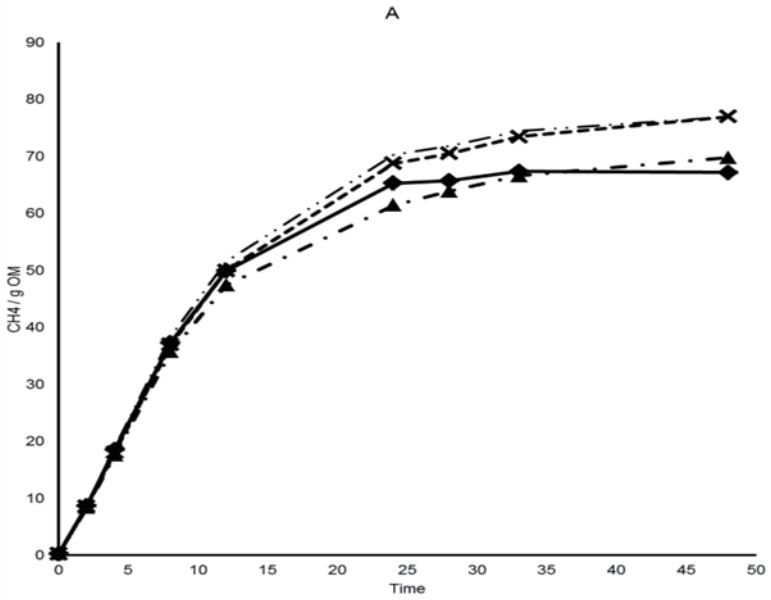

Figure 1. Comulative gas production (ml g^{-1} OM) curves of C+WKS ( ), C+KO ( ), C+KSR ( ), C+KO+KSR ( ), 48 h incubation.

Table1. *Total VFA concentration and individual VFA after incubations 48 h.*

Fatty acid	C	C+WKS	C+KO	C+KO+ KSR	C+KSR	SEM	P-value
Low fat 8.5%							
Acetic acid	60.44^{ab}	60.32^{ab}	59.34^{b}	60.99^{a}	61.19^{a}	0.466	0.020
Propionic acid	18.21	17.02	17.08	17.57	17.81	0.365	0.040
iso Butyric acid	0.987^{a}	0.925^{b}	0.919^{b}	0.936^{b}	0.986^{a}	0.010	>0.000
Butyric acid	12.06	11.77	11.89	12.14	11.66	0.325	0.584
iso Valeric acid	1.819^{a}	1.693^{b}	1.696^{b}	1.758^{ab}	1.795^{a}	0.025	0.002
Valeric acid	1.524^{a}	1.469^{ab}	1.454^{b}	1.477^{ab}	1.511^{ab}	0.02	0.028
Total	95.05^{a}	93.19^{ab}	92.39^{b}	94.87^{a}	94.95^{a}	0.767	0.017
Methane in total gas (%) 48 h	11.35	12.11	10.24	11.27	10.19	0.756	0.388
Methane / g OM 48 h	82.06	67.18	76.96	76.96	69.75	4.818	0.257

REFFERENCES

Cone, J.W and Becker, P.M. 2012. Fermentation kinetics and production of volatile fatty acids and microbial protein by starchy feedstuffs. Animal Feed Science and Technology. 1-2: 34-41.

Mitsumori, M and Sun, W. 2008. Control of rumen microbial fermentation for mitigating methane emissions from the rumen. Asian-Aust.J. Anim. Sci. 21, 144-154.

Panyakaew, P., Goel, G., Lourengo, M., Yuangklang, C., Fievez, V., 2013a. Medium-chain fatty acids from coconut or krabok oil inhibit in vitro rumen methanogenesis and conversion of non-conjugated dienoic biohydrogenation intermediates. Animal Feed Science and Technology. 180: 18-25.

Panyakaew, P., Goel G., Yuangklang C., Boon N., Schonewille.J. TH., Hendriks W.H., Fievez, V., 2013b. Effect of supplementing coconut or krabok oil, rich in medium chain fatty acids on ruminal fermentation, protozoa and archaeal population of bulls. Animal. 1950 – 1958.

Pellikaan, W.F., Hendriks, W.H., Uwimana, G., Bongers, L.J.G.M., Becker, P.M. & Cone,
J.W. 2011. A novel method to determine simultaneously methane production during in vitro.

O-41-1

The effect of nomadic and transhumant animal production to environmental stability

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Introduction

The most important milestone in the one-million year history of humanity has been the transition from hunter-gathering to farming and herding. Humankind (*homo sapiens*) have spent the last 990,000 years of this period hunting, gathering food, and fishing. "Settled" life began around 10000 BC, initially with farming and later by domesticating animals. Climate change, lack of resources and dependency of production on natural resources has forced migration of human being between continents, thus a nomadic lifestyle emerged. Transhumance, also referred to as mobile pastoralism has been practiced since the Neolithic, the late stone age period (Nandris, 1985 Arnold and Greenfield, 2006). With the onset of agricultural evolution among prehistoric humans, people have started to move for better and more feed for their domesticated animals, so called nomadic /transhumant practices. The term ''Transhumance'' can be described as "a way of living which practice seasonal and cyclical movement of people with their animals between different ecological zones/pasture lands. Also could be denoted as ''mobile pastoralism'', transhumance can sometimes be mistaken for nomads who are in contrast, communities that continually live in different locations, moving from one place to another with no real base. Transhumant systems have been common for centuries in many regions of the Old World, and it is a traditional farming practice that shapes the landscape and benefits ecosystem conservation (Ruiz and Ruiz, 1986 MacDonald et al., 2000 Fortina et al., 2001 Garzo ´ n, 2001). Today they are still the main livelihood, alleviating rural poverty, in certain regions of the world (Moktan et al. 2008). The transhumant system, has become a traditional farming practice now in the New World. Since its evolution in the Mediterranean region the practice has maintained interesting ecosystems shaping a unique landscape and weaving the social fabric for a rich culture. The system is one of many customary practices developed by ancient Mediterranean societies to cope with the unpredictable and highly fluctuating climate of the region. It creates a cultural landscape that includes a complex mosaic of habitats, each varying in extent and productivity during the year (Oteros-Rozas et al., 2012).

However, despite this practice being present in many European countries, it is currently a declining activity in Europe (Ruiz and Ruiz, 1986 Liberatori and Penteriani, 2001) and the Mediterranean basin. The progressive loss of this land-use model means the decrease of economic and natural resources, as well as the disappearance of an entire folklore, which was specially developed over centuries around the world. Many mountain areas in Europe where transhumant livestock spend most of the year have developed highly diverse ecosystems that play a significant role in conserving biodiversity (MacDonald et al., 2000).

It is difficult to assume that our present day sophisticated modern techniques, which have so dramatically altered our landscape and affected the global ecological stability in less than a century, could assume to hold light to these traditional practices which over the millennia have maintained and managed the natural environment on which they have solely relied on, to perform within its capacity. In addition, the transhumant has created an enduring social fabric which has resulted in sound cultural resilience. As such we have come to accept that human and natural systems cannot be economically viable unless they are also ecologically sound and socially responsible.

In this study we aim to highlight social and environmental dynamics that transhumant communities provide and introduce their holistic methodology on preserving social and cultural resilience which is another approach for understanding the benefits of those communities for natural resource management.

Methodology

All of the data for the study was collected in 2016 by interviewing anthropologists, govt. employees, historians and most importantly by paying routine visits to the summer and winter locations of various Turkish Mediterranean and Central Anatolian transhumant populations during their migration route and settlement areas. During the study, 15 different families were evaluated. Interviews, for data collection varied from family to family from a few hours to some lasting a week. Majority of the interviews were held with each member of the family in their single tent which constituted their entire living space **(Fig. 1)**. Some of the interviews were walk and talk carried

out during migration period. The interview topics and the subsequent discussions were related to the geography of the region, determining factors for the migration routes, constraints and opportunities, animal husbandry skills they practiced including mating programs and grazing strategy and finally their income sources. All demographic, ethnic, and sociological data presented in the study has been IP approved by the owners of the knowledge. All photographs are original and been taken by the author.

Discussion

Anatolian lands today are ''home'' to many different nomadic groups with the Laz and Georgian nomads in the Black Sea region the Turkic, Turkomen, and Tahtaci in Western Anatolia and Central Taurus mountains predominantly Kurds in Eastern Anatolia and the Turkomen, Turkic, and Tatar nomads in Central Anatolia (Buyukcan Sayilir, 2012). Turkish transhumance living in the Mediterranean region starts to move in early April to the summer settlement areas in Central Anatolian highlands for two consecutive months covering 400-500 km every year **(Fig 2)**. The main factor determining the migration route these days is solely water availability. For today's mobile pastoralist, goats are the livestock of preference and even though compared to other livestock they are more resilient to dehydration, water still remains a basic necessity. Transhumant, needs to ensure that reliable fountains or wells, along the route **(Fig. 3).** Welfare and proper nutrition of the animals is very important to the tribe members on the long and arduous migration route. Overnight rests are a must during the migration process and are determined by the availability of water and nearby pasture-shrubs- heather land. Turkish transhumance are very aware of their natural environment and conscious about damaging the habitat with over grazing and therefore keep their stays with the large herd fairly short in their resting spells somewhat similar to practicing rotational grazing principals.

The mobile pastoralist during their migration uses black goat hair woven tents in fact it has become a symbol of the transhumant, often being referred to as the **''Black Tent''** The thick and heavy tent while protecting its inhabitants from heat during the summer, is also an admirable insulator from the cold winds during winter **(Fig. 4).** The tents are usually set reasonably apart (500 m) from each other both for privacy and to keep the animals distant enough to utilize resources efficiently without too much competition with minimal impact on the environment.

The special thick weave pattern is only transparent from one side, allowing those
inside to see out, but not the other way. Besides these bare necessities for their own survival, handicrafts such as rug weaving and other skills have become a thing of the past for the modern transhumant mainly due to the heavy workload due to large livestock numbers. Nowadays the most unique product that the families produce is the ''tuluk'' cheese **(Fig. 5).**

Pastures occupy around 18.6 % of the total land surface of Turkey, present day Anatolia (Fig. 6). They can be classified into 6 main ecological regions or biomes (1) Steppes (2) High steppes or High pastures, (3) Anthropic steppe, (4) Alpine and sub-alpine pastures, (5) Forest and (6) Marquis Scrubland or Garrigue (Atalay, 2000). Aiming to maximize the utilization of resources within these eco-regions through grazing, the transhumant moves livestock between winter and summer pastures showing that the transhumant has practiced agro ecological principles as part of their grazing process for centuries in the region. There is strong proof that, soil enrichment and native pasture improvement has definitely resulted through natural urine and manure deposits constantly left behind by the migrating herds. Resultantly, good vegetation cover and no overgrazing has ensured soil conservation due to lack of erosion helping to improve water quality in the respective catchment areas. On the migration route which the authors travelled together with the transhumant families, they were also allowed to graze the stubble of harvested crops on various fallow land. This mutually beneficial arrangement has resulted in natural and cost effective fertilizing for the agriculturalist keeping the rivalry between the two antagonists relatively calm over the years.

Periodic livestock movements and the successive occupation of different territories have helped develop various interesting fauna-flora symbiosis in theAnatolian steppes. For instance one of the main shrub species *Quercus coccifera*the kermes oak, has developed spiny-serrated coriaceous leaves limiting the forage browsing by goats and sheep and yet for a short period early in its development can provide valuable nutrient intake. The same applies for the various perennial *Euphorbia spp*. the spurges, which again are toxic to most herbivores but in small quantities at certain times of the year, has anti-inflammatory properties for various small ruminant ailments, the goats seem to know exactly how many bites to take without reaching the toxic threshold. The Anatolian alpine pasture system is a product of the ancient livestock vegetation interaction that has evolved with the transhumance

lifestyle through many millennia. The ingested pasture seeds that are later deposited within the fertilizer rich manure of the travelling sheep or goat have meant higher germination ensuring a better spread of the established species. If we are to assume that we can identify potential areas of intervention for sustainable natural resource management for the livestock sector in Turkey, we must first accept that there is much to be learned from existing knowledge on transhumance in present day Anatolia. *Pinus brutia*, commonly referred to as 'Turkish pine' a very common forest tree in the East Mediterranean steppes and very pone to debilitating forest fires has performed far better in the historical transhumant routes than in other regions where livestock interaction was abated. By browsing the undergrowth and low scrub, the herd maintains the brush and heath to manageable levels where fires are far less intense if they occur due to natural causes. As well by constant stomping whilst browsing the understory, the herd buries the volatile pine needles into the soft forest soil, further deterring the likelihood of forest fires.

Transhumance has made use of this rich resource over the years and has paid particular attention to preserving the ecology. Yet unwarranted bad press has often been attributed to the mobile pastoralists for contributing to deforestation. This was experienced first hand by the authors where, during the migration whilst conducting the interviews, on numerous occasions as the herd was grazing near or in forest zones, the regional forest rangers warned and unjustly "informally" fined the pastoralists for trespass and damage to govt. property. This was carried out despite obvious care and attention being observed by the herdsman to the grazing habits of the herd. Most of the claims related to forest damage have never been scientifically backed, as was the case on that occasion. There has been few incidents of recorded damage to newly planted forest seedlings in and around the Anatolian basin but this has primarily been the result of sedentary farmers constantly grazing their animals illegally on sensitive re-vegetation sites, without any regards for seasonal effect and damage caused during collection of timber for heating or cooking fuel purposes.

KEYWORD：transhumance, sedentism, animal production, global warming, environment

Fig 1.

Fig 2.

Fig 3.

Fig 4.

Fig 5.

REFFERENCES

Atalay, I. (2000). Les principaux facteurs de dégradation des pâturages en Turquie. http://medias.obs-mip. fr/www/Reseau/Lettre/13bis/fr/12.TURQUIE.PDF. Accessed July 2011.

Buyukcan Sayilir, S. (2012). The nomadism and semi-nomadism issue and differentiation of the nomads in terms of geography. Journal of Turkish World Studies, 1, 563–580.

Fortina, R., Battaglini, L. M., Tassone, S., Mimosi, A., Ripamonti, A. 2001. The shepherd's road: pastoralism and tourism in Piedemonte (NW Italy). In Recognising european pastoral farming systems and understanding their ecology. Proceedings of the 7th European Forum on Nature Conservation and Pastoralism. EFNCP Occasional Publication no. 23. Kindrochaid, Isle of Islay, UK.

Garzon, J. 2001. Importancia de la trashumancia para la conservación de los ecosistemas en España. Boletín de la Institución Libre de Enseñanza, 40, 35–60.

Liberatori, F., Penteriani, V. 2001. A long-term analysis of the declining population of the Egyptian vulture in the Italian peninsula: distribution, habitat preference, productivity and conservation implications. Biological Conservation, 101, 381–389.

Nandris, J. G. 1985. The Stîna and the Katun: Foundations of a research design in European Highland Zone Ethnoarchaeology. World Archaeology, 17, 256– 268.

MacDonald, D., Crabtree, J. R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P. 2000. Agricultural abandonment in mountain areas of Europe: Environmental consequences and policy response. Journal of Environmental Management, 59, 47–69.

Moktan, M. R., Norbu, L., Nirola, H., Dukpa, K., Rai, T. B., Dorji, R. 2008. Ecological and social aspects of transhumant herding in Bhutan. Mountain Research and Development, 28, 41–48.

Oteros-Rozas, E., Gonzalez, J. A., Martın-Lopez, B., Lopez-Santiago, C. A., Montes, C. 2012. Ecosystem services and social-ecological resilience in transhumance cultural landscapes: Learning from the past, looking for a future. Cambridge: Cambridge University Press. doi:10.1017/ CBO9781139107778.018.

Ruiz, M., Ruiz, J. P. 1986. Ecological history of transhumance in Spain. Biological Conservation, 37, 73–86.

O-41-2

Effects of high ambient temperature on physiological responses and brain oxidative condition in growing chickens

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Objective

Heat stress that places the birds above their thermo-neutral zone causes serious physiological dysfunction including a reduced feed intake, body weight gain, and increased mortality (Azad et al., 2010 Mujahid et al., 2009). Hot climate also produce reactive oxygen species (ROS) within the body. Surplus ROS induced by heat stress can cause oxidative injury, such as lipid peroxidation (Mujahid et al., 2007). Moreover, high environmental temperatures also affect metabolic alterations in chickens. Some papers showed that heat challenge elevated (Zulkifli et al., 2009) or did not affect (Chowdhury et al., 2012) plasma corticosterone concentrations. Although production of ROS in some tissue (Azad et al., 2010 Del Vesco et al., 2014) mitochondria of chickens increases under high environmental temperatures, there has been little work evaluating ROS in the brain of chickens subjected to high environmental temperature for several hours.

The present study was carried out to examine the effect of acute exposure of high environmental temperature (34℃ , 8 hours) on a performance, plasma metabolites and brain hydroxyl radical in egg-type growing chickens.

Methodology

All procedures involving the use of animals were performed according to the guidelines for the care and use of animals, as approved by the Animal Experimentation Committee of Utsunomiya University.

Male one-day old egg-type chicks were reared in a temperature-controlled (32℃) room with a 12:12h light/dark cycle. They were given free access to a commercial starter and *ad- libitum* water until the experiment. At 7 d of age, chickens were weighed and selected so that the average body weight was as uniform as possible and they were housed in individual cages in an experimental room at a temperature of 28℃ . Chickens were transferred to another room at 24ºC on 3 days before the heat treatment.

A pair of chickens aged 21-d-old was used, one was for control, CT 24ºC and another heat treatment, HT 34ºC. The HT chicken was placed in a cage set at a temperature-controlled incubator, whilst CT chick was housed in the cage at room. Food and water intake were individually recorded every hour for 8 h. Body weight was recorded at 0, 4 and 8 h, and rectal temperature was measured at 0, 2, 4, 6, and 8 h using a digital thermistor thermometer. Blood samples were taken from the wing vein of each chicken at 0, 4, and 8 h. Plasma was obtained and stored at -20℃ . The plasma samples were divided into three sets to measure the corticosterone, malondialdehyde (MDA) and some metabolites of plasma. Each set included 6 chickens in each group. The corticosterone concentration was determined using an ELISA kit (Cayman chemical, USA). A TBARS assay kit (Cayman chemical, USA) was used for assaying MDA. The metabolites (glucose, total cholesterol, total protein, glutamic oxaloacetic transaminase (GOT), uric acid, and calcium) were determined using an automated analyzer (SPOTCHEM EZ SP-4430, Arkray, Japan).

Brain hydroxyl radical was determined in a microdialysis technique. Microdialysis experiments were carried out on a pair of CT and HT chickens aged ranging 21 from 23 days in accordance with Tachibana et al. (2000). Microdialysis probes were inserted into the lateral ventricle (Kuenzel and Masson, 1988) via previously implanted guide cannula. The temperature for the HT group was changed from 24 to 34ºC after the level of 3, 4-dihydroxybenzoic acid (3,4-DHBA) became stable. 3, 4-DHBA is a product of reaction of ROS and 4-hydroxybenzoic acid (4-HBA), and has been proved to be useful to trap hydroxyl radical in the dialysate from the brain (Liu et al., 2002). Dialysate sample was collected every 30 min for 9 h. 3, 4-DHBA in the dialysate was analyzed using a high performance liquid chromatograph with an electrochemical detector. After completion of the dialysis, serial coronal sections of the brain were made to determine microscopically the location of the dialysis probe.

All results were expressed as mean±SEM. Data were analyzed using a two-way ANOVA and t-test. The 3, 4-DHBA in the dialysate was expressed as a percentage of the baseline. A probability level of $P < 0.05$ was considered to be

significant.

Results

Effect of HT on body weight, rectal temperature, food and water intake in chickens

Body weight was not significantly affected *(P>0.05)* by the heat treatment in the two groups. Rectal temperature increased *(P<0.05)* at 2 h of exposure to HT, and the difference between the HT and CT groups was maintained until the end of experiment (Fig. 1A). Heat treatment showed strong suppression of food intake in HT group from 1 to 7 h (Fig. 1B). No significant difference of water intake was observed between HT and CT chicks (data not shown). These responses of rectal temperature and food intake are partly similar to previous studies (Chowdhury et al., 2012), which shows the temperature and during of heat exposure was enough to affect the body temperature and food intake.

Effect of HT on plasma corticosterone, MDA and plasma metabolites

Neither plasma MDA nor corticosterone was affected by heat exposure (data not shown). Plasma metabolites except for uric acid did not differ between the control and HT groups. The concentration of uric acid was significantly lower in HT than control group at 4 h (4.9 and 7.7 mg/dl) and 8 h (7.2 and 9.5 mg/dl) *(P<0.05)*. The concentration of plasma metabolites varied with studies previously reported (Chowdhury et al., 2012, Del Vesco et al., 2014).

Changes in 3, 4-DHBA in chickens exposed to high environment temperature

Fig. 2 shows the time course concentration of 3, 4-DHBA in the dialysate collected from a microdialysis probe implanted in the lateral ventricle of HT and CT chickens. Microscopic observations showed that microdialysis probes were located in the lateral ventricle in five birds for CT and four for HT. No significant difference in 3,4-DHBA between the groups was observed throughout the heat treatment, although the concentration of 3,4-DHBA continued to increase during the course of heat treatment. In this connection, MDA in the diencephalon of chickens exposed to the temperature of 35°C for 48 hours increased (Chowdhury et al., 2014). The effect of high environmental temperature on the brain oxidative condition likely depends on the duration of exposure. It is not clear what made similarly progressive increase of 3,4-DHBA of the dialysate from the two groups.

Conclusion

Rectal temperature increased and food intake reduced in growing chickens within several hours when they were individually housed under the temperature of 34°C. Plasma metabolites hardly differ in 8 hours of heat treatment. Hydroxyl radical in the dialysate from the lateral ventricle did not differ even when chickens were exposed to acute HT. It is likely that physiological, biochemical and thermoregulatory responses occurred without the induction of oxidative damage in the brain when chickens were exposed for 8 hours.

Acknowledgements

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KEYWORD：high ambient temperature, brain hydroxyl radical, growing chicken

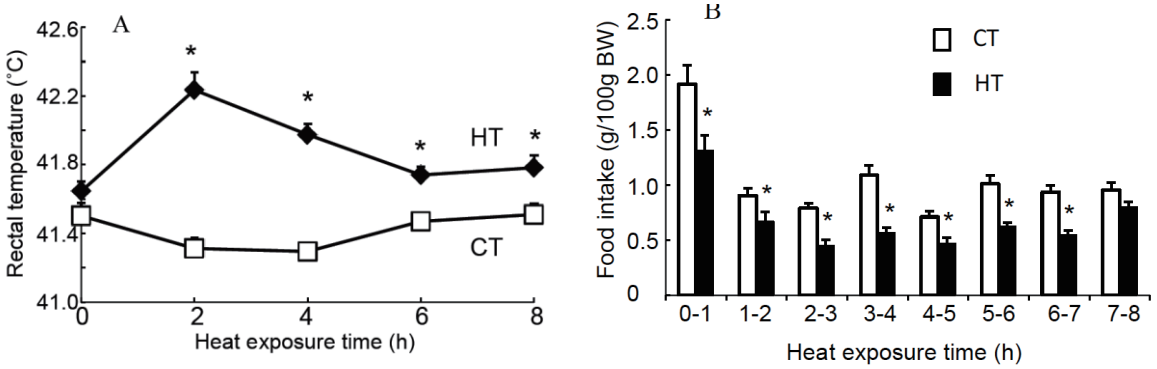

Fig. 1 Rectal temperature (A), and food intake (B) in growing chickens exposed to a control temperature (CT; 24ºC) or a high ambient temperature (HT; 34ºC) for 8 h. Values are means ± SEM (n= 16 in each group). Food intake expressed as g/100 g body weight. *Significantly different from the CT group ($P < 0.05$).

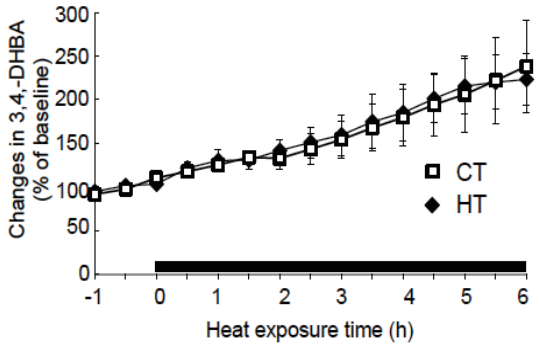

Fig. 2 Changes in 3, 4-DHBA concentrations in response to heat treatment in chickens. The horizontal bar indicates the heat treatment. Data are expressed as means±SEM (n = 5, CT; n=4, HT).

REFFERENCES

Azad MAK, Kikusato M, Sudo S, Amo T and Toyomizu M. 2010. Time course of ROS production in skeletal muscle mitochondria from chronic heat-exposed broiler chicken, Comparative Biochemistry and Physiology, Part A 157: 266-271.

Chowdhury VS, Tomonaga S, Nishimura S, Tabata S and Furuse M. 2012. Physiological and behavioral responses of young chicks to high ambient temperature. Journal of Poultry Science, 49, 212-218.

Chowdhury VS, Tomonaga S, Ikegami T, Erwin E, Ito K, Cockrem JF and Furuse M. 2014. Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature. Comparative Biochemistry and Physiology, Part A 169: 70-76.

Del Vesco AP, Gasparino E, Grieser DO, Zancanela V, Gasparin FRS, Constantin J and Oliveira Neto AR. 2014. Effect of methionine supplementation on the redox state of acute heat stress-exposed quails. Journal of Animal Science, 92:806-815.

Kuenzel WJ and Masson MA. 1988. Stereotaxic Atlas of the Brain of the Chick (Gallus domesticus) Baltimore, The Johns Hopkins University Press.

Liu M, Liu S, Peterson SL, Miyake M, Liu KJ. 2002. On the application of 4-hydroxybenzoic acid as a trapping agent to study hydroxyl radical generation during cerebral ischemia and reperfusion. Molecular and Cellular Biochemistry 234/235: 379–385.

Mujahid A, Akiba Y, Warden CH, and Toyomizu M. 2007. Sequential changes in superoxide production, anion carriers, and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. FEBS letter, 581:3461-3467.

Mujahid A, Akiba Y, and Toyomizu M. 2009. Olive oil supplemented diet alleviates acute heat stress-induced mitochondrial ROS production in chicken skeletal muscle. American Journal of Physiology, 297(Suppl.3): R690-R698.

Tachibana, T., Utimura D., Kato H, Kubo T, and Sugahara K. Extracellular norepinephrine in the medial hypothalamus increases during feeding in chicks: a microdialysis study. 2000. Comparative Biochemistry and Physiology, Part A 127: 331-338.

Zulkifli I, Al-Aqil A, Omar AR, Sazili AQ, and Rajion MA. 2009. Crating and heat stress influences blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poultry Science, 88: 471-476.

O-41-7

Effect of Fishery Related Activities on the Food and Nutrition Situation of Indigenous Community : A Study of Adolescent girl

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INTRODUCTION

There are more than two million *Adivasi* (ethnic people) living mostly on the margins of Bangladeshi territory and society. Population pressure, resource depletion and shrinking aquatic habitats have undermined their traditional livelihoods of hunting and gathering of food in wetlands. The *Adivasi* Fisheries Project (AFP) set out in 2007 to help these people find new and more sustainable livelihoods. The livelihood options made available to AFP beneficiaries were pond aquaculture, rice-fish culture, fingerling production in cages, habitat restoration, forming netting teams, and fingerling and food fish treading.

Monitoring surveys found all of the project's fisheries-related livelihood options profitable in the first year of the intervention and likelihood to become more profitable with time. The AFP lifted the average income of participating pHH from Taka 44,075 (US$ 647) in 2007 to Taka 52,035 (US$ 763) in 2008, largely by quadrupling the contribution of fish[2]. The increased income improved the food security of pHH, reducing their food deficit period from 1.7 months in 2007 to 1.4 months in 2008. By the end of 2008, the AFP had improved the livelihoods of 3,594 pHH[2].

Food intake being directly attributable to nutritional levels has continued to remain a critical socio-economic goal of any development programme. The monetized indicator of well-being, income or expenditure, has been viewed since last decade as denoting only limitary explanation in the welfare analysis of poverty reduction programms. Using child or adolescent anthropometry, in this regard, emerged as a better welfare measure[3]for the followings advantages: (i) it measures net nutritional status i.e., inputs of nutrients minus claims made by body maintenance, physical activity, and disease (ii) data on body size may be used as a proxy measure of household income or food consumption and (iii) it provides information on individuals. Moreover, unlike economic data, anthropometric data can be compared directly across regions and countries.

From the foreground, it is clear that any welfare programme like AFP need to be evaluated through nutrition indicators to assess its impact on individuals and society.

MATERIALS AND METHODS

Study design: A cross-sectional survey was conducted to evaluate the socio-economic, anthropometric and food consumption situations in ethnic households received various aquaculture related inputs from AFP. A cohort of adolescent girls (10-19 years) from pHH were identified and followed up for anthropometric and food frequency data after 7 months. Non-project ethnic households were also surveyed for comparisons.

Sample size and sampling design: The list of the farmers' field school (FFS) collected from the WorldFish center databank was considered as the sampling frame for household selection. This list contained the name of household head, name and age of children, adolescent girls, and other family member, household identification number, village name, *Upzilla* (sub-district) name, and other necessary information.

The total number of adolescent girls (10-19 years) in 3650 pHH under AFP was 909[4]. Ten percent of these adolescent girls (i.e., 91) were considered as the minimum number of statistically sufficient study subjects.

Adolescent girls were selected from four purposively selected household clusters of four main ethic groups *(Santal, Garo, Oraon*, and *Hajong*) by quota-sampling design (minimum 20 girls per household cluster) using the FFS as the sampling frame. A 4x60 cluster design was used to select pHH assuming an average of one adolescent girl

per household. Available all npHH having adolescent girls in each cluster were included in the study for impact comparison.

Data collection methods: Socioeconomic and opinion data was collected by face-to-face interview of the HH head using a validated structured questionnaire. Anthropometric data was collected by standard measuring instruments and using WHO procedure. Traditionally the 24-hour recall is undertaken in chronological order of consumption. Instead of it, a multiple pass recall (MPR) approach was used for this study with in-depth probing interview covering 24-h dietary recall conducted among selected adolescent girls in both pHH and npHH.

Data Processing and Analysis: Data processing operation consisted of editing, coding, classification and tabulation followed by analysis using SPSS 16 software package programme. Besides SPSS other well-known package like MSWord and MS Excel were also used for graphical representation and calculation.

Data analysis scheme included survey statistics (percent, means, standard deviations, Chi-squares) and advanced statistics (t-test, one way ANNOVA, correlation co-efficient for the association of variables). Cross tables for different variables were constructed to observe whether there is any significant relationship between two variables. Chi-square test was used for the association between non-parametric variables corresponding to their frequency distribution. On the other hand, for the degree of association among the study variables (both measured on study subjects and found normally distributed), Pearson's (product-moment) correlation coefficient (r) was determined.

RESULTS AND DISCUSSION

Socioeconomic and demographic features of the participants

The following Tables show the distributions of study households and participant adolescent girls by Table 1 describes the socioeconomic and demographic features of the study participants. Majority of the study participants belonged to the *Garo* (37.3%) ethnic community. Majority of both pHH and npHH belonged to middle income group (Taka 3001 -10000) in terms of rural economic condition of Bangladesh at the time of survey. The pHH, however, showed relatively

higher income-expenditure parity than their npHH counterparts. Nevertheless, monthly income and expenditure of the pHHs were found approximately similar thus indicating that only few families can save money for other purposes than spending on food and related commodities.

Nutritional status of the adolescent girls

The growth performances of the study subjects were further elaborated by using nutritionals status indicators of BMI revealed severe underweight (16BMI) among npHH compared to pHH girls(Figure 1). At the follow-up stage, determination of nutritional status revealed that the prevalence of severe malnutrition in pHH girls was reduced whereas it was increased in npHH girls. This increase in nutritional status of pHH adolescent girls could be a result of increased dietary intake pHH families.

The household food consumption behavior

Table 2 revealed the calorie and nutrient differences between pHH and npHH. The family members of pHH have found to consume higher amount of calorie and protein than npHH. This increased in intake of dietary was reflected in better nutritional status of the adolescent girls both in baseline and follow up survey. Especially, the 15-19 yrs. girls have been found to consume more calorie and nutrients than girls of similar age in npHH.

In conclusion, it could be seen from the study that any income generation activity of marginal people like ethnic population generally increases the total expenditure on food. This increase in expenditure on food causes increase intake of energy and nutrient. Thus, any minor incase in dietary consumption nutrients in terms of quantity and quality when cumulated in the course of time, could achieve the daily requirement fulfillment for ideal growth which intern brings improvement in nutrition and health of the household members.

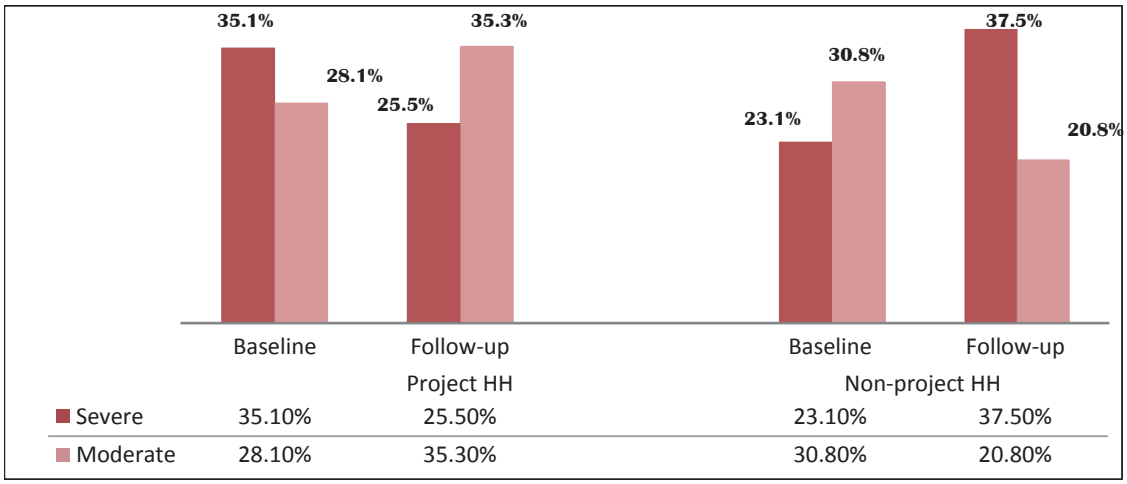
The present study, therefore, disclosed that the aquaculture and related activities under AFP among *Adivasi*(ethnic) people of Bangladesh had produced some positive impact to reduce existing household malnutrition as indicated by the improved nutritional status of the household adolescent girls.

IMPLICATIONS

Quantifying household and individual welfare and investigating the impact of safety net programmes like aquaculture related activities requires various sorts of data that measure living standards. The adolescent

anthropometric data generated in this study will be complementary to that notion.

KEYWORD : Adolescent girl, Nutritional status, Nutritional Assessment, Body Mass index, Indigenous population (Adivashi)



HH Type	Baseline		Follow-up	
	Severe (<16.5)	Moderate (<18.5)	Severe (<16.5)	Moderate (<18.5)
Project HH	20 (n=57)	16 (n= 57)	13 (n=51)	18 (n= 51)
	Total Underweight = **63.2%** (n=36)		Total Underweight = **60.8%** (n=31)	
Non-Project HH	6 (n=26)	8 (n= 26)	9 (n=24)	5 (n= 24)
	Total Underweight = **53.8%** (n=14)		Total Underweight = **58.3%** (n=14)	

Figure 1. Prevalence of severe and moderate underweight by BMI in adolescent girls

Table 1. The socioeconomic features of the study subjects

Sample Features	PP (57)	NPP (26)	Total (83)	
	No	No	No	%
Age				
10-14 yrs	41	16	57	**68.7**
15-19 yrs	16	10	26	31.3
Ethnicity				
Garo	18	13	31	**37.3**
Santal	15	6	21	25.3
Oraon	20	4	24	28.9
Hajong	4	3	7	8.4
Religion				
Hindu	1	1	2	2.4
Sanaton	27	7	34	41.0
Christian	29	18	47	**56.6**
Education				
Illiterate	1%	11%	…	…
Primary	42%	26%	….	…
Secondary	56%	61%	….	….
Monthly Income (Taka)*				
≤ 3000	9	1	10	13.7
3001-5000	19	12	31	42.5
5001-10,000	18	10	28	38.3
>10,000	1	3	4	5.5
Monthly expenditure(Taka)				
≤ 3000	5	4	9	12.3
3001-5000	21	12	33	45.2
5001-10,000	19	8	27	37.0
>10,000	2	2	4	5.5

*72 Taka = US$ 1 at time of survey

Table 2. Mean Per capita Nutrient intake of pHH and npHH

Calorie and nutrients	10-14 yrs.		15-19 yrs.	
	pHH (Mean ± SD)	npHH (Mean±SD)	pHH (Mean±SD)	npHH (Mean±SD)
Energy(Kcal)	1653.83±615.77	1629.03±630.79	2043.91±718.03	1532.13±645.12
Protein(g)	38.5±15.69	39.95±25.87	47.87±21.31	31.52±14.02
Fat(g)	5.19±7.42	6.5±1.31	3.61±2.41	2.81±2.64
Carbohydrate(g)	366±135.02	349.38±139	451±159.38	341.71±145.33
Vitamin A(IU)	4596 ± 2842	5316 ± 360	3389 ± 2202	2211 ± 395
Thiamin(mg)	1.11 ± 0.46	1.16 ± 0.57	1.42 ± 0.6	1.01 ± 0.45
Riboflavin(mg)	0.34 ± 0.22	0.71 ± 1.39	0.47 ± 0.23	0.24 ± 0.13
Niacin(mg)	17.86 ± 8.07	17.31 ± 6.31	21.37 ± 7.69	16.63 ± 7.29
Vitamin C(mg)	37.28 ± 34.83	31.19 ± 18.93	37.26 ± 26.43	31.35 ± 44.9
Calcium	268.97 ± 97	206.05 ± 267.67	292.44 ± 246.32	213.43 ± 386.32
Iron(mg)	10.23 ± 5.73	12.54 ± 11.02	18.95 ± 17.30	8.73 ± 5.14
Zinc(g)	6.28 ± 2.61	7.56 ± 5.33	8.95 ± 5.2	5.17 ± 2.5

REFERENCES

Bangladesh National Nutrition Survey,1995-96 (BNNS) 1999. INFS, University of Dhaka, Dhaka, Bangladesh.

Gillespie S, Haddad L. 2003. Nutrition and the MDGs: The Relationship between Nutrition and the Millennium Development Goals: A Strategic Review of the Scope for DFID's Role. IFPRI Report. International Food Policy Research Institute, Washington.

Margen S. 1984. Energy Protein Malnutrition: The Web of Causes and Consequences In: Malnutrition and Behavior: Critical Assessment of Key Issues, Brozek J and Schurch B, Eds., Nestle Foundation Publication Series Vol. 4, Lausanne, pp. 20-31.

UNS. 2004. 5th Report on the World Nutrition Situation.UN System Standing Committee on Nutrition, Geneva.

World Bank. 2000/2001. World Development Report: Attacking Poverty, Oxford University Press, Oxford.

Khandkar SR and Chowdhury OH .1996. Targeted Credit Programmes and Rural Poverty in Bangladesh, World Bank Discussion Paper 336.

Chowdhury MJA .2004. Micro-credit and sustainability of poverty alleviation: a case study of Grameen Bank in Bangladesh. Social Science Review .The Dhaka University Studies, Part-D]; 21 (1):105-20.

Mitra SN, Sabir AA, Saha T, and Kumar S. 2001.Bangladesh demographic and Health Survey 1999 – 2000. NIPORT,Mitra and Associates, Dhaka, Macro International, USA.

ACC/SCN . 2000. Nutrition throughout the Life Cycle. Fourth Report on the World Nutrition Situation. Geneva: ACC/SCN in collaboration with IFPRI, p. 18.

INFS .1983.Nutrition survey of rural Bangladesh 1981-82. Institute of nutrition and food science, Dhaka university.

Karim, R .and Hassan, N. 1989.Changes in food consumption in Bangladesh.Bangladesh journal of nutrition. 2 :17-20.

Anderson, J. B .1991.The status of Adolescent Nutrition. Nutrition Today, March-April,7-10

Latham, M. C.1997. Human Nutrition in the Developing World.FAO. PP.432-37

[2]WorldFish Center Factsheet 1954.Available at: http://pubs.iclarm.net/resource_centre/WF2486.pdf

3Micklewright and Ismail 2001 4WF center database 2007.

Raper N, Perloff B, Ingwersen L, Steinfeldt L, Anand J. An overview of USDA's Dietary Intake Data System.pdf.J Food Compos Anal. 2004;17:545–55. Available from: http://ac.els-cdn.com/S0889157504000511/1-s2.0-S0889157504000511-main.pdf?_tid=38ca7396-288a-11e4-b96f-00000aacb360&acdnat=1408553665_17df9f4984c2d96ac22e899bb5f9a25d.

O-41-10 Performance of Hissar cattle (Bos indicus) in the Dry Tropics of Sumbawa, Eastern Indonesia

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Introduction

In 1920, the Dutch introduced 202 (15 males, 138 females, and 49 calves) Hissar cattle (*Bos indicus*) from Punjab India into Sumbawa island to be used as draught animals. Since then, Sumbawa island was designated as the centre for purification of Hissar cattle. Hissar cattle in Sumbawa is characterized by longer horns of females than males, white or dark grey coat, good temperament, and large withers with hump. By 1938, population of Hissar cattle declined to only 8 (2 intact bulls, 2 castrated bulls, 2 cows and 2 calves). They were the germplasm from which the current Hissar cattle which have been well adapted to the dry tropics of Sumbawa (Dilaga, 2014).

Based on information from the key farmers the highest number of Hissar cattle occurred in 1960-1970 but there was no official data available. In 1976/1977 the government introduced Bali cattle to Sumbawa to purify this cattle breed and to increase Bali cattle population in Indonesia (Sudradjat and Pambudy, 2003). To successfully implement the Bali cattle purification program, Hissar cattle which have been in the island for 56 years should be eliminated. However, farmers raised Hissar cattle illegally because they really like this cattle breed. Hissar cattle have good temperament and tolerant to diseases.

Based on the Decree of Ministry of Agriculture Republic of Indonesia no: TN 220/18/A/0299 dated 7 February 1999, the national government declared Penyaring Village in the northern part of Sumbawa island, as the region for Hissar cattle development. This period was the starting point of the rapid growth of Hissar cattle population in Sumbawa. Due to the rapid increase in population of Hissar cattle, the Indonesian government declared Hissar cattle as the local germplasm and called "Sumbawa cattle" (decree of Ministry of Agriculture Republic of Indonesia no: 2909/Kpts/OT.140/1/2011, dated 17 June 2011). Since then, Hissar cattle in Indonesia are known as "Sumbawa cattle".

By 2015, population of Hissar cattle in Sumbawa reached 10,541 and scater all across Sumbawa Island. The highest population occurred in Sumbawa district (6,763) and 70% of the Hissar cattle in this district are raised by farmers in Penyaring village (Anonymous, 2015). Hissar cattle are now known as dual purpose cattle to produce beef and milk and have not been studied intensively.

Performance of Hissar cattle in Sumbawa

Adaptation ability to harsh environment

Hissar cattle are able to grow well and have quite high milk production under the humid tropics of Sumbawa probably because they have white skin that can reflect ultraviolet light from the sun. The large hump enables them to adapt to low quality diets as the hump functions as the fat store. According to Sutardi (1981) when an animal does not eat, the energy demand is met from oxidation of body fat. In addition to energy, fat metabolism also results in metabolic water, therefore Hissar cattle are efficient in using water in the dry areas (Dilaga, 2014). Husnainy (2005) reported that Hissar cattle consume smaller amount of water (159 ml/kg $B^{.75}$/day) than FH cross (268 ml/kg $B^{.75}$/day) and Bali cattle (332 ml/kg $B^{.75}$/hari).

Growth and beef production

Hissar cattle are mostly raised in semi extensive system. They are allowed to graze night and day on communal grazing land and collected only when they are registered or to be sold. Consequently their growth rate is slow (0.5 kg/day in average). Growing Hissar bulls supplementation with 1 kg rice bran per head per day before grazing increased the growth rate to 0.7 kg/day (Dilaga et. al., 2002).

Supplementation of cows during late pregnancy with 1 kg fresh leucaena per day increased birth weight from

22.83±2.40 kg to 24.67±1.63 kg (Dilaga, et. al., 2015). Results of an ongoing experiment, growth rate of weaned male Hissar cattle supplemented with leucaena at 1% dry matter of body grew at 0.4 kg/day compared to the ones fed 100% grass growing at 0.1 kg/day. Similar results of leucaena feeding were also reported in other studies. Panjaitan et. al. (2013) reported that Bali bulls fed leucaena as the main component of the diet grew at 0.42±0.12 kg/day, twice the rate of similar bulls fed grass only. Dahlanuddin et. al. (2014) also reported that young Bali bulls fed leucaena as the sole diet grew at 0.47±0.05 kg/day.

The potential of Hissar cattle to produce beef can be compared with other *Bos indicus* breed (Sumba Ongole and Peranakan Ongole (Ongole cross) (Table 1).

Table 1. Average weight and percentage of body parts of medium frame *Bos indicus* (Dilaga, 2014)

No	Items	Hissar	Sumba ongole (SO)	Peranakan ongole (PO)
1.	Warm carcass, kg	125.4	180.0	136.0
2.	Carcass, %	47.5	44.9	45.3
3.	Head, kg	16.0	19.9	15.2
4.	Hide, kg	16.0	26.8	18.4
5.	Legs, kg	6.0	7.5	5.8
6.	Heart and lung, kg	3.3	6.7	3.8
7.	Liver, kg	2.7	5.2	3.3
8.	Spleen, kg	1.5	1.0	0.7
9.	Digestive system, kg	28.0	26.0	15.5

Hissar cattle has higher carcass percentage compared to Sumba Ongole and Peranakan Ongole. This is because Hissar cattle have smaller proportion of hide, head and leggs. Under free grazing condition, the slaughter weight of Hissar cattle can reach 450 kg. Recently, farmers cross male Hissar cattle with Bali cows to increase beef production. The birth weight of this Hissar x Bali cross (called Hisbal crossbred) is 18-20 kg and grow faster than Bali cattle (Dilaga, 2014). However, information on ADG, slaughter weight and carcass weight of Hissar x Bali bulls are not available and open for more detailed study.

Milk production

Hissar cattle grazing on native pasture has low milk production. Supplementation of lactating cows (4th lactation) with 1 kg fresh leucaena and 1 kg rice bran, increased milk production from 2.7 ± 0.8 liter/day to 3.9 ± 0.9 liter/day and increase income from milk by IDR 14,000 per litre of milk (Dilaga, *et al*., 2015). This increased milk production was due to additional protein from leucaena to balance the energy rich rice bran. Tudsri *et al*. (1998) also reported that milk production of dairy cattle grazing on pasture improved with leucaena and brachiaria grass increased from 11.9 litre per day to 13.6 litre per day. Maasdorp dan Dzowela (1998) also reported that milk production of cows supplemented with leucaena, *Acasia boliviana*, *Caliandra calothyrsus* and grass were 13.6, 11.94, 11.14 and 11.36 litre per day respectively.

Conclusion

Hissar cattle have been well adapted to the humid tropics of Sumbawa. Under traditional rearing system, Hissar cattle bull grow at an average of 0.5 kg/day. Supplementation with energy or protein rich supplements can increase liveweight gain to 0.7 kg/day, and increase milk production from less that 3 litres per day to about 4 litres per day.

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KEYWORD : Hissar cattle, Dry tropics, Productivity

REFERENCES

Anonymous (2015). Annual Report of Dinas Peternakan dan Kesehatan Hewan NTB.

Dahlanuddin , O. Yanuarianto , D. P. Poppi , S. R. McLennan and and S. P. Quigley (2014) Liveweight gain and feed intake of weaned Bali cattle fed grass and tree legumes in West Nusa Tenggara, Indonesia. Animal Production Science 54(7) pp 915-921

Dilaga, S.H. 2014. Sapi Sumbawa, Sumber Daya Genetik Ternak Indonesia. Penerbit Pustaka Reka Cipta, Bandung.

Dilaga, S.H., Hasyim, C. Arman, Lestari, and M. Dahlik. 2002. Pengembangan Sapi Hissar di Wilayah Moyo Hilir Sumbawa. Laporan Penelitian Hibah Bersaing IX/II Perguruan Tinggi, Ditbinlitabmas Ditjen Dikti Depdiknas RI.

Dilaga, S.H., Imran, Santi Nururly, and Padusung. 2015. Pengembangan Sumber Daya Lar Sapi Sumbawa Guna Meningkatkan Produksi Daging dan Susu Nasional (Penelitian kaji Terap). Prosiding Seminar Nasional Peternakan Berkelanjutan 7. Sumedang Indonesia, 11 November 2015. Hal 6-10.

Husnany, B.H. 2005. Perbandingan Konsumsi Pakan dan Air Minum, serta Laju Penguraian Penguraian Feses Sapi Hissar, Bali, dan Peranakan Friesian Holstein (PFH). Skripsi Fakultas Peternakan Unram.

Maasdorp, R.V. and B.H. Dzowela. 1998. Comparison of *Leucaena leucocephala* and Other Tree Fodders as Supplements for Lactating Dairy Cows. In *Leucaena* Adaptation, Quality and Farming Systems. Proc. Of Workshop Held in Hanoi, Vietnam 9-14 February 1998.

Panjaitan, T., M. Fauzan, Dahlanuddin, M.J. Halliday, and H.M. Shelton., 2013. Growth of Bali bulls with forage tree legumes in Eastern Indonesia: *Leucaena leucocephala* in Sumbawa. Tropical Grasslands – *Forrajes Tropicales* (2014) Volume 2, 116 — 118.

Sudardjat, S.D. dan R. Pambudy. 2003.Menjelang Dua Abad Sejarah Peternakan dan Kesehatan Hewan Indonesia: Peduli Peternak rakyat. Penyunting A. Syarief. Penerbit Yayasan Agrindo Mandiri, Jakarta.

Sutardi, T. 1981. Landasan Ilmu Nutrisi. Departemen Ilmu Nutrisi Fakultas Peternakan IPB-Bogor.

Tudsri, S., S. Prasanpanich, and S. Swasdiphanich. 1998. Milk Production from Ruzi mixed with *Leucaena, Ruzi* alone and *Ruzi* supplemented with *Lablab purpureus*. In *Leucaena* Adaptation, Quality and Farming Systems. Proc. Of Workshop Held in Hanoi, Vietnam 9-14 February 1998.

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Growth pattern of young progeny test steers and relationship between the carcass measurements in Japanese Black (Wagyu)

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ABSTRACT

To date, the selection of the Japanese Black breed has put emphasis on intramuscular fat resulting in highly marbled beef. However, there is need to improve meat production ability and efficiency. The purpose of this study is to determine the growth pattern of young progeny test steers and the relationship between the common carcass traits and new carcass measurements in Japanese Black cattle. Carcass measurements that were measured at the 7th thoracic vertebra area were *longissimus* muscle area (LMA), *trapezius* muscle area (TMA), rib thickness (RT), *longissimus* muscle thickness (LMT), *trapezius* muscle thickness (TMT), *subcutaneous* fat thickness (SFT), intermuscular fat thickness (IMFT), etc. The steers were slaughtered at the end of the test (20 months of age). Ultrasound measurements were taken at 11, 14, and 18 months of age. Ultrasound traits were *longissimus* muscle area (ULMA), rib thickness (URT), *subcutaneous* fat thickness (USFT), etc. High correlation coefficients were observed between LMA and LMT, TMA and TMT. Intramuscular fat had a higher correlation with IMFT compared to SFT which had lower values. Despite the fact that the steers in this study were slaughtered at 20 months of age, the results obtained were similar to a study that evaluated steers slaughtered at 30 months of age. Moderate to high partial correlation coefficients were obtained between carcass LMA and ULMA, carcass SFT and USFT. Carcass RT and URT had low correlation. The Growth patterns observed in this study are similar to those of progeny test steers slaughtered at 30 months of age. On a positive note, LMA in the current study was slightly larger than the previous study. Furthermore, SFT did not show any increase. This study determined the growth pattern of young progeny test steers and the relationship between the common carcass traits and new carcass measurements in Japanese Black cattle.

INTRODUCTION

To date, the selection of the Japanese Black breed has put emphasis on intramuscular fat resulting in highly marbled beef. However, there is need to improve meat production ability and efficiency. Evaluation of meat production ability in live animals is possible though a number of techniques. Among them, ultrasound techniques have been commonly used in the field of livestock production because they have many advantages over other techniques (Tokunaga et al., 2013. Hwang et al., 2014. Nade et al., 2014). A number of studies have reported growth patterns and carcass traits of steers slaughtered at around 30 months of age. However, there is need to evaluate growth patterns of young cattle and determine efficacy of shortening the fattening period to increase production efficiency.

The purpose of this study is to determine the growth pattern of young progeny test steers and the relationship between the common carcass traits and new carcass measurements in Japanese Black cattle.

MATERIALS AND METHODS

Animals and traits. The data were collected from 172 young Japanese Black steers that were under progeny testing at the Livestock Improvement Association of Miyazaki from April 2012 to March 2014. The steers were progeny of 22 sires. The datasets comprised of carcass and real-time ultrasound measurements. Carcass measurements that were measured at the 7th thoracic vertebra area were: *longissimus* muscle area (LMA), *trapezius* muscle area (TMA), rib thickness (RT), *longissimus* muscle thickness (LMT), *trapezius* muscle thickness (TMT), *subcutaneous* fat thickness (SFT), intermuscular fat thickness (IMFT), *longissimus* muscle fat ratio (LMF), and *trapezius* muscle fat ratio (TMF). The steers were slaughtered at the end of the test (20 months of age); each carcass was graded at the 6^{th} to 7^{th} rib cross section area by official Japanese graders following Japanese Grading Standards (JMGA, 1989). Ultrasound measurements were taken at 11, 14, and 18 months of age. Ultrasound measurements were done using a B-mode real-time ultrasound device (HS-2000, FHK Co. Ltd, Japan) with a 12 cm, 2 MHz linear probe. Scanning was made by positioning the probe vertically along the dorsal-ventral line

parallel to the ribs between 6th and 7th thoracic vertebra on the left side. Ultrasound traits were *longissimus* muscle area (ULMA), *trapezius* muscle thickness (UTMT), *subcutaneous* fat thickness (USFT), and intermuscular fat thickness (UIMFT). Furthermore, the ultrasound estimate for rib thickness (URT) were measured at 3.45 cm from the *musculus iliocostalis* (the point for measuring USFT and UIMFT), this is a different position with actual carcass RT measurement. *Trapezius* muscle thickness (UTMT) was the thickness of the *trapezius* muscle at 10 cm from the ventral end of the *trapezius* muscle (Figure 1). High-definition digital images of the 6th to 7th rib of the carcass were taken using the mirror type photographic equipment (HK-333, Hayasaka Rikoh Co. Japan). The image analysis software used was WinROOF2015 (Mitani Co. Japan).

Statistical analyses. Descriptive statistics and correlation coefficients were calculated using JMP® 12.1.0 (SAS Institute Inc., Cary, NC, USA). To estimate the growth patterns, the following equations were fitted: Gompertz, Bertalanffy, Brody, and Logistic. Ultimately the best fitting equations were used.

RESULTS AND DISCUSSION

Results of the simple correlation analysis of carcass measurements are shown in Table 1. High correlation coefficients were observed between LMA and LMT. To add to that, High correlation coefficients were observed between TMA and TMT. Intermediate correlation was observed between TMA and TMF however, a lower correlation was observed between LMA and LMF. Intramuscular fat (i.e. LMF and TMF) had a higher correlation with IMFT (0.57 and 0.33 respectively) compared to SFT which had lower values of 0.30 and 0.20.

Results of the partial correlation coefficient of carcass and ultrasound traits are shown in Table 2. Despite the fact that the steers in this study were slaughtered at 20 months of age, the results obtained were similar to a study that evaluated steers slaughtered at 30 months of age (Tokunaga et al., 2013). Moderate to high partial correlation coefficients were obtained between carcass LMA and ULMA, carcass SFT and USFT. Carcass RT and URT had low correlation. The reason is that carcass RT is measured further down at the mid-point of the rib.

Growth patterns of LMA and SFT are shown in Figure 2 and Figure 3. Ultimately the best fitting equations used were Gompertz for LMA and Bertalanffy for SFT. The Growth patterns observed in this study are similar to those of progeny test steers slaughtered at 30 months of age (Tokunaga et al., 2013). On a positive note, LMA in the current study was slightly larger than the previous study. Furthermore, SFT did not show any increase.

This study determined the growth pattern of young progeny test steers and the relationship between the common carcass traits and new carcass measurements in Japanese Black cattle. The growth patterns observed in this study highlight the possibility of reducing slaughter age to increase production efficiency however more studies need to be done.

Keywords: Carcass measurements, Growth pattern, Japanese Black cattle, Ultrasound

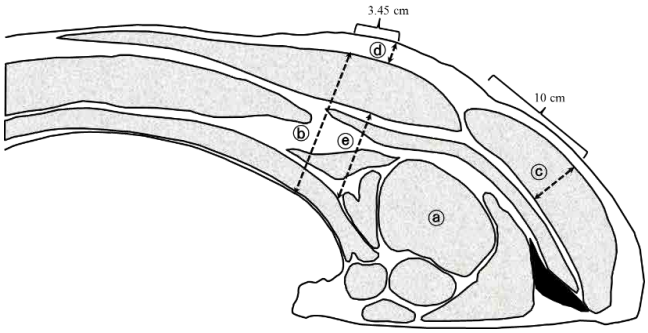

Figure 1. Schema showing positions of the ultrasound traits measurements at 6th and 7th thoracic vertebra area
ⓐ: *longissimus* muscle area (ULMA), ⓑ: rib thickness (URT),
ⓒ: *trapezius* muscle thickness (UTMT),ⓓ: *subcutaneous* fat thickness (USFT),
ⓔ: intermuscular fat thickness (UIMFT)

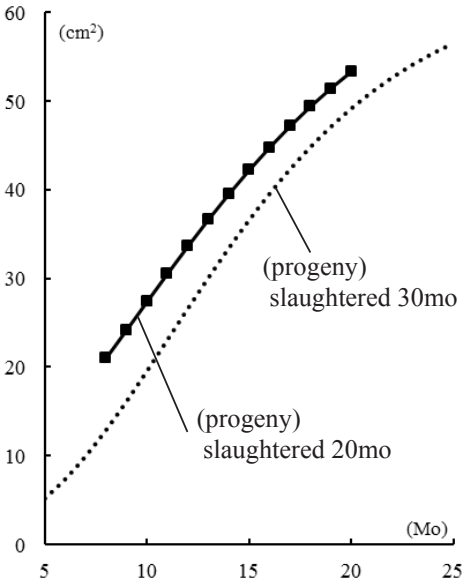

Figure 2. Growth pattern of *longissimus* muscle area (LMA)

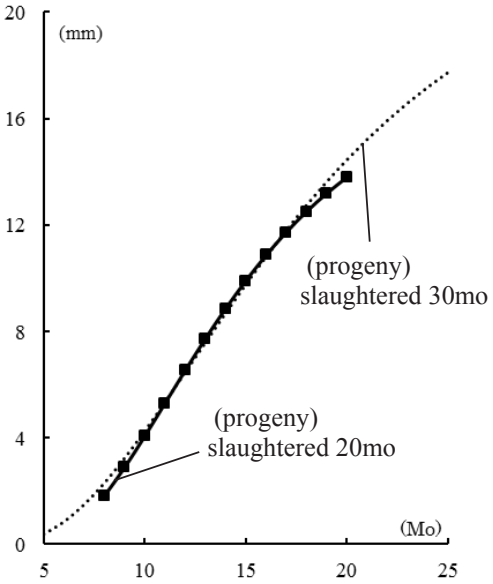

Figure 3. Growth pattern of *subcutaneous* fat thickness (SFT)

Table 1. Simple correlation analysis of carcass measurements[1]

	LMA		TMA		RT		SFT		IMFT	
LMT	0.75	**	0.57	**	0.45	**	0.17	*	0.21	**
LMF	0.38	**	0.26	**	0.37	**	0.30	**	0.57	**
TMT	0.37	**	0.89	**	0.35	**	0.25	**	0.21	*
TMF	0.19	*	0.54	**	0.32	**	0.20	*	0.33	**

[1] LMA: *longissimus* muscle area, TMA: *trapezius* muscle area, RT: rib thickness, SFT: *subcutaneous* fat thickness, IMFT: intermuscular fat thickness, LMT: *longissimus* muscle thickness, LMF: *longissimus* muscle fat ratio, TMT: *trapezius* muscle thickness, TMF: *trapezius* muscle fat ratio. ** P<0.01, * P<0.05.

Table 2. Partial correlation coefficient of carcass and ultrasound traits

Ultrasound Traits[1]	Age (Mo.)	Carcass Traits[2]									
		LMA		RT		TMT		SFT		IMFT	
ULMA	11	0.62	**	0.25	**	0.36	**	0.06		0.34	**
	14	0.75	**	0.28	**	0.41	**	0.02		0.36	**
	18	0.93	**	0.29	**	0.39	**	0.03		0.40	**
URT	11	0.17	*	0.20	*	0.17	*	0.01		0.19	*
	14	0.35	**	0.29	**	0.23	**	0.12		0.24	**
	18	0.47	**	0.33	**	0.35	**	0.19	*	0.36	**
UTMT	11	0.05		0.02		0.12		0.01		0.04	
	14	0.13		0.15		0.48	**	0.01		0.01	
	18	0.21	*	0.21	*	0.49	**	0.17		0.17	
USFT	11	-0.11		0.29	**	0.11		0.31	**	0.16	
	14	-0.06		0.33	**	0.23	*	0.40	**	0.26	**
	18	0.01		0.49	**	0.21	*	0.70	**	0.28	**
UIMFT	11	0.14		0.16		-0.07		0.08		0.11	
	14	0.21	*	0.21	*	0.08		0.09		0.21	*
	18	0.05		0.31	**	0.21	*	0.17	*	0.40	**

[1] ULMA: ultrasound *longissimus* muscle area, URT: ultrasound rib thickness, UTMT: ultrasound *trapezius* muscle thickness, UIMFT: ultrasound intermuscular fat thickness. [2] LMA: *longissimus* muscle area, RT: rib thickness, TMT: *trapezius* muscle thickness, SFT: *subcutaneous* fat thickness, IMFT: intermuscular fat thickness. ** P<0.01, * P<0.05.

REFERENCES

Hwang, J. M., J. K. Cheong, S. S. Kim, B. H. Jung, M. J. Koh, H. C. Kim and Y. H. Choy. 2014. Genetic analysis of ultrasound and carcass measurement traits in a regional Hanwoo steer population. Asian Australas. J. Anim. Sci. 27:457-463.

JMGA. 1989. New Beef Carcass Grading Standards. Japan Meat Grading Assoc. Tokyo.

Nade, T., Y. Kataoka, K. Hoshi, I. Maeda and K. Saito. 2014. Estimation of beef marbling in the Longissimus muscle with computer image analysis of ultrasonic pictures of the iliocostalis muscle area. Anim. Sci. J. 85:247-253.

Tokunaga, T., T. Ishida, T. Takami, S. Oyama, H. Harada and T. Morita. 2013. Estimation of the growth patterns of carcass composition by use of ultrasound measurements in fattening Japanese Black cattle. Society of Beef Cattle Science. 94:9-15. (in Japanese).

PO-01-2 The multiple regression analysis to estimate the unit price of Hanwoo (Bos taurus coreanae) meat

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INTRODUCTION

Contribution analysis of Unit price is important because unit price of Hanwoo directly correlate with farmer income. Exact contribution analysis of factors on unit price gives assistance to farmer income through production cost decrease and efficient improvement breeding plan. Thus estimation for unit price and genetic parameters on carcass traits have studied like contribution analysis of carcass traits on unit price in Hanwoo (Sun et al. 2012, Park et al. 2015) and the estimation of genetic parameters on carcass traits (Koo et al. 2011, Yoon et al. 2002 and Sun et al. 2010). These contribution analyses of carcass traits on unit price in Hanwoo were important because farmers can utilize them to improvement direction.

Contribution analysis method of carcass traits is multiple regression analysis using dependent variable for unit price and independent variable for carcass traits. Existed research methods have been studied numeric variables for backfat thickness, eye muscle area, carcass weight, and marbling score (Kim et al. 2015, Sun et al. 2012 and Kong et al. 2014). But marbling score was set 1~9 grade, therefore treating marbling score as numeric variable is more appropriate than categorical variables at contribution analysis.

The objectives of this study were to estimate contribution for carcass traits on unit price, analyze marbling score as categorical variable not numeric variable and develop optimal model include holiday effect and raising period additionally.

MATERIALS AND METHODS

Data

The data for this study was acquired from Korea Institute for Animal Products Quality Evaluation. The data consisted of trading records on 1,613,699 heads (Steer 956,276 heads, cow 657,423 heads) at the 12 wholesale markets from 2010 to 2014.

Statistical analysis methods

• Contribution analysis of factors

The main factors affecting the unit price of Hanwoo meat were carcass traits (backfat thickness, eye muscle area, carcass weights, and marbling scores) and environmental sources such as selling time and raising period. To analysis the contribution of each factor, this study used multiple regression analysis using linear model.

Model 1: $Y = a + b_1\ BF + b_2\ EMA + b_3\ CW + b_4\ MS + e$

Model 2: $Y = a + b_1\ BF + b_2\ EMA + b_3\ CW + b_4\ MS + b_5\ RP + e$

Y: Unit price (Won/kg)

BF: Backfat Thickness (mm)

EMA: Eye muscle Area (cm^2)

CW: Carcass Weight (kg)

MS: Marbling Score

RP: Raising period (day)

e: Random error

Thus, Y is unit price as dependent variable, carcass traits (backfat thickness, eye muscle area, carcass weights and marbling scores) are dependent variable, b_1, b_2, b_3, b_4 and b_5 are regression coefficients of each trait and *e* is random error. In order to compare contributions of variables, squared semi-partial correlation was used for this study because squared semi-partial correlation has been ordinary used. Squared semi-partial correlation is not only considering effect of other independent variable but also indicating relatively contribution in regression analysis model.

• Contribution analysis of factors using dummy variable

Marbling score was treated as categorical value using dummy variable in this model.

Model 3: $Y = a + b_1 BF + b_2 EMA + b_3 CW + b_4 MS\_dum_i + e$
Model 4: $Y = a + b_1 BF + b_2 EMA + b_3 CW + b_4 MS\_dum_i + b_5 HD + b6 RP + e$
Y: Unit price for dependent variable
BF: Backfat Thickness
EMA: Eye muscle Area
CW: Carcass Weight
MS: Marbling Score
MS_dum_i: i grade marbling score (i = 2, 3, 4, 5, 6, 7, 8, 9)
HD: Holiday effect
RP: Raising period
e: Random error

When contribution analysis of unit price using dummy variable, it could be more accurate analysis than existed method. When marbling score is processed numeric variables, differences between each grade are no difference. But when marbling score is processed categorical variables, differences between each grade are different.

MS_dum_i means the difference of unit price between marbling score 1 and i. MS_dum_i variable consist of 0 and 1. For example, if marbling score of one of record is 2, MS_dum_2 is 1 and the others are 0 and it means the difference of unit price between marbling score 1 and 2. Holiday effect was used for estimating the effect of selling Hanwoo meat from 30days before until Korean traditional holidays (e.g. New Year's Day, Thanksgiving). When raising period effect was used independent value, analysis was divided by sex because there is big different in raising period by sex.

RESULTS AND DISCUSSION

Contribution analysis of carcass traits

Table 2 represents contribution analysis of factors. Parameters to estimate the unit price of Hanwoo were –83.09 Won/mm, 11.51 Won/cm^2, 5.30 Won/kg, 918.00 Won/score at backfat thickness, eye muscle area, carcass weight, and marbling score respectively. Squared semi-partial correlation to estimate the unit price of Hanwoo were 0.023, 0.001, 0.008 and 0.38 at backfat thickness, eye muscle area, carcass weight, and marbling score respectively. Changing squared semi-partial correlation to percentage value, percentages were 5.58%, 0.24%, 1.94% and 92.235%, respectively. Marbling score has the highest contribution. R-Square was 0.57 in this model.

Park et al. (2015) reported that parameters of backfat thickness, eye muscle area, carcass weight and marbling score to estimate the unit price of Hanwoo were –59.90 Won/mm, 18.71 Won/cm^2, 3.06 Won/kg and 1187.49 Won/score respectively. Also contributions of each trait were 3.08%, 0.83%, 0.66% and 95.41%. Comparing with this study, Hanwoo meat unit price in Gyeongsangnam-do was more affected than unit price in whole country by marbling score. Choi et al. (2011) reported that partial R-Squares were 0.0004, 0.0000, 0.0035, 0.3463 at backfat thickness, eye muscle area, carcass weight, and marbling score respectively and backfat thickness and eye muscle area were not affected unit price. That result was different with this study because result of this study was there were effect of both traits. Research of Sun et al (2012) said that marbling score effect was absolutely high. Result of Sun et al (2012) was similar with our result.

Contribution analysis of factors by sex

Table 3 represents contribution analysis of factors by sex. Parameters to estimate the unit price of cow were –50.56 Won/mm, 8.78 Won/cm^2, 6.76 Won/kg, 1,058.65 Won/score, -0.97 Won/day at backfat thickness, eye muscle area, carcass weight, marbling score and raising period respectively. And R-Square of this model was 0.55. Parameters to estimate the unit price of steer were –89.06 Won/mm, 19.43 Won/cm^2, 1.75 Won/kg, 80.89 Won/score, -1.78 Won/day at backfat thickness, eye muscle area, carcass weight, marbling score and raising period respectively. R-Square was 0.54. Contributions in cow were 1.50%, 0.21%, 1.29%, 89.89% and 7.10% at backfat thickness, eye muscle area, carcass weight, marbling score and raising period respectively and Contributions in steer were 0.23%, 1.17%, 7.73%, 89.93% and 0.94 at backfat thickness, eye muscle area, carcass weight, marbling score and raising period respectively.

When analyzing by sex, parameter of marbling score of cow was higher than Model 1, parameter of backfat thickness of cow was lower than Model 1, parameter of carcass weight of steer was lower than Model 1 and parameter of eye muscle area of steer was higher than Model 1. Also parameter of raising period in steer was higher than cow. This like, when analyzed separately cow and steer, more accurate model was estimated.

Contribution analysis of carcass traits using dummy

Table 4 represents contribution analysis of carcass traits using dummy. Parameters to estimate the unit price of Hanwoo were –86.71 Won/mm, 12.35 Won/cm^2, 4.99 Won/kg at backfat thickness, eye muscle area and carcass weight respectively. Parameters for marbling score dummy variables 1038.99, 1286.40, 3361.15, 3564.02, 4895.19, 5123.53, 6615.73, 7336.12 Won/kg. Parameter of MS_dum_2 means price difference between marbling score 1 and 2. In case of MS_dum9, difference of unit price between marbling score 1 and 9 was 7336.12 Won. Contribution of Hanwoo in model were 4.79%, 0.38%, 1.34% and 93.50% at backfat thickness, eye muscle area, carcass weight and marbling score respectively. R-Square was 0.59.

When analysis of marbling score with numeric variables, price difference between score was fixed 918.00 Won. However, when analysis of marbling score with categorical variables, we could see detail unit price difference. According to this result, the method using dummy variables was better than using numeric variables. The reason why unit price of 2~3, 4~5, 6~7 and 8~9 grade were similar seemed that 2~3, 4~5, 6~7 and 8~9 are graded as a group in final grading service as product.

Contribution analysis of factors using dummy by sex

Table 5 represents contribution analysis of factors using dummy by sex. Parameters to estimate the unit price of cow were –52.50 Won/mm, 8.93 Won/cm^2, 7.20 Won/kg, -1.04 Won/day at backfat thickness, eye muscle area, carcass weight and raising period respectively. Parameters for dummy variables at marbling score 1~9 were 0, 531.19, 703.84, 2947.24, 3271.88, 4858.43, 5232.94, 7397.39, 8328.74 Won/kg which means each MS grade had differed price values. The price of meat sold when traditional holidays were significantly higher 827.71 Won/kg for cow than those sold at non-holiday. R-Square was 0.59 in this study. Parameters to estimate the unit price of steer were –92.12 Won/mm, 20.22 Won/cm^2, 1.30 Won/kg, -1.72 Won/day at backfat thickness, eye muscle area, carcass weight and raising period respectively. Parameters for dummy variables at marbling score 1~9 were 0, 1719.36, 1850.90, 3713.50, 3749.51, 4995.58, 5189.18, 6551.40, 7338.80 Won/kg which means each MS grade had differed price values. The price of meat sold when traditional holidays were significantly higher 645.15 Won/kg for cow than those sold at non-holiday. R-Square was 0.58.

The model 4 used in Table 5 was added holiday effect and raising period effect to model 3 in Table 4. As a result of model 4, marbling score was lower and R-Square was higher than model 3. Therefore model 4 of table 5 was more accurate than model 3 of table 4. In summary, the model 4 that multiple regression analysis including carcass traits, raising period and holiday effect was most accurately estimated contribution of Hanwoo meat unit price among all models.

Table 1. Number of records of Hanwoo by sex and auction price

Sex	Number of records	Auction price
Cows	657,423	12735.92±2,823.78
Steers	956,276	14707.14±2,339.91
Total	1,613,699	13,721.54±2,581.85

Table 2. Squared semi partial regression coefficients of carcass traits on auction price

	Carcass traits	Parameter (won)	Squared semi partial correlation	Contribution (%)
Auction Price	Backfat thickness	-83.09**	0.023	5.58
	Eye muscle area	11.51**	0.001	0.24
	Carcass weight	5.30**	0.008	1.94
	Marbling score	918.00**	0.380	92.23
	Intercept	7614.81**	R-Square	0.57

R-Square: Coefficient of determination for the multiple regression model.

** : p<0.01

Table 3. Squared semi partial regression coefficients of carcass traits on auction price

	Carcass traits	Parameter (won)	Squared semi partial correlation	Contribution (%)
Auction Price (only cow)	Backfat thickness	-50.56**	0.007	1.50
	Eye muscle area	8.78**	0.001	0.21
	Carcass weight	6.76**	0.006	1.29
	Marbling score	1,058.65**	0.418	89.89
	Raising period	-0.97**	0.033	7.10
	Intercept	7,861.34**	R-Square	0.55
Auction Price (only steer)	Backfat thickness	-89.06**	0.001	0.23
	Eye muscle area	19.43**	0.005	1.17
	Carcass weight	1.75**	0.033	7.73
	Marbling score	808.89**	0.384	89.93
	Raising period	-1.78**	0.004	0.94
	Intercept	10781**	R-Square	0.54

R-Square: Coefficient of determination for the multiple regression model.

** : p<0.01

Table 4. Squared semi partial regression coefficients of carcass traits on auction price

	Carcass traits	Parameter (won)	Squared semi partial correlation	Contribution (%)
Auction Price	Backfat thickness	-86.71**	0.025	4.79
	Eye muscle area	12.35**	0.002	0.38
	Carcass weight	4.99**	0.007	1.34
	MS_dum2	1038.99**	0.004	0.77
	MS_dum3	1286.40**	0.006	1.15
	MS_dum4	3361.15**	0.040	7.66
	MS_dum5	3564.02**	0.043	8.24
	MS_dum6	4895.19**	0.080	15.33
	MS_dum7	5123.53**	0.080	15.33
	MS_dum8	6615.73**	0.115	22.03
	MS_dum9	7336.12**	0.120	22.99
	MS_dum_all		0.488	93.50
	Intercept	8568.30**	R-Square	0.5907

R-Square: Coefficient of determination for the multiple regression model.

MS_dum_all: Subtotal of MS_dumi(i=2, 3, 4, 5, 6, 7, 8, 9)

** : $p<0.01$

Table 5. Squared semi partial regression coefficients of carcass traits on steer auction price

	Carcass traits	Parameter (won)	Squared semi Partial correlation	Contribution (%)		Carcass traits	Parameter (won)	Squared semi partial correlation	Contribution (%)
Auction Price (only cow)	Backfat thickness	-52.50**	0.008	1.34	Auction Price (only steer)	Backfat thickness	-92.12**	0.035	8.84
	Eye muscle area	8.93**	0.001	0.17		Eye muscle area	20.22**	0.005	1.34
	Carcass weight	7.20**	0.007	1.18		Carcass weight	1.30**	0.000	0.10
	Raising period	-1.04**	0.037	6.22		Raising period	-1.72**	0.004	0.92
	Holyday effect	827.71**	0.015	2.52		Holyday effect	645.15**	0.013	3.36
	MS_dum2	531.19**	0.002	0.34		MS_dum2	1719.36**	0.006	1.39
	MS_dum3	703.84**	0.003	0.50		MS_dum3	1850.90**	0.007	1.66
	MS_dum4	2947.24**	0.049	8.24		MS_dum4	3713.50**	0.027	6.89
	MS_dum5	3271.88**	0.055	9.24		MS_dum5	3749.51**	0.028	6.98
	MS_dum6	4858.43**	0.108	18.15		MS_dum6	4995.58**	0.049	12.40
	MS_dum7	5232.94**	0.096	16.13		MS_dum7	5189.18**	0.052	13.01
	MS_dum8	7397.39**	0.129	21.68		MS_dum8	6551.40**	0.079	19.76
	MS_dum9	8328.74**	0.085	14.29		MS_dum9	7338.80**	0.093	23.35
	MS_dum_all		0.527	88.57		MS_dum_all		0.527	88.57
	Intercept	9380.47	R-Square	0.59		Intercept	10784	R-Square	0.58

R-Square: Coefficient of determination for the multiple regression model.

MS_dum_all: Subtotal of MS_dumi(i=2, 3, 4, 5, 6, 7, 8, 9)

** : p<0.01

Reference

Yoon, H. B., Kim, S. D., Na, S. H., Chang, U. M., Lee, H. K., Jeon, G. J. and Lee, D. H. 2002. Estimation of Genetic Parameters for Carcass Traits in Hanwoo Steer. J. Anim Sci. & Technol. (kor). 44(4) 383-390.

Choi, S. D., Lee, J. J., Dang, C.W., Kang, S.N. and Kim, N.S. 2011. The Effect of Carcass Traits on Economic Values in Hanwoo. Korean J. Food Sci. Ani. Resour. Vol. 31, No. 4, pp. 603-608.

Koo, Y. M., Kim, S. D., Kim, J. I., Song, C. E., Lee, K. H., Jung, Y. H., LEE, J. Y., Jang, H. G., Park, B. H., Choi, T. J., Cho, K. H., Lee, S. S., Lee, J. G. and Kim, H. S. 2011. Research of Statistical Model for Genetic Evaluation of Hanwoo Carcass Traits. JAST. 53(4) 283-288.

Kong, J. S. 2014. The Empirical Analysis on Economic effects of Hanwoo Improvement. Ph.D. Thesis, KonKuk National University, Seoul, Korea.

Kim, H. G. 2015. Trend on Contribution of Carcass Traits Influencing Auction Price in Hanwoo. M.S. Thesis, GyeongSang National University, JinJu, GyeongSangnam-do, Korea.

Park, H. R., Eum, S. H., Park, J. H., Seo, J. K., Cho, S. K., Shin, T. S., Cho, B. W., Park, H. C., Lee, E. J., Sun, D. W., Lim, H. T., Lee, J. G. and Kim, B. W. 2015. Contribution Analysis of Carcass Traitson Auction Price in Gyeongsangnam-do Hanwoo. M.S. Thesis, Pusan National University, Miryang, GyeongSangnam-do, Korea.

Sun, D. W., Kim, B. W., Moon, W. G., Park, J. C., Park, C. H., Koo, Y. M., Jeoung, Y. H., Lee, J. Y., Jang, H. G., Jeon, J. T., Lee, J. G. 2010. The Estimation of Environmental Effect and Genetic Parameters on Carcass Traits in Hanwoo. Journal of agriculture & Life Science. 44(6) pp. 83-89.

Sun, D. W. 2012. Estimation for environmental effect and genetic parameters on carcass traits of Hanwoo and analysis of its contribution to price. Ph.D. Thesis, GyeongSang National University, JinJu, GyeongSangnam-do, Korea.

Sun, D. W., Kim, B. W., Park, J. C. and Lee, J. G. 2012. Effects of Carcass Traits on Auction Price in Hanwoo. JAST 54(2) 77-82.

PO-01-5

Comparison of the growth curve models of Hanwoo steer (Bos taurus coreanae)

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INTRODUCTION

Study about growth characteristic is very important factor of domestic animals like Hanwoo (Korean native cattle; *Bos Taurus coreanae*) depending on individual characteristic like specific breed and gender. Optimal selection of rearing management method and shipping date through the grasp of the growth characteristics of weight and body type according to the characteristics of objects, such as particular breeds or gender is essential to livestock improvement. It would be impracticable to collect continuous data from birth to shipping date in order to determine the weight by age and it follows many difficulties, therefore nonlinear model is often used to estimate growth curve as one way of estimating unmeasured value (Brown et al. 1976). Studies for estimating the growth curve of cattle using nonlinear models like Gompertz (Winsor, 1932), von Bertalanffy (von Bertalanffy, 1957), Logistic (Nelder, 1961) and Brody (Brody, 1945) were conducted from abroad (Brown et al., 1976; Nelsen et al., 1982; Menchaca et al., 1996) and Korea (Cho et al., 2002; Lee et al., 2003).

The objective of this study was to estimate growth curve of Hanwoo steer using Gompertz, von Bertalanffy and Logistic nonlinear models and compare the three models. In addition, by comparing the estimated growth curve and the actual data, to identify the problems of the estimated growth curve therefore intended to be used as basic data for further study.

MATERIALS AND METHODS

Data

Using total 20,509 steer's growth records (weight per month) from Hanwoo Improvement Center. National Agricultural Cooperative Federation in Korea to estimate Hanwoo steer's growth curve. Each animal in the data was born between from 2003 to 2014, castrated at 5-6 months of age and took weight from 6 to 24 months (for each individuals, there was some difference in the time of measurement). Weight data according to age by months were converted to age by day through calculating difference between measured period and birthday.

Data were used without considering number of measurements per individual. Outliers which excessively deviate from the average of measured month were removed. And a measured period is so vigorous growth phase of Hanwoo, reduced records than just previous records in a steer were removed because they were considered errors. After these processes, finally 20,509 weight-age (day) records were used for analysis of growth curve.

Both measured age (day) and weight are summarized in Table 1. As recorded age is increase, it seems like standard deviation of measure weight increases due to influence of environment, but coefficient of variation doesn't.

Nonlinear model for Growth Curve & Statistical analysis

There were 3 nonlinear models (Gompertz, von Bertalanffy and Logistic) used for estimating Hanwoo steer's growth curve. All models show estimated weight by age(day) and sigmoid curve with an inflection point exists. Equation of each model is as follows.

Gompertz Model: $W_t=Ae^{-be^{\wedge(-kt)}}$ (Winsor, 1932)

von Bertalanffy model: $W_t=A(1-be^{-kt})^3$ (von Bertalanffy, 1957)

Logistic model: $W_t=A(1 + be^{-kt})^{-1}$ (Nelder, 1961)

W_t= weight at age t(day)

A= asymptote for weight; mature weight

b= constant of integration

k= intrinsic growth rate

e= natural logarithm

The infection point is a point where the shape of growth curve changes from being concave downward to convex upward. In other words, the point where daily gain changes being increase section to decrease section is inflection point and daily gain becomes highest value at that point in growth model. t is age at inflection point (t_i) when solution of the twice differentiated growth curve equation becomes 0 ($d/dt(dW_t/dt)$)=0, W_{ti} is weight at inflection

point and slope at t_i is daily gain of inflection point.

Weight-age data was fitted to 3 kinds of nonlinear models using SAS 9.4 (SAS Institute, USA) NLIN nonlinear regression procedure with Gauss-Newton method.

RESULTS AND DISCUSSION

Growth parameters

Estimated growth parameters are shown in Table 2. Mature weight parameter A was highest at von Bertalanffy model and lowest at Logistic model and MSE was estimated lowest value by Gompertz model. Mean square error (MSE) is one of the factor that how much nonlinear model appropriate for data, the lower MSE value of growth curve could be better fit for the sample population.

Growth curves

Estimated growth curve by Gompertz, von Bertalanffy and Logistic models were $W_t=1062.0e^{-2.8227e^{\wedge}-0.00239t}$, $W_t=1306.6(1-0.6576e^{-0.00158t})^3$and $W_t=812.9(1 + 8.823e^{-0.00482t})^{-1}$,respectively. Estimated growth curve and observed weight are shown in Figure 1.

According to fitted curve in Figure 1, there were minor differences between three models inside observed range (113~764 days) but outside of that range, it appears significant differences between the growth curves. Each birth weight(W_0)estimated to 63.1 kg by Gompertz, 52.5 kg by von Bertalanffy and 82.8 kg by Logistic. Estimated birth weight was excessively estimated than normal birth weight of Hanwoo bull in all models. Estimated weights by Logistic model at birth and mature period were more and less estimated and this result were same with Brown (1976) has done. Weight at shipping age (about 31 months to Hanwoo steer, t=930) was estimated to 781.4 kg by Gompertz, 798.4 kg by von Bertalanffy and 739.0 kg by Logistic. Again, weight at shipping age also estimated higher than actual market data 720.6 kg (Korean institute for animal products quality evaluation, 2016).

For comparison between observed weight and estimated weight, weight at main measured period is listed in Table 3. Unlike the outside of observed range in Figure 1, 3 types of estimated weight were similar to observed weight. Gompertz weight was less similar than other models to mean of observed weight in those main measured periods at Table 3.

Inflection point

Characteristic of inflection point is summarized at Table 4. Inflection points from Gompertz, von Bertalanffy and Logistic were 434.4 days, 430.3 days and 452.0 days. And weights at inflection point from each model were 390.7 kg, 387.1 kg and 406.4 kg. Daily gain at inflection point was highest with 0.979 kg/day in Logistic model and lowest with 0.917 kg/day in von Bertalanffy model.

Kim (2002) estimated inflection point of Hanwoo steer which were raised from 1996 to 2001 at 444 days using Gompertz model which was about 10 days slower than this study result but there was no big difference.

In most of the period whichis within the data measure range, Gompertz, von Bertalanffy and Logistic all models were satisfactorily fitted. But out of that range, birth weight was over estimated and shipping weight was over estimated especially in Logistic in model. Age at inflection point was similar with passed study. With these results, in order to develop a more suitable growth curve for Hanwoo steer, the data from wider measured range from birth to shipping age would be necessary.

Table 1. Simple statistic of measured age(day) and weight by recorded age(month).

Value	Recorded age (month)	N	Mean±SD	Min	Max	Coefficient of variation
Measured age (day)	6	3980	181.2±20.36	113	245	11.24
	9	1744	262.4±20.37	203	317	7.76
	12	3969	356.2±20.36	281	412	5.72
	15	1550	445.3±20.95	385	502	4.70
	18	3966	534.6±21.39	463	596	4.00
	21	1359	630.6±20.64	566	685	3.27
	24	3941	703.4±19.66	645	764	2.79
Measured weight (kg)	6	3980	170.9±30.68	76.0	271.0	17.95
	9	1744	234.5±32.03	137.5	359.0	13.66
	12	3969	319.0±38.10	186.0	455.0	11.95
	15	1550	394.7±37.65	262.0	525.0	9.54
	18	3966	486.3±49.20	338.5	650.5	10.12
	21	1359	561.5±51.81	394.0	748.0	9.23
	24	3941	628.3±60.35	451.0	820.5	9.61

Table 2. Estimated growth curve parameters, standard error and mean square error.

Growth Model	Parameters			*MSE
	A	b	k	
Gompertz	1062.0±9.9791	2.8227±0.00632	0.00239±0.000025	1677.5
von Bertalanffy	1306.6±18.5638	0.6576±0.000932	0.00158±0.000024	1677.8
Logistic	812.9±3.8440	8.8231±0.0443	0.00482±0.000029	1689.3

*MSE: mean square error

Table 3. Observed weight and estimated weight by growth models at main measured period

Age(day)	N	Mean of observed weight (kg)	Estimated weight (kg)		
			Gompertz	von Bertalanffy	Logistic
170-190	1457	171.7	169.3	168.4	172.7
260-280	680	241.9	241.5	242.8	238.9
350-370	1564	323.6	321.6	322.9	317.8
440-460	612	398.4	405.3	405.2	404.5
530-550	1426	492.3	488.3	487.0	491.3
620-640	501	560.7	567.5	566.4	570.8
710-730	1237	642.9	640.6	641.8	637.6

Table 4. Characteristics at inflection point by three growth models of Hanwoo steer

Type	Model	Inflection point (day)	Weight at inflection point (kg)	Daily gain at inflection point (kg/day)
Steer	Gompertz	434.4	390.7	0.933
	von Bertalanffy	430.3	387.1	0.917
	Logistic	452.0	406.4	0.979

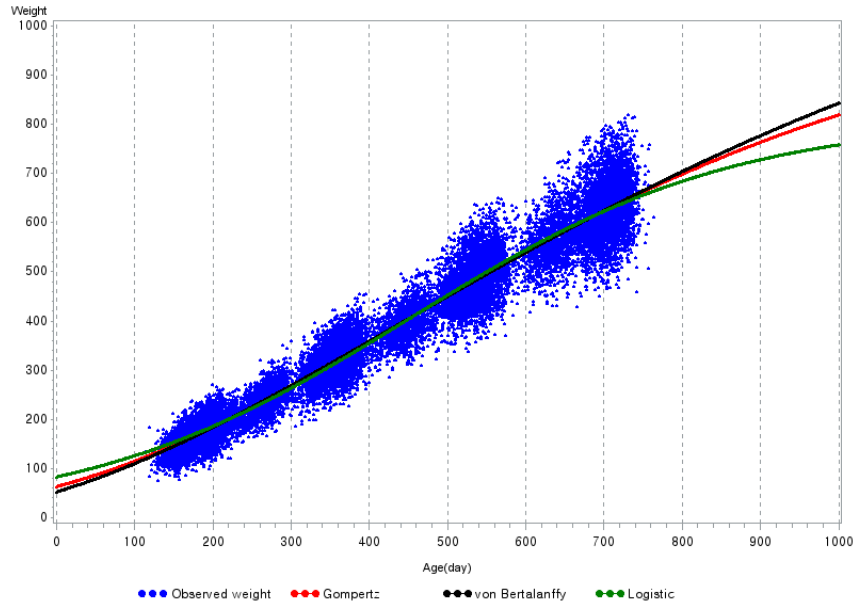

Figure 1. Observed weight at age(day) and estimated growth curves of Hanwoo steer

REFERENCES

Brody, S. 1945. Bioenergetics and growth; with special reference to the efficiency complex in domestic animals. Rheinhold Publishing, New York. USA.

Brown, J. E., H. A. Fitzhugh, Jr. and T. C. Cartwright. 1976. Comparison of Nonlinear Models for Describing Weight-Age Relationships in Cattle. J. Anim. Sci 42:810-818.

Korean Institute for animal products quality evaluation. 2016. 2015 Animal products grading statistical yearbook. 11th Ed. Korean Institute for animal products quality evaluation, Sejong, Rep. of Korea.

Kim, N. S., Ju, J. C., Song, M. K., Chung, C. S., Choi, Y. I. and Park, C. J. 2002. Growth Curve Characteristics of Bull and Steer of Hanwoo (Korean Cattle). Journal of Animal Science and Technology 44:519-522.

Lee, C. W., Choi, J. G., Jeon, K. J., Na, K. J., Lee, C. Y., Yang, B. K. and Kim, J. B. 2003. Estimation of growth curve for evaluation of growth characteristics for Hanwoo cows. Journal of Animal Science and Technology 45:509-516.

Menchaca, M., C. Chase Jr, T. Olson and A. Hammond. 1996. Evaluation of Growth Curves of Brahman Cattle of Various Frame Sizes. J. Anim. Sci 74:2140-2151.

Nelder, J. A. 1961. The Fitting of a Generalization of the Logistic Curve. Biometrics 17:89-110.

Nelsen, T. C., C. R. Long and T. C. Cartwright. 1982. Postinflection Growth in Straightbred and Crossbred Cattle. I. Heterosis for Weight, Height and Maturing Rate. J. Anim. Sci 55:293-304.

Von Bertalanffy, L. 1957. Quantitative laws in metabolism and growth. Quarterly Review of Biology:217-231.

Winsor, C. P. 1932. The Gompertz curve as a growth curve. Proceedings of the national academy of sciences 18:1-8.

PO-01-6

Efficiency of Genomic Selection to Improve Meat Quality in Pigs Using ZPLAN +

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INTRODUCTION

Korean consumers show very strong preferences for particular parts of the pig that is higher in fat (Seo et al., 2012). Hence, intramuscular fat (IMF) is one of the most considered traits in pork carcass grading in South Korea and some breeding companies have included meat quality traits as fundamental part of breeding selection programs. Genetic improvement for meat quality under conventional quantitative selection has not been effective because these traits have a low-to-moderate heritability and also these traits are very difficult and expensive to measure (Miar et al., 2014). Therefore, it is indispensable to assess the potential of using genomic information in selection of animals. Nowadays, genomic selection (GS) is discussed as a potential method to improve breeding goal traits where the accuracy of selection is low, such as low heritable traits and traits that can only be recorded directly in one sex or slaughtered animals such as meat quality traits. GS simultaneously estimate the effects of dense genetic markers and summing up these effects across all marker loci used to predict the breeding value of selection candidates (Meuwissen et al., 2001). Herein, the prediction of genetic merit obtained through GS in this way is termed the genomic estimated breeding value (GEBV).

The objective of this study was to compare designs for implementation of genomic selection with the scenarios using correlated phenotypes with/or correlated GEBV of traits which are traditionally used for routine genetic evaluation. Also, this paper aims to assess the potential of ultrasound intramuscular fat (phenotype and GEBV) as selection criteria to improve meat quality in pigs. Scenarios were compared in terms of selection accuracy and genetic gain.

MATERIALS AND METHODS

Modeling software

The computer program ZPLAN + (Täubert et al., 2010), a user-friendly interfaces software was used to simulate and evaluate the different breeding schemes. This deterministic software allows modeling all related breeding structures, genetic and economic parameters to account complex breeding programs with special emphasis on genomic information. ZPLAN + was developed based on the gene flow method (Hill, 1974), selection index procedure for predicting reliabilities by Hazel and Lush (1942), and as well as on a complex economic modeling. Schemes were compared in terms of accuracy of the selection index, monetary genetic gain, breeding costs, returns and discounted profit. The costs and returns in this study are not accounted due to complexity of their determination.

Breeding scenarios

Traits in the breeding goal considered in this simulation were average daily gain (ADG), feed conversion rate (FCR), ultrasound backfat depth (UBF), and ultrasound intramuscular fat (IMF) as one of the main traits determining meat quality. Heritability, phenotypic standard deviations, phenotypic and genetic correlations among traits are presented in Table 1. All the genetic parameter estimates were adopted from the study of Miar et al. (2014). The simulated breeding population used in this study consists of 400 sows with an annual sow replacement rate of 40 percent. The breeding sows were mated naturally to 30 active boars with 65 percent replacement rate every year.

Five scenarios were modeled in ZPLAN + as shown in Table 2. The first and second scenarios genetic evaluation was based entirely on phenotypic information without considering any of the marker information. The first scenario reflected the current conventional selection program where selection index is composed of ADG, FCR and UBF. Methods of measuring these traits were discussed by Cabling et al. (2015) and Miar et al. (2014) in their same study about genetic associations between production and meat quality traits. The main information source for selection is based only from their own performance. In the second scenario, UIMF was added into the basic selection index as an indicator trait for meat quality. The heritability estimate of UIMF is 0.26 and it is shows strong genetic correlation with ADG (0.69) and UBF (0.48). In scenario 3, UIMF was also incorporated into the current selection index; however a type of information used was not phenotype but a GEBV. This genomic trait

will be denoted as gUIMF. This scenario was modelled to assess the impact of genomic value of UIMF alone in the overall genetic gain and accuracy of the selection index. In the scenario 4 and 5, the selection was strictly based on GEBV and the traits on the scenario 4 were only the basic selection index traits denoted as gADG, gFCR and gUBF while scenario 5 was added by gUIMF.

For all the scenarios, the accuracy of the selection index was measured ranging the accuracy of GEBV from 0.1 to 0.9. The accuracy of the GEBV is defined as the correlation between the GEBV and the true breeding value for the corresponding trait. Two approaches were used in this study to insert the genomic trait in the program as described in VIT (2011) user's manual and briefly described below. All traits had an economic weight of 1 monetary unit per genetic standard deviation of the trait.

RESULTS AND DISCUSSION

Accuracy of the selection index

The accuracy of selection index (r_{TI}) for different scenarios depending on the accuracy of GEBV is presented in Table 3. The target trait under selection in this simulation was UIMF. The accuracy of selection index was 0.56 and 0.57 for scenario 1 and 2, respectively. The difference on the accuracy of scenario 1 and 2 were small, indicating that inclusion of UIMF in the selection index had no large influence on the $r_{TI.}$ This result explains by low heritability estimate of UIMF (0.26). As expected, the accuracies of scenario where GEBV information was included were increased with increasing $r_{GBV.}$ The r_{TI} of scenario 3 ranges from 0.56-0.86 depending on the value of $r_{GBV.}$ The trends of scenario 4 and 5 where information source was based mainly on GEBV were changed more rapidly as the r_{GBV} increase compared to scenario 3. The r_{TI} of scenario 4 and 5 can increase as high as 0.92 when the r_{GBV} was 0.9. Moreover, the accuracy of scenario 4 and 5 is higher than scenario 1 and 2 when the r_{GBV} was ≥0.6. In comparison of scenario 4 and 5, the accuracy of scenario 5 was higher than scenario 4 when r_{GBV} is 0.1-0.7 but had the same accuracy when the r_{GBV} was 0.8-0.9. These results on the accuracies were analogous in the study of Pimentel and Konig (2012).

Annual genetic gain

The main target in each breeding program is to maximize the genetic gain per generation and per year. The overall and per trait monetary genetic gain depending on the accuracy of GEBV (0.1-0.9) of the breeding goal per year is shown in Table 4. The overall annual monetary genetic gain (AMGG) of scenario 1, and 2 was 0.15 and 0.36, respectively. The scenario 2 was 40 percent higher than scenario 1 because of the inclusion of UIMF in the selection index. In terms of per trait, the ADG was able to improve by 0.02 while the FCR and UBF were reduced by 0.07 and 0.05, respectively, in scenario 1. The genetic gain of ADG in scenario 2 is higher than scenario but the reduction in the FCR was lower. Moreover, the UBF in scenario 2 shows unfavorable genetic trends as it increase by 0.02. The addition of UIMF in the selection index favors the genetic gain of ADG because these two traits were positively genetic correlated and positive economic value. In the case of UBF, this trait was genetically positive correlated with UIMF but their economic weight was opposite.

Applying the GEBV of UIMF in the selection index as in scenario 3 may create an AMGG of 0.36-0.55 depending on the accuracy of GEBV. It shows that scenario 3 can further enhanced the AMGG up to 53 percent compared to scenario 2. Also, the ADG is further improved from 0.02 in scenario 1 to 0.06-0.07 in scenario 3. However, the UBF also shows unfavorable result like in scenario 2. Conducting scenario 3 would create an annual increase of 0.02-0.03 inch in BF. In the case of UIMF, it is enhanced from 0.29 in scenario 2 to 0.30-0.49 in scenario 3 depending on the accuracy of GEBV. This confirms the results of former studies that the value of genetic gain increases when incorporating genomic information in the selection index (Schaeffer, 2006; Täubert et al., 2011). In scenario 4, the AMGG was 67 percent higher than scenario 1. All traits in the selection index were also showed favorable results. The FCR and UBF can further reduced up to 0.15 and 0.06, respectively. The highest AMGG was in scenario 5 with 0.59 when the accuracy of GEBV was 0.9. The ADG can improved up to 0.08 while 0.47 for UIMF. Though, scenario 3 can improve the UIMF 4 percent higher than scenario 5. The FCR shows higher favorable response in scenario 5 than scenario 3 with up to 0.06 kg annual reduction in the feed intake realized per kg body mass gain. The UBF also shows unfavorable response same with all scenarios were UMIF was added on the selection index. The overall results on genetic gain show that genomic selection significantly increases the AMGG. Simianer (2009) reported a 36.7 percent increase on genetic gain in litter size in pig under genomic selection when compared to conventional.

CONCLUSION

The addition of UIMF in the selection index shows favorable results in terms of accuracy and genetic gain. Moreover, higher results are to be expected if genomic selection is implemented. The results show that scenario 5 was the most advisable strategy followed by scenario 3. The inclusion of UIMF in the selection index increases the genetic gain of ADG and FCR. However, this positive result also brings unfavorable result to the UBF. UIMF and UBF were genetically positive correlated but inverse in economic weight. Thus, UBF must carefully consider when improving UIMF. This is still depends on the capability of the breeding company and also whether the customers are willing to pay more for improved genetic quality.

Table 1. Heritabilities (diagonal), phenotypic standard deviation, phenotypic (above diagonal) and genotypic (below diagonal) correlations among simulated traits

Trait	ADG	FCR	UBF	UIMF
ADG, kg/d	0.30	0.27	-0.31	0.32
FCR	-0.19	0.20	0.28	0.00
UBF, in	0.26	0.39	0.45	0.34
UIMF	0.69	0.48	0.00	0.26
SD	0.145	3.17	0.125	0.83

ADG = average daily gain, UBF = ultrasound backfat depth, FCR = feed conversion ratio, UIMF = ultrasound intramuscular fat, SD = standard deviation

Table 2. Information sources of different simulated scenarios

Scenario	Information Sources
1	ADG + FCR + UBF
2	ADG + FCR + UBF + UIMF
3	ADG + FCR + UBF + gUIMF
4	gADG + gFCR + gUBF
5	gADG + gFCR + gUBF + gUIMF

Table 3. Accuracy of the index for each scenario depending on the accuracy of GEBV

Scenario	Accuracy of GEBV								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
2	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
3	0.56	0.57	0.59	0.61	0.64	0.68	0.73	0.79	0.86
4	0.11	0.23	0.34	0.45	0.55	0.65	0.75	0.84	0.92
5	0.13	0.25	0.37	0.48	0.58	0.68	0.76	0.84	0.92

Table 4. Annual monetary genetic gain for different information sources

Scenario		Accuracy of GEBV								
		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	AMGG	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	ADG	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	FCR	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	UBF	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
2	AMGG	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
	ADG	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
	FCR	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
	UBF	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	UIMF	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
3	AMGG	0.36	0.37	0.38	0.39	0.42	0.44	0.47	0.51	0.55
	ADG	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07
	FCR	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
	UBF	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03
	UIMF	0.30	0.30	0.32	0.33	0.35	0.38	0.41	0.45	0.49
4	AMGG	0.03	0.06	0.09	0.12	0.15	0.18	0.20	0.23	0.25
	ADG	0.00	0.01	0.01	0.02	0.02	0.03	0.03	0.03	0.04
	FCR	-0.02	-0.04	-0.06	-0.07	-0.09	-0.11	-0.12	-0.14	-0.15
	UBF	-0.01	-0.02	-0.02	-0.03	-0.04	-0.04	-0.05	-0.05	-0.06
5	AMGG	0.08	0.16	0.24	0.31	0.37	0.44	0.49	0.54	0.59
	ADG	0.01	0.03	0.04	0.05	0.06	0.07	0.07	0.08	0.08
	FCR	-0.01	-0.01	-0.02	-0.03	-0.03	-0.04	-0.05	-0.06	-0.06
	UBF	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.02
	UIMF	0.07	0.13	0.20	0.25	0.31	0.35	0.39	0.43	0.47

REFERENCES

Cabling, M. M. et al. 2015. Estimation of genetic associations between production and meat quality traits in Duroc pigs. Asian-Australasian Journal of Animal Sciences 28: 1061-1065.

Hazel, L. N., and J. L. Lush. 1942. The efficiency of three methods of selection. Journal of Heredity 33: 393-399.

Hill, W. G. 1974. Prediction and evaluation of response to selection with overlapping generations. Animal Science 18: 117-139.

Meuwissen, T. H. E., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-1829.

Miar, Y. et al. 2014. Genetic and phenotypic correlations between performance traits with meat quality and carcass characteristics in commercial crossbred pigs. PLoS ONE 9: e110105.

Pimentel, E. C., and S. Konig. 2012. Genomic selection for the improvement of meat quality in beef. Journal of Animal Science 90: 3418-3426.

Schaeffer, L. R. 2006. Strategy for applying genome-wide selection in dairy cattle. Journal of Animal Breeding and Genetics 123: 218-223.

Seo, S. H., E. M. Kim, and Y. B. Kim. 2012. Preferences and consumption patterns of consumer to develop processed pork products for export. Korea Journal for Food Science of Animal Resources 32: 18-32.

Simianer, H. 2009. The potential of genomic selection to improve litter size in pig breeding programmes 60th Annual Meeting of the European Association for Animal Production. p 210, Barcelona.

Täubert, H., F. Reinhardt, and H. Simianer. 2010. ZPLAN+ A new software to evaluate and optimize animal breeding programs. In: Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany

Täubert, H., S. Rensing, and F. Reinhardt. 2011. Comparing conventional and genomic breeding programs with ZPLAN+. In: Interbull Bulletin. p 162-168.

VIT. 2011. User's Manual for ZPLAN+. A software to evaluate and optimise animal breeding programs. In: V. I. T. w. V. (ed.), Verden, Germany.

PO-01-8

Identification of a Visual Appraisal Tool for Evaluating Pork Belly Quality in Yorkshire Pigs

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Introduction

Visual appraisal or the eye test is the first and most effective method of a combination of methods that includes production testing, progeny testing, and marker-assisted selection useful in making productive animal breeding decisions (Haddy and Lammers, 2015). Visual appraisal is best used to evaluate the phenotype of an animal when compared to its contemporaries in instances where traits are highly heritable and animals compared are of a similar age or stage of life (Haddy and Lammers, 2015).

The cost and value of quality pork belly as an economically principal part of the pig cannot be overemphasised (Hermesch, 2008). Pork belly represents approximately 15-17% of a pig's total carcass value (Choe et al., 2015). The feasibility of maximising the profitability of a pig production venture through pork belly meat lies in the ability to determine variance components and correlations for traits associated with pork belly in pigs. According to Kim et al. (2006) most of the pork belly meat in Korea comes from Yorkshire crossbred pigs. There is need to constantly evaluate the quality of pork belly and to improve its quality to meet market demands. Phenotypic correlations for pork belly traits and muscles can be used to evaluate pork belly quality using belly muscles as visual appraisal tool. Scope exists to use the visual appraisal tool in combination with other breeding methods to make sound decisions on selection for pork belly improvement. There is need to compute genetic correlations for some pork belly muscles and traits in Yorkshire to determine a visual appraisal tool for pork belly quality in the breed.

Materials and method

Data utilized

Data for the study was collected from a Great Grand Parent (GGP) farm in Korea from pork bellies of 550 Yorkshire finishing pigs constituted by 70 females and 480 castrates. The data was collected over a 6 months period from 2012 to 2013. In 2012, there were 367 belly observations and in 2013 there were 183 belly observations.

Belly traits included loin muscle area (LMA), backfat thickness (BF), belly weight (BW), total belly muscle (TBM), and muscle percent (MP). Major belly muscles were used in the study, and these include pectorals profundi (PM), latissimus dorsi (LM), cutaneous trunci (CM), rectus abdominis (RM), and external abdominal oblique (EM) muscles. Procedure for measurements followed that described by Kang et al. (2015).

Statistical analysis

SAS version 9.3 was used for data analysis. Genetic correlations with standard errors were estimated using Restricted Maximum Likelihood (REML) Method in WOMBAT (Meyer, 2007). Bivariate analysis was used to establish genetic correlation between traits. The following multiple regression model was applied:

$$y = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \ldots + \beta_n X_n + e$$

Where y = observation, β_0 = intercept, β_i = i^{th} regression coefficient of traits, X_i = i^{th} independent variable trait, and e = error

Results and discussion

Table 1 shows phenotypic correlations for pork belly traits and pork belly muscles. The best trait for use in identifying an indicator for belly quality evaluation and improvement in Yorkshire pigs was TBM. TBM had the highest positive and significant ($p<0.01$; $p<0.05$) correlations with other belly traits, LMA, BW, and MP. The phenotypic correlations were moderate to strong and ranged between 0.33 - 0.58. TBM and BF had a significant ($p<0.01$; $p<0.05$) and positive but weak phenotypic correlation (0.14). Phenotypic correlations of TBM with belly

muscles, PM, LM, CM, RM, and EM, were strongest and positive, with a range of 0.58-0.87. CM showed the highest and strongest phenotypic correlation with TBM (0.87) when compared to other belly muscles (Table 1). With the exception of the phenotypic correlation between PM and CM (0.48) that was lower than that of PM and LM (0.65), CM had the strongest positive phenotypic correlations with other belly muscles (0.48-0.78) (Table 1).
Analyses of phenotypic correlations for belly muscle sections and TBM were all positive and significant ($p<0.01$; $p<0.05$). CM belly sections had the strongest phenotypic correlations with TBM of all muscle sections (0.38 - 0.64) (Table 2). The strongest phenotypic correlation for TBM was found with CM at the 11th area of the belly section.

Implication

CM at the 11th belly section can be used to evaluate quality of pork belly in Yorkshire. The more enhanced the CM measure at the 11th belly section, the better the quality of pork belly. As a visual appraisal tool, CM at the 11th belly muscle section can be used to aid production testing at farm level, and progeny testing and marker-assisted selection at GGP level, as a practical tool to identify Yorkshire pigs with quality belly for belly improvement programs. The moderate to high heritability estimates for belly traits will ensure genetic gain in belly traits through selection and the cascading of quality belly traits to crossbred Yorkshire pigs meant for the Korean market. Selection for increase in CM size at the 11th belly section selects for increase in TBM with corresponding improvement in other belly traits. This selection process will be associated with a small increase in BF. The small increase in pork belly BF would most likely be welcome by the Korean consumer market that strongly prefers high-fat cuts.

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Table 1. Phenotypic correlations amongst area pork belly traits and pork belly muscles

	LMA	BF	BW	TBM	MP	PM	LM	CM	RM	EM
LMA	1	0.11*	0.29**	0.33**	0.02	0.27**	0.23**	0.28**	0.38**	0.29**
BF		1	0.52**	0.14**	-0.49**	0.17**	0.10**	0.22*	0.05	0.08
BW			1	0.51**	-0.47**	0.48**	0.41**	0.49**	0.42**	0.37**
TBM				1	0.38**	0.58**	0.68**	0.89**	0.78**	0.87**
MP					1	0.07	0.18**	0.28**	0.28**	0.39**
PM						1	0.65**	0.48**	0.36**	0.39**
LM							1	0.60**	0.44**	0.55**
CM								1	0.72**	0.78**
RM									1	0.68**
EM										1

LMA: Loin muscle area, BF: Backfat thickness, BW: Belly weight, TMB: Total belly muscle, MP: Muscle percent, PM: Pectorales profundi muscle, LM: Latissimus dorsi muscle, CM: Cutaneous trunci muscle, RM: Rectus abdominis muscle, and EM: External abdominal oblique muscle.

**: $p<0.01$, *: $p<0.05$.

Table 2. Phenotypic correlations between cutaneous trunci muscle and pork belly traits

CM Belly Muscle Section	LMA	BF	BW	TBM	MP
CM 5th	0.23**	0.11*	0.22**	0.38**	0.17**
CM 6th	0.27**	0.17**	0.28**	0.53**	0.25**
CM 7th	0.30**	0.13**	0.23**	0.52**	0.27**
CM 8th	0.33**	0.19**	0.32**	0.57**	0.23**
CM 9th	0.33**	0.17**	0.32**	0.63**	0.30**
CM 10th	0.33**	0.15**	0.32**	0.63**	0.31**
CM 11th	0.29**	0.14**	0.31**	0.64**	0.35**

LMA: Loin muscle area, BF: Backfat thickness, BW: Belly weight, TMB: Total belly muscle, MP: Muscle percent, CM: Cutaneous trunci muscle, and CM 5th, CM 6th, CM 7th, CM 8th, CM 9th, CM 10th, CM 11th: Belly sections.

**: $p<0.01$, *: $p<0.05$.

REFERENCES

Chloe, J. H., H. S. Yang, S. H. Lee, and G. H. Go. 2015. Characteristics of pork belly consumption in South Korea and their health implication. *J. Anim. Sci. Technol.* 57:22 DOI: 10.1186/s40781-015-0057-1

Hady, A. A. and P. Lammers. 2015. Introduction to Swine Genetics for Small Beginning Farmers. Pork Information Gateway Fact Sheet. Available online at http://porkgateway.org/resource/introduction-to-swine-genetics-for-small-and-beginning-farmers/

Kang, H. S. B. M. Lopez, T. H. Kim, H. S. Kim, H. S. Kim, K. C. Nam, and K. S. Seo. 2015. Estimation of Genetic Parameters for Pork Belly Components in Yorkshire Pigs. *Asian Australas. J. Anim. Sci.* 28(7):922-925.

Kim, J. H., B. Y. Park, Y. M. Yoo, S. H. Cho, I. H. Hwang, P. N. Seong, K. H. Hah, and M. J. Lee. 2006. Characteristics of carcass and meat quality for Landrace, Yorkshire, Duroc and their crossbreeds. *J. Anim. Sci. Technol.* (Korea). 48:101-106.

Hermesch, S. 2008. Genetic relationships between composition of pork bellies and performance, carcass and meat quality traits. *Animal.* 2:1178-1185.

Meyer, K. 2007. WOMBAT – A tool for mixed model analyses in quantitative genetics by Restricted Maximum Likelihood (REML). *J. Zhejiang Univ. Sci. B* 8:815-821.

PO-01-10

Association of SNPs on the GH and PRL genes with egg production traits in Brown Tsaiya ducks

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Introduction

In Taiwan, the egg consumption mainly consists of chicken and duck eggs, and the production and value of chicken eggs were 15 and 13 times of duck eggs, respectively; however, duck eggs still take a consistent proportion in the market with higher unit price (Statistic Office of C. O. A., 2014). The local breed, Brown Tsaiya duck (*Anas platyrhynchos domestica*), has excellent laying performance due to the selection based on phenotypic criteria such laying rate and egg weight. Yet the heritability of these criteria is quite low, leading the limited efficiency of traditional breeding strategy. On the other hand, the restricted population size of can cause some degree of inbreeding depression. In addition to advise the producers to make pedigree and to estimate breeding values, the selection and genetic monitoring should be assisted with molecular markers. Furthermore, the efficiency could be elevated by involving the molecular markers in the duck industry. So far the molecular markers for duck egg production and their relevant information are less available.

Growth hormone (GH) is involved in the generation of reproductive organs and facilitates hen ovary follicular development (Ahumada-Solorzano et al*.,* 2012). Effect of single nucleotide polymorphisms (SNPs) of *GH* gene have significantly associated with duck (*Anas platyrhynchos domestica*) and Muscovy duck (*Cairina moschata*) reproductive traits including the highest clutch days, double-yolk percentage, fertility rate, maximum duration of fertility, egg numbers at 59 weeks and 300 days of age, and egg weight at 30 weeks of age (Chang et al., 2012, Li et al., 2009; Wu et al., 2012; Zhang et al., 2015). Prolactin (PRL) has been considered as a negative regulator of avian reproductive activities such as expression of incubating behavior and atresia of ovarian follicles (Li et al., 2011). Previous studies revealed that the SNPs of *PRL* gene had influences on egg weight at 30 weeks of age and double-yolk percentage in duck (*Anas platyrhynchos domestica*), age of the first laying and egg numbers at 59 weeks and 300 days of age in Muscovy duck (*Cairina moschata*), and egg production/numbers in goose (*Anser cygnoides* and *Anser anser*), respectively (Jiang et al., 2009; Li et al., 2009; Wang et al., 2011; Zhang et al., 2015).

The aim of this study was to investigate the genetic markers suitable for the Brown Tsaiya duck through the association analysis between the SNPs on *GH* and *PRL* genes and egg production. The experimental animals were composed of three populations of the Brown Tsaiya duck kept in the Ilan Branch of Livestock Research Institute: the conserved Brown Tsaiya (with no selection; GBT), the Brown Tsaiya LRI 1 (selected on egg number; L105), and the Brown Tsaiya LRI 3 (selected on shell color; L106).

Materials and Methods

Animals, Genomic DNA Preparation and Trait Collection

A total of 298 Brown Tsaiya ducks were used in this present study including GBT (72 females and 50 males), L105 (115 females), and L106 (61 females). Genomic DNA was extracted from whole blood using EasyPure Genomic DNA Purification Kit (BIOMAN Scientific Co., Ltd., Taiwan). The concentration and purity were determined by measuring the absorbance at 260 and 280 nm and the ratio of absorbance at 260 and 280 nm (260/280), respectively.

The traits were collected including body weight at 20 and 40 wks of age (BW20 and BW40), age of the first laying (AFE), egg numbers at 26, 27, 28, 29, 30, 40 and 52 wks of age (EN26, EN27, EN28, EN29, EN30, EN40 and EN52), egg weight at 40 weeks of age (EW40), and strength of eggshell at 40 weeks of age (ES40).

SNP Identification and TaqMan® SNP Genotyping Assay

Nine to 14 samples from each experimental population were randomly chosen for preliminary SNP identification. Polymerase chain reaction (PCR) was used to amplify the target fragments of the *GH* and *PRL* genes. PCR was performed on ABI thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA) in a 40-μL mixture containing 50 ng genomic DNA as template, 0.2 μM forward and reverse primers, and 1X Taq DNA Polymerase Master Mix Red (with 1.5 mM Mg_2Cl; AMPLIQON III, Denmark). The PCR condition was 95°C/5 min for initial

denaturation, following 30 cycles of 94°C/30 sec for denaturation, 50-60°C/45 sec for annealing, and 72°C/10 min for elongation; thereafter a final step was carried out at 72°C for 10 min. Agarose gel electrophoresis was used to check the size of PCR products. Sequencing was carried out by Mission Biotech Co., Ltd. (Taiwan) and the software CLC Sequence Viewer 7 was applied to align the multiple sequences for SNP identification.

TaqMan® SNP Genotyping Assay (Applied Biosystems, USA) was used for genotyping of the target SNPs at *GH* intron 2 and *PRL* exon 5. The primers and probes were designed according to the results of multiple alignments. PCR was performed on StepOne™ Real-Time PCR System (Applied Biosystems, USA) in a 10-μL mixture containing 25 ng genomic DNA as template, 1X master mix (KAPA PROBE FAST ABI Prism MasterMix, KAPA Biosystems, USA), and 1X primer/probe. The reaction condition was set as initial step of 60°C/30 sec, followed by 40 cycles of 95°C/1 sec, 60°C/20 sec and 60°C/30 sec. The genotypes were analyzed using the software StepOne_Software_v 2.1 (Applied Biosystems, USA).

Statistical Analysis

Statistical analysis was carried out using Microsoft EXCEL (2010) and the statistical software package SAS 9.3 (SAS Institute Inc., 2011). Frequencies of genotypes and alleles were calculated with each population. The GLM procedure was applied with the fixed effect of genotype of each SNP. The results were expressed as least square means and standard error (LSMean ± SE).

Result and Discussion

Genotypic and allelic frequencies

Four SNPs on the *GH* gene and two SNPs on the *PRL* gene chosen from literature were used in the preliminary SNP identification in our experimental populations (Chang et al., 2012; Li et al., 2009; Wang et al., 2011). Two polymorphic SNPs were identified in the examined Brown Tsaiya ducks — — g.3081C>T at the inton 2 of *GH* gene and g.5961C>T at the exon 5 of *PRL* gene (Figure 1). All experimental ducks were genotyped for these two SNPs using TaqMan® SNP Genotyping Assay**.** The genotypic and allelic frequencies were calculated within each population (Table 1). For g.3081C>T of *GH* gene, frequencies of the *CC*, *CT* and *TT* genotypes were 0.69 (0.50-0.63), 0.27 (0.09-0.43) and 0.04 (0-0.07), respectively; for g.5961C>T of *PRL* gene, frequencies of the *CC*, *CT* and *TT* genotypes were 0.02 (0-0.08), 0.28 (0.16-0.43), and 0.70 (0.49-0.84), respectively. The wild allele *C* was predominant (frequency=0.71~0.96) in g.3081C>T of *GH* gene whereas the mutant allele *T* (frequency=0.70~0.92) was predominant in g.5961C>T of *PRL* gene.

Effect of the SNPs on egg production traits

Egg production traits among different genotypes of g.3081C>T of *GH* gene is showed in Table 2. The comparison was discussed only between the *CC* and *CT* genotypes since only 13 *TT* ducks were identified. The *CC* genotype had heavier BW20 and BW40 than the *TT* genotypes (P<0.05; 1213.6 ± 14.8 *vs.* 1158.4 ± 16.1 g and 1262.5 ± 16.0 *vs.* 1198.5 ± 17.4 g, respectively). EW40 and ES40 were not affected by this mutation. For EN26, EN27, EN28, EN29 and EN30, the *CC* genotype had more egg production than the *CT* genotype in the L105 population (P<0.05). However, EN40 and EN52 were not significantly different between the *CC* and *CT* genotypes either in the L105 population or in the pooled population. Effect of SNPs of *GH* gene on reproductive traits had been examined and the results showed that the highest clutch days, double-yolk percentage, fertility rate, maximum duration of fertility (MDF), egg numbers at 59 weeks and 300 days of age, and egg weight at 30 weeks of age (Chang et al., 2012, Li et al., 2009; Wu et al., 2012; Zhang et al., 2015). Chang et al. (2012) revealed that the g.3169C>T in exon 3, g.3700C>T in exon 4 and g.5058C>G in exon 5 were associated with MDF and had no influence on egg number and egg weight in Brown Tsaiya LRI 2, selected based on MDF. The SNP g.3081C>T was also identified by Chang et al. (2012), but there was no further association analysis due to its position in intron 2.

Table 3 showed the comparison of egg production traits between the *CT* and *TT* genotypes of *PRL* gene since only seven *CC* ducks were identified. In contrast to g.3081C>T of *GH* gene, g.5961C>T of *PRL* gene had significant influence on AFE and ES40 (P<0.05) where the *TT* genotype was older at the first laying and had stronger eggshell strength at 40 wks. For EN26, EN27, EN28, EN29 and EN30, the *TT* genotype produced more eggs than the *TC* genotype in the L105 population (P<0.1). In addition, there was no difference of EN40 and EN52 between the *CT* and *TT* genotypes in the L105 population (data not shown); however, EN40 and EN52 from pooled records of were higher in the *CT* genotype (P<0.05). Contrary to our study, Wang et al. (2011) reported that the *C* allele was predominant (frequency=0.581-0.939) in g.5961C>T of *PRL* gene and this mutation had effect on annual egg production which the *CC* genotype was superior to the *CT* genotype. In the present study, the EN40 and

EN52 of the *CC* genotype were 146.0 ± 15.3 and 218.7 ± 19.8, respectively (data not shown); however, the statistical analysis excluded the *CC* genotype due to its minor frequency (Table 1). In summary, the *C* allele could have advantageous effect on long-term egg production and the effect should be further examined in the Brown Tsaiya duck populations. Additionally, other SNPs of *PRL* gene also had effect on egg production in waterfowls. In Chinese Wan-xi White geese (*Anser cygnoides)* and European Rhine geese (*Anser anser)*, A-401G, G-268A, and T-266A in the 5'-proximal region of *PRL* gene were significantly associated with egg production (Jiang et al., 2009). Zhang et al. (2015) identified the effect of T-884C and T-335C in the 5'-flanking sequence on egg number at 59 wks and 300 d of age in Muscovy ducks.

Conclusion

In the present study, genotype-trait association was carried out between the SNPs of *GH* and *PRL* genes and egg production of the Brown Tsaiya duck, the native breed in Taiwan. The *CC* genotype in g.3081C>T of *GH* gene was superior in body weight at 20 and 40 wks of age and egg numbers at 26, 27, 28, 29 and 30 wks of age while the *C* allele could be advantageous in egg numbers at 40 and 52 wks of age but disadvantageous in strength of eggshell at 40 wks of age. The further investigation should be conducted to verify those association including the additive and dominant effects.

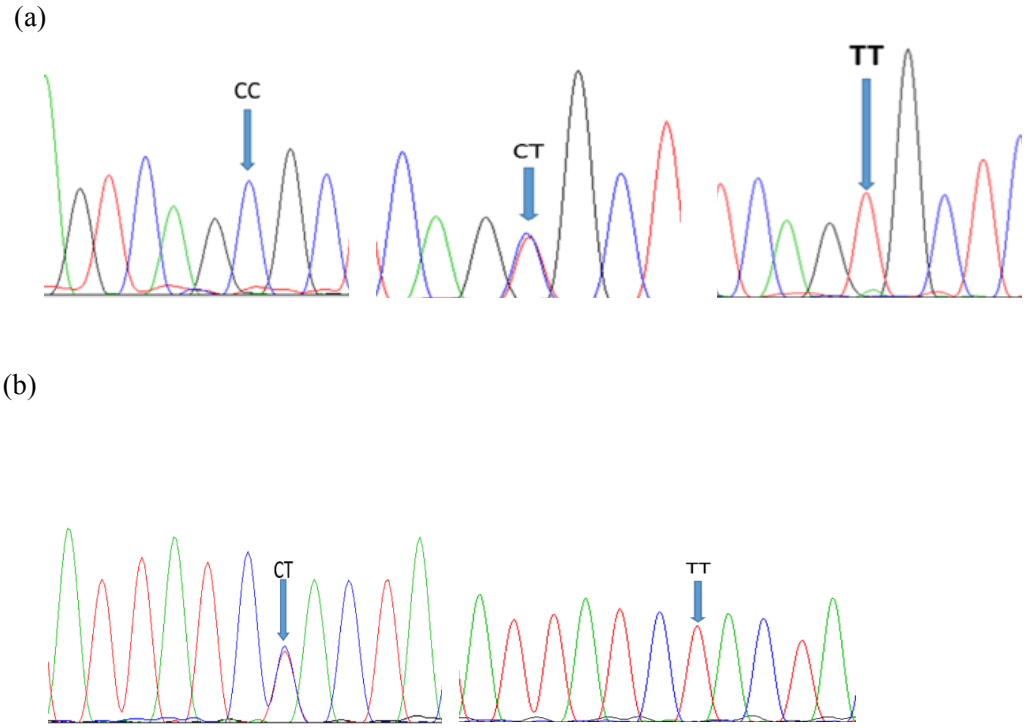

Figure 1 Polymorphic SNP identification: (a) *CC*, *CT*, and *TT* genotypes of g.3081C>T in the intron 2 of *GH* gene and (b) *CT* and *TT* genotypes of g.5961T>C in the exon 5 of *PRL* gene.

Table 1 Genotypic and allelic frequencies of g.3081C>T of *GH* gene and g.5961T>C of *PRL* gene in the experimental populations

	SNP[1]									
	GH: g.3081C>T					*PRL*: g.5961C>T				
Population[2]	CC	CT	TT	C	T	CC	CT	TT	C	T
GBT	0.50 (61)	0.43 (52)	0.07 (9)	0.71	0.29	0.02 (2)	0.32 (39)	0.66 (81)	0.18	0.82
L105	0.91 (103)	0.09 (10)	0 (0)	0.96	0.04	0 (0)	0.16 (18)	0.84 (95)	0.08	0.92
L106	0.63 (39)	0.30 (18)	0.07 (4)	0.79	0.21	0.08 (5)	0.43 (26)	0.49 (30)	0.30	0.70
Total	0.69 (203)	0.27 (80)	0.04 (13)	0.82	0.18	0.02 (7)	0.28 (83)	0.70 (206)	0.16	0.84

Number of ducks expressed in parentheses.
[1] *GH*: *growth hormone* gene (Chang et al., 2012); *PRL*: *prolactin* gene (Li et al., 2009).
[2] GBT: conserved Brown Tsaiya duck (n=122); L105: Brown Tsaiya LRI 1 (n=113); L106: Brown Tsaiya LRI 3 (n=61).

Table 2 Comparison of egg production traits among different genotypes of g.3081C>T of *GH* gene in the experimental Brown Tsaiya ducks[1]

	g.3081C>T of *GH* gene	
Trait[2]	*CC*	*CT*
Age at the 1st laying (day)	115.8±1.4	113.5±2.4
Body weight at 20 wks (g)	1213.6±14.8[a]	1158.4±16.1[b]
Body weight at 40 wks (g)	1262.5±16.0[a]	1198.5±17.4[b]
Egg weight at 40 wks (g)	60.9±0.5	60.4±1.1
Strength of eggshell at 40 wks (kg/cm^2)	4.70±0.10	4.60±0.22
Egg number		
at 26 wks	45.9±2.3[a]	27.8±7.4[b]
at 27 wks	51.7±2.5[a]	32.1±7.9[b]
at 28 wks	56.9±2.6[a]	36.5±8.3[b]
at 29 wks	61.3±2.7[a]	40.4±8.8[b]
at 30 wks	66.6±2.9[a]	45.3±9.2[b]
at 40 wks	124.1±3.1	124.2±5.3
at 52 wks	188.5±4.0	184.7±6.9

[1] *GH*: *growth hormone* gene. Data expressed as LSMean±SE.
[2] Age of 1st laying and egg numbers at 40 and 52 wks were calculated from the conserved Brown Tsaiya duck (GBT), Brown Tsaiya LRI 1 (L105) and LRI 3 (L106); body weights at 20 and 40 wks were calculated only from GBT; egg weight and strength of eggshell at 40 wks were calculated from L105 and L106; egg numbers at 26-30 wks were calculated only from L105.
[a,b] LSMeans with different superscript are significantly different among the three genotypes ($P<0.05$).

Table 3 Comparison of egg production traits between different genotypes of g.5961C>T of *PRL* gene in the experimental Brown Tsaiya ducks[1]

	g.5961C>T of *PRL* gene	
Trait[2]	*CT*	*TT*
Age at the 1st laying (day)	111.0±2.4[b]	116.8±1.4[a]
Body weight at 20 wks (g)	1178.8±18.4	1194.7±12.8
Body weight at 40 wks (g)	1227.9±20.3	1229.7±14.1
Egg weight at 40 wks (g)	61.3±0.9	60.7±0.53
Strength of eggshell at 40 wks (kg/cm^2)	4.35±0.17[b]	4.85±0.10[a].
Egg number		
at 26 wks	35.6±5.6[y]	45.9±2.4[x]
at 27 wks	40.3±5.9[y]	51.8±2.6[x]
at 28 wks	45.2±6.3[y]	56.9±2.7[x]
at 29 wks	49.3±6.6[y]	61.4±2.9[x]
at 30 wks	54.4±7.0[y]	66.7±3.0[x]
at 40 wks	136.8±5.1[a]	119.0±3.0[b]
at 52 wks	203.8±6.6[a]	180.3±4.0[b]

[1] *PRL*: *prolactin* gene. Data expressed as LSMean±SE.
[2] Age of 1st laying and egg numbers at 40 and 52 wks were calculated from the conserved Brown Tsaiya duck (GBT), Brown Tsaiya LRI 1 (L105) and LRI 3 (L106); body weights at 20 and 40 wks were calculated only from GBT; egg weight and strength of eggshell at 40 wks were calculated from L105 and L106; egg numbers at 26-30 wks were calculated only from L105.
[a,b] LSMeans with different superscript are significantly different among the three genotypes (P<0.05).
[x,y] LSMeans with different superscript are significantly different among the three genotypes (P<0.1).

REFERENCES

Ahumada-Solorzano, S. M., M. E. Carranza, E. Pedernera, A. J. Rodriguez-Mendez, M. Luna, and C. Aramburo. 2012. Local expression and distribution of growth hormone and growth hormone receptor in the chicken ovary: Effects of GH on steroidogenesis in cultured follicular granulosa cells. Gen. Comp. Endocrinol. 175:297-310.

Chang, M.-T., Y.-S. Cheng, M.-C. Huang. 2012. The SNP genotypes of growth hormone gene associated with reproductive traits in Tsaiya ducks. Reprod. Dom. Anim. 47:568-573.

Jiang, R. S., L. L. Zhang, Z. Y. Geng, T. Tang, S. S. Zhang. 2009. Single nucleotide polymorphisms in the 5'-flanking region of the prolactin gene and the association with reproduction traits in geese. S. Afr. J. Anim. Sci. 39:83-87.

Li, H. F., W. Q. Zhu, W. T. Song, T. J. Zhang, W. J. Xu, and K. W. Chen. 2009. Genetic Effects of Prolactin (PRL) and Growth Hormone (GH) Genes on Egg Performance in Gaoyou Duck. J. Agric. Biotech. 17:263-268.

Li, W. L., Y. Liu, Y. C. Yu, Y. M. Huang, S. D. Liang, and Z. D. Shi. 2011. Prolactin plays a stimulatory role in ovarian follicular development and egg laying in chicken hens. Domest. Anim. Endocrinol. 41:57-66.

Statistic Office of C. O. A. 2014. Livestock Production, in: Yearly Report of Taiwan's Agriculture, pp. 118-119, Council of Agriculture, Executive Yuan, Taipei, Taiwan.

Wang, C., Z. Liang, W. Yu, Y. Feng, X. Peng, T. Gong, and S. Li. 2011. Polymorphism of the prolactin gene its association with egg production traits in native Chinese ducks. S. Afr. J. Anim. Sci. 41:64-69.

Wu, X., M. J. Yan, L. P. Liu, S. T. Lian, X. Y. Fu, G. Y. Wang, and A. Li. 2012. Genetic Effects of Gonadotropin-releasing Hormone (GnRH) and Growth Hormone (GH) Genes on the Egg Performance in Muscovy Duck (Cairina moschata). J. Agric. Biotech. 20289-295.

Zhang, D. X., Z. Q. Xu, J. He, C. L. Ji, Y. Zhang, and X. Q. Zhang. 2015. Polymorphisms in the 5'-flanking regions of the GH, PRL, and Pit-1 genes with Muscovy duck egg production. J. Anim. Sci. 93:28-34.

PO-01-13 Effect of Days to Parturition and Locality of Hanwoo Pregnant Cows on Ultrasound Scan Measures by Parity

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Introduction

In the beef industry, it is very important to breed cows for producing outstanding calves for profit of famers in Korea. This has led to study genetic and phenotypic characteristics (Lee et al, 2003a; Lee et al, 2003b; Lee et al, 2014; Ha et al, 2009) of Hanwoo cows. Nevertheless, data on productive traits and reproductive traits on Hanwoo cows are lacking. Therefore, this study was undertaken that genetic evaluation of ultrasound measure traits would be an efficient tool to make genetic improvement of Hanwoo cows for beef production only if exact breeding records and locality are to be provided along with ultrasound measures.

MATERIALS AND METHODS

Data

A total of 1,596 pregnant cows and heifers born form 2010 to 2014 and raised on 9 regions were scanned with ultrasound machines owned and operated by local agents. Scanning position was common at vertical application on longissimus dorsi muscle area between 13th thoracic vertebrae and first lumbar vertebrae. And the ultrasound scan images were analyzed to measure backfat thickness (UBF), eye muscle area (UEMA) and visually appraised score of marbling (UMS, 1 to 27 points, higher the score higher the marbling). Table 1 shows the number of cows by birth-year, location and age groups of days to parturition

Statistical analyses

Data were fitted go general linear models to check the significances of fixed effects on the traits under study (SAS Institute Inc., Cary, NC, 2002). For ultrasound measure traits, effect birth-year, location and age group at ultrasound measurements were all significant sources of variation

Result

The analysis of variance for ultrasound measurements of Hanwoo cows for parity is given in Table 2. Locality of cow farm was a significant source of variation for all ultrasound scan measures of all parity ($p < 0.01$). Nine age groups of days to parturition by 15 days interval were insignificant at all parity. Least square means of ultrasound measurements by birth year are given in Table 3. Except for UBF and UEMA of second-parity, and UEMA of third-parity, all of measurements had a significant effect ($p < 0.05$). The result of least square means of ultrasound measurements by location is reported in Table 4. It was showed a significant difference in each region ($p < 0.05$). Table 5 provides least square means of ultrasound measurements by age groups of days to parturition. There was no significant difference in the UBF, UEMA and UMS of all of age groups for all parity

Table1. Number of cows by birth-year, location and age groups of days to parturition

Items	Group	Parity		
		1st	2nd	3rd
Brith-year	2010			76
	2011		136	178
	2012	169	391	15
	2013	557	46	
	2014	28		
Locality	A	69	68	35
	B	52	42	16
	C	185	62	14
	D	33	34	12
	E	64	51	32
	F	16	19	8
	G	90	72	43
	H	128	124	57
	I	117	101	52
Age groups of days to parturition	1(1~15days)	152	76	30
	2(16~30days)	116	86	46
	3(31~45days)	132	79	49
	4(46~60days)	99	79	36
	5(61~75days)	68	48	25
	6(76~90days)	75	79	22
	7(91~105days)	47	58	27
	8(106~120days)	38	44	19
	9(121~135days)	27	24	15
Total		754	573	269

Table 2. Analysis of variance for ultrasound measurements of hanwoo cows for parity

Parity	Source	df	UBF	UEMA	UMS
1st	Birth-year	2	2.357	79.992	8.602
	Locality	8	21.395**	558.701**	48.966**
	Age groups of days to parturition	8	4.616	125.228	4.130
	error	735	3.785	86.828	1.899
	RMSE		1.945	9.318	3.606
2nd	Birth-year	2	31.138**	345.420*	7.884
	Locality	8	46.380**	813.951**	25.697**
	Age groups of days to parturition	8	8.644	91.815	2.923
	error	554	5.307	92.197	3.006
	RMSE		2.304	9.602	1.734
3rd	Birth-year	2	5.383	357.951*	4.636
	Locality	8	23.484**	593.674**	10.795**
	Age groups of days to parturition	8	4.947	106.704	3.138
	error	250	5.797	95.788	3.640
	RMSE		2.408	9.787	1.908

UBF : ultrasound back fat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
RMSE : root mean-square error
* p<0.05, **p<0.01

Table 3. Least square means of ultrasound measurements by year at birth for parity

Year at Brith	UBF	UEMA	UMS
	---------- 1st parity ----------		
	ns	ns	ns
2012	3.641	59.498	5.203
2013	3.390	57.503	4.547
2014	3.039	57.186	4.495
	---------- 2nd parity ----------		
	**	*	ns
2011	4.745	61.487	5.163
2012	3.563	59.119	4.559
2013	3.843	55.002	4.568
	---------- 3rd parity ----------		
	ns	*	ns
2010	4.777	64.704	5.218
2011	4.522	62.095	5.007
2012	3.576	54.693	4.118

UBF : ultrasound back fat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
* p<0.05 ** p<0.01

Table 4. Least square means of ultrasound measurements by locality for parity

Location	UBF	UEMA	UMS
	---------- 1st parity ----------		
	**	**	**
A	4.037	53.529	5.023
B	2.261	52.067	3.412
C	3.786	61.031	3.671
D	3.751	61.936	6.234
E	3.665	56.260	5.823
F	3.958	64.814	5.484
G	1.879	53.219	3.952
H	3.477	59.793	4.729
I	3.398	59.909	4.408
	---------- 2nd parity ----------		
	**	**	**
A	3.779	53.841	4.318
B	3.887	52.941	3.967
C	3.864	61.522	3.607
D	4.837	61.612	6.043
E	4.516	55.412	5.465
F	4.468	69.306	5.164
G	1.697	50.315	4.416
H	4.630	60.092	5.248
I	4.776	61.784	4.642
	---------- 3rd parity ----------		
	**	**	**
A	4.113	52.292	4.110
B	4.033	59.501	4.811
C	4.136	59.374	4.178
D	5.175	67.159	5.490
E	4.495	57.023	5.785
F	5.337	69.146	4.800
G	1.980	52.812	4.350
H	5.010	65.245	5.319
I	4.347	61.925	4.187

UBF : ultrasound back fat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
** $p<0.01$ * $p<0.05$

Table 5. Least square means of ultrasound measurements by age of days to parturition

Group[1]	UBF	UEMA	UMS
	---------- 1st parity ----------		
	ns	ns	ns
1	3.630	57.097	4.874
2	3.295	57.789	4.963
3	3.421	56.200	4.709
4	3.349	56.870	4.474
5	3.205	58.040	4.896
6	3.574	59.851	4.992
7	2.740	57.548	4.254
8	3.678	58.295	4.916
9	3.319	60.868	4.656
	---------- 2nd parity ----------		
	ns	ns	ns
1	3.800	57.432	5.038
2	4.600	59.384	4.967
3	3.700	58.101	4.768
4	3.807	56.738	4.660
5	3.801	58.317	4.687
6	4.127	58.090	4.480
7	4.593	60.054	4.973
8	3.763	57.499	4.444
9	4.264	61.211	4.854
	---------- 3rd parity ----------		
	ns	ns	ns
1	3.431	56.528	4.196
2	4.648	61.877	4.972
3	4.110	58.921	4.752
4	4.406	58.920	4.782
5	3.971	60.098	4.229
6	4.318	61.966	4.647
7	4.324	61.532	4.884
8	5.062	62.838	5.244
9	4.356	61.795	5.322

UBF : ultrasound back fat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
[1]Group : age groups of days at parturition
** $p<0.01$ * $p<0.05$

Reference

Ha, J. J., J. B. Kim, C. W. Lee and Y. H. Song. 2009. Studies on live-carcass trait of parity and region in Hanwoo cows measured by real-time ultrasound. J. Anim. Sci. & Technol(Kor.) 51:3255~262.

Lee, C. W., J. G. Choi, K. J. Jeon, K. J. Na, C. Lee, B. K. Yang, and J. B. Kim. 2003a. Estimation of growth curve for evaluation of growth characteristics for Hanwoo cows. J. Anim. Sci. Technol. (Kor.) 45:509-516.

Lee, C. W., J. G. Choi, G. J. Jeon, K. J. Na, C. Lee, J. M. Hwang, B. W. Kim, and J. B. Kim. 2003b. Estimation of parameters for individual growth curves of cows in *Bos taurus Coreanae*. J. Anim. Sci. Technol. (Kor.) 45:689-694.

Lee, J. H., S. H. Oh, Y. M. Lee, Y. S. Kim, H. J. Son, D. J. Jeong, N. C. Whitley, and J. J. Kim. 2014. Study on growth curves of longissimus dorsi muscle area, backfat thickness and body conformation for Hanwoo (Korea Native) cows. Asian-Aust. J. Anim. Sci. 27(9): 1250-1253.

SAS. 2002. SAS/STAT User's Guide. V. 9.1 SAS Institute Inc., Cary, NC, USA

PO-01-16

JAK/STAT signaling pathway related genes differentially expressed in Necrotic Enteritis induced Fayoumi Chicken Lines

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1. Introduction

Necrotic enteritis (NE) disease that affects broiler chickens is a serious problem for poultry industries. The causative agent of NE disease is a gram-positive bacterium *Clostridium perfringens* (Long, 1973). NE disease causes intestinal mucosal damage leading to poor digestion and absorption in infected birds (Timbermont, 2001; Broussard, 1986). Therefore, the experimental induction of NE disease in chickens by *C. perfringens* alone, or by coinfection with *C. perfringens* and *E. maxima*, has become prevalent in veterinary studies (Shojadoost, 2002; Jang, 2012). NE disease has been increasing interest in studying the pathophysiological conditions of NE disease, the etiology of NE-associated diseases, and the development of control measures. Gene expression analyses of the tissues from NE-afflicted chickens showed alterations in the local immunity (Hong, 2012). However, the understanding of signalling pathway regulation of immune response to NE infected has been unclear.

The JAK/STAT pathway is activated by more than 40 cytokines and growth factors and is involved in multiple cell functions such as differentiation, proliferation, and apoptosis. In this pathway, the cytokines induce phosphorylation of the Janus tyrosine kinases (JAK1, 2 and 3, TYK2) (Kiu and Nicholson, 2012), followed by activation of STAT1-6 (Signal transducers and activators of transcription) (Kiu and Nicholson, 2012). Signalling through the JAK/STAT pathway is initiated when a cytokine binds to its corresponding receptor. This leads to conformational changes in the cytoplasmic portion of the receptor, initiating activation of receptor associated members of the JAK family of kinases. The JAKs, in turn, mediate phosphorylation at the specific receptor tyrosine residues, which then serve as docking sites for STATs and other signalling molecules (Schindler and Strehlow, 2000; Zgheib et al., 2013).

In contrast with the large function of JAK-STAT pathway related genes in mammalian, limited information has been known about the expression of JAK-STAT signalling pathway related genes in chicken. In this study, we hypothesized that the differences between two chicken lines result from JAK-STAT signalling pathway cascade that mediates the intestinal mucosal function and innate responsiveness to bacterial and protozoan co-infected in chicken. Therefore, we evaluated JAK-STAT pathway related genes expression between intestinal mucosal of line M5.1 and line M15.2 with *E. maxima* and *C. perfringens* (CP) co-infection using a Next General Sequencing (NGS) and bioinformatics analysis.

2. Materials and Methods

2.1. Experimental Animals and Sample Collection

The strain of *Eimeria maxima* (EM, 41A) and *Clostridium perfringens* strain Del-1(CP) was prepared according to protocols of the Animal Biosciences and Biotechnology Laboratory of the Agriculture Research Service, USDA. Fayoumi chicken M5.1 and M15.2 line were obtained from Iowa State University. The method used to produce NE chicken described (Kim et al., 2015). Intestinal mucosa samples were collected at 20-day post-hatching as described (Hong et al., 2012).

2.2. RNA Extraction and Quality Analysis

Total RNA was extracted from intestinal mucosa using Trizol RNA extraction kit (Invitrogen, CA, USA) and purified using the RNeasy Mini Kit (Qiagen, Germantown, MD, USA) and treated with DNase I (Promega, Madison, WI, USA) following the manufacturer's instructions. The RNA concentration and quality were further determined using the Agilent 2100 bioanalyzer (Agilent Technologies, San Diego CA, USA), Tecan F2000 (Tecan group Ltd., Männedorf, Switzerland) and the samples with RNA integrity number (RIN) above 7.0 and high-quality RNA (28S/18S>1) were only used for the next experiments.

2.3. RNA-Sequencing, mapping reads and identifying DEGs contents

The total RNA of all samples were pooled prior to library preparation in each experimental group. The mRNA libraries for Illumina sequencing constructed from total RNA as described (Trapnell et al., 2010). Sequencing was performed at Theragen Bio Institute (Suwon, Korea) on an Illumina HiSeq 2000 high throughput sequencer

according to the manufacturer's specifications and following RNA-Seq data analysed performed (Trapnell et al., 2012). To identify the differentially expressed genes (DEG) between two conditions, we used the EdgeR package (Robinson et al., 2010). The data were normalized by fragments per kilobase of exon per million mapped reads (FPKM) method (Mortazavi et al., 2008) and the RPKM data were used to quantify the relative gene expression. The threshold for calling a DEGs was false discovery rate (FDR) < 0.01. The FDR < 0.01 and |log2Ratio| ≥ 2 were used to identify DEGs.

2.4. Expression Pattern and Gene Ontology (GO) analysis

The genes identified were analysed and subjected to hierarchical clustering using Cluster (MeV v4.9: www.tm4.org) and Java Treeview software (http://jtreeview.sourceforge.net/). Cluster map analysis of genes identified between two lines using Euclidean distance. The p values calculated using the right-tailed Fisher's exact test. GO functional enrichment analysis was carried out using Blast2GO (version 2.7.1) (http://www.blast2go.org/). Kyoto Encyclopedia of Genes and Genomes (KEGG: http://www.kegg.jp/) pathway analyses were performed using DAVID Bioinformatics Resources version 6.7, NIAID/NIH (http://david.abcc.ncifcrf.gov/tools.jsp).

3. Results

3.1. Transcription of JAK/STAT signalling pathway genes between two chicken lines

Using RNA-seq, we determined 94 genes in the JAK/STAT signalling pathway expression between two chicken lines before and after co-infection with *Eimeria maxima* (EM) and *Clostridium perfringens* (Figure 1). Based on the p values < 0.001 and fold-change ≥ 2, several genes were significantly upregulated and downregulated in intestinal mucosal of two chicken lines compared with control. Of these genes, the expression of 23 genes (*AKT3, CSF2, CSF3R, IFNB, IL10, IL19, IL20RA, IL22, IL22RA2, IL23R, IL2RA, IL7R, IL9, JAK1, JAK2, LIF, LIFR, PIK3CD, PIM1, SOCS1, SOCS3, STAT1* and *STAT3*) was significantly increased by 2.0- to 5.6-fold, and 3 genes (*CSF3, IL12B* and *IL9R*) was significantly decreased by 3.2- to 4.6-fold with EM/CP co-infected in chicken line M5.1 (Figure 1).

Similarly, three genes of line M15.2, (*IL10, IFNB* and *IL9R*) were markedly increased by 2.1- to 2.5-fold, but 8 genes (*IL19, IL20RA, IL22, IL22RA2, IL9, MPL*, *OSMR* and *SPRY4*) were markedly decreased by 2.4- to 4-fold after co-infection with EM/CP (Figure 1). Compared with line M15.2, the expression of 13 genes (*AKT3, CSF2, CSF3R, IFNG, IL19, IL20RA, IL22, IL9, JAK2, LIFR, MPL, OSMR* and *SPRY4*) was upregulated by 2.1- to 3.3-fold and three genes (*CSF3, IL12B* and *IL9R*) was downregulated by 3.3- to 4.7-fold in line M5.1 (Figure 1).

3.2. Identification of the role of JAK-STAT signalling pathway genes in intestinal mucosal of two chicken lines

We investigated and mapped the position of 94 JAK-STAT signalling pathway genes that expressed in intestinal mucosal in KEGG *Gallus gallus* JAK-STAT signalling pathway. In NE-induced chicken line M5.1, about 69 genes were significantly increased, and 25 genes were markedly decreased than control groups. In NE induced chicken line M15.2, about 64 genes were upregulated, and 30 genes were downregulated ($p < 0.01$, compared treatment to control). All these 94 DEGs were critically positioned in the KEGG JAK-STAT signalling pathway. Figures 2 and 3 showed the position of 94 genes that differentially expressed in intestinal mucosal of NE-induced chicken line M5.1 and M15.2, respectively.

3.3. Gene ontology (GO) analysis of differentially expressed genes

All of the upregulated and downregulated genes in intestinal mucosal of two lines were categorized into three major functional groups including, molecular function, cellular component, and biological process according to GO. Each major group was further divided into several subcategories. We first evaluated the GO in line M5.1, all of the up-and downregulated genes were categorized into two major functional groups including biological process and molecular function. The biological process was divided into 22 subcategories such as regulation of immune system process, regulation of cell differentiation, immune response, immune system process, intracellular signal transduction. The molecular function was divided into 7 subcategories including: cytokine receptor binding and activity, protein binding, signal transducer activity, leukemia inhibitory factor receptor activity (Figure 4).

Totals of 32 GO terms were found from up-and downregulated genes in line M15.2. The biological process was divided into 21 subcategories such as regulation of protein metabolic process, regulation of multicellular organismal process, intracellular signal transduction, response to chemical, immune system process. The cellular component was divided into 4 subcategories including extracellular region, extracellular space, phosphatidylinositol 3-kinase complex and extracellular region part. The molecular function was divided into 7 subcategories such as signal transducer activity, cytokine receptor binding, and protein binding (Figure 5).

4. Conclusion

The JAK-STAT signalling pathway regulates the adaptive and innate immune of immunity as well as epithelial repair and regeneration. We herein report the differential transcriptional expression of members of the JAK/STAT pathway in intestinal mucosal of two lines NE-induced. This NGS and bioinformatics study provided abundant data that will lead to future studies in JAK-STAT pathways associated with two genetic chicken lines differentiation and function.

Acknowledgements

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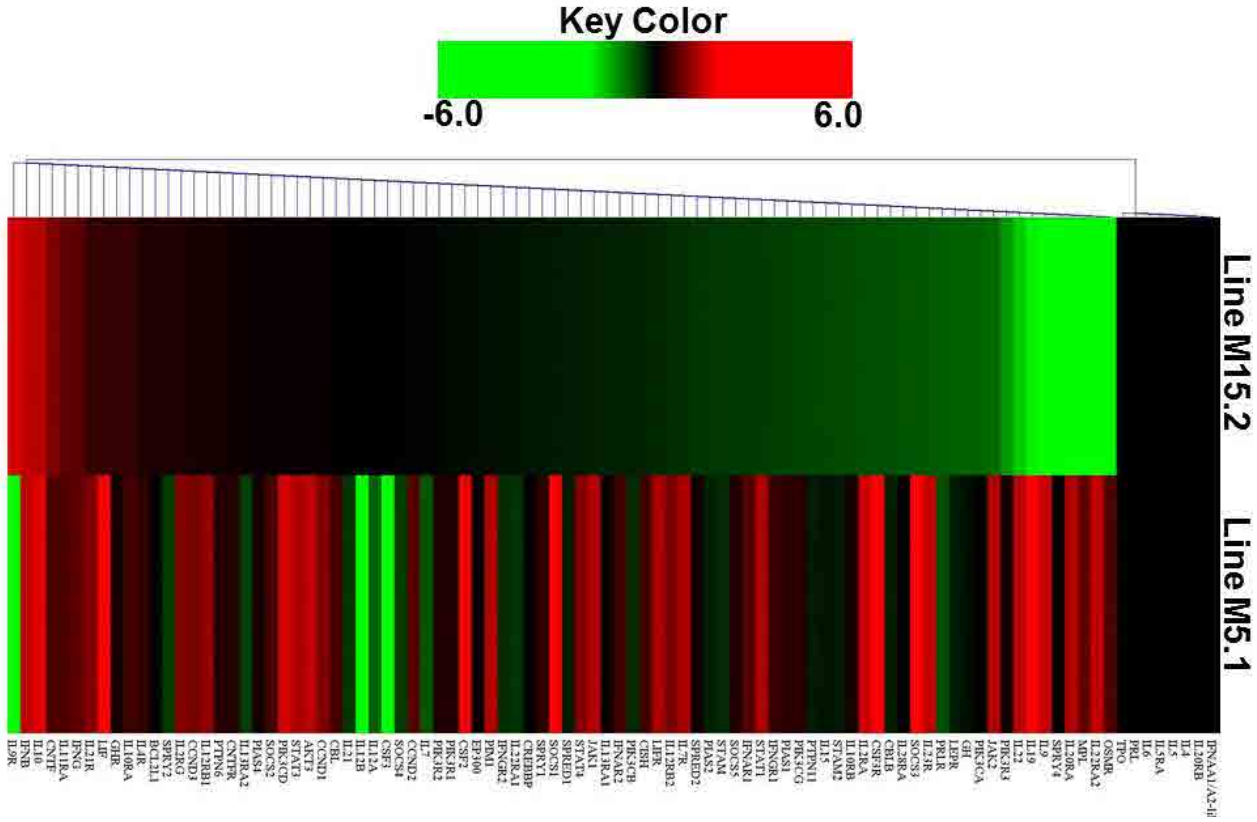

Figure 1

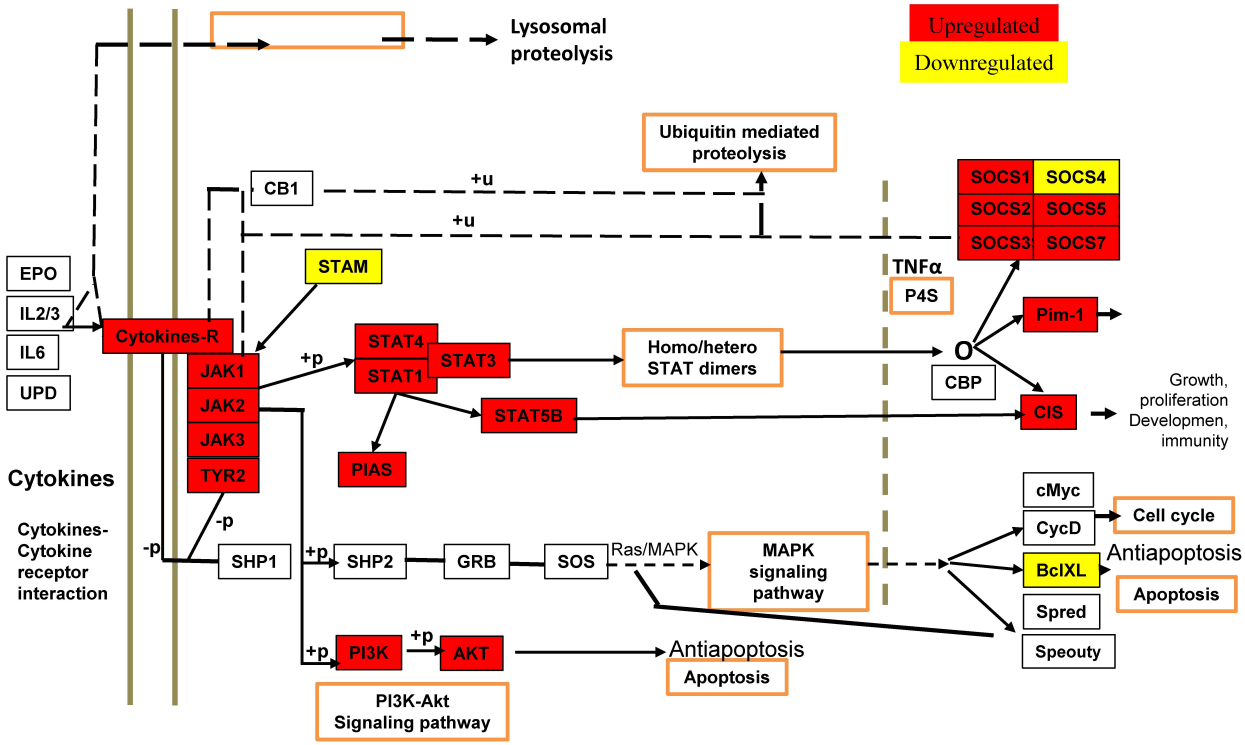

Figure 2

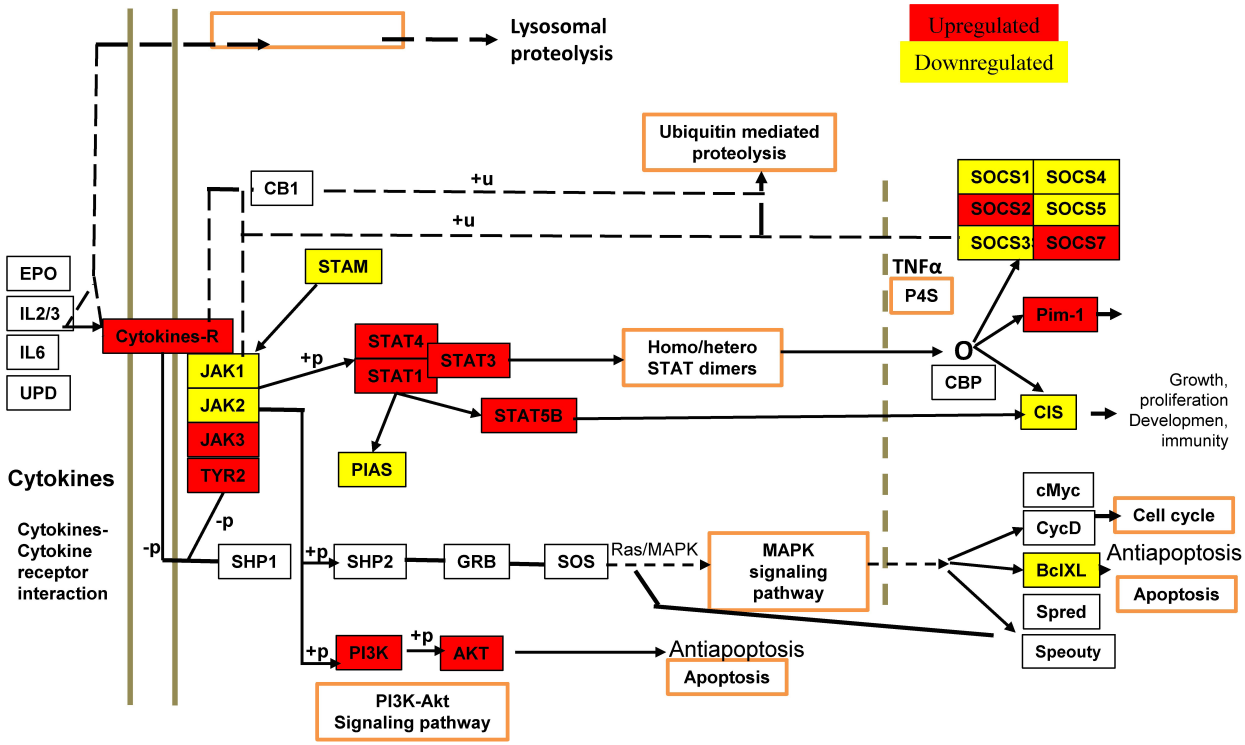

Figure 3

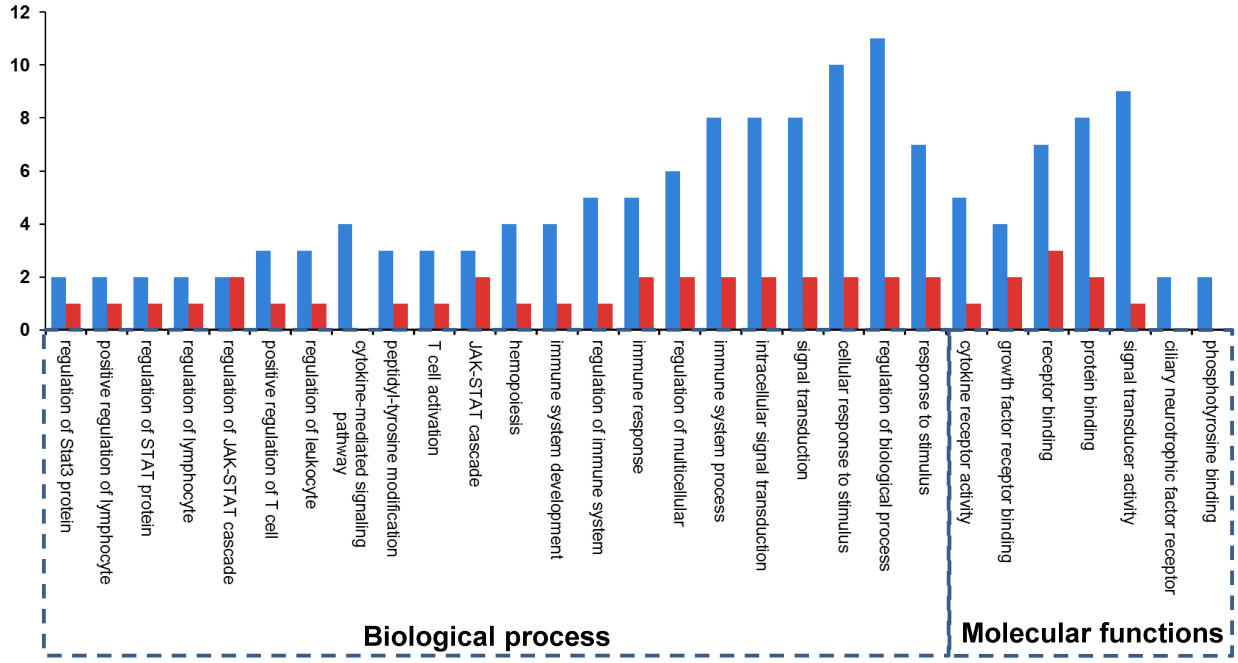

Figure 4

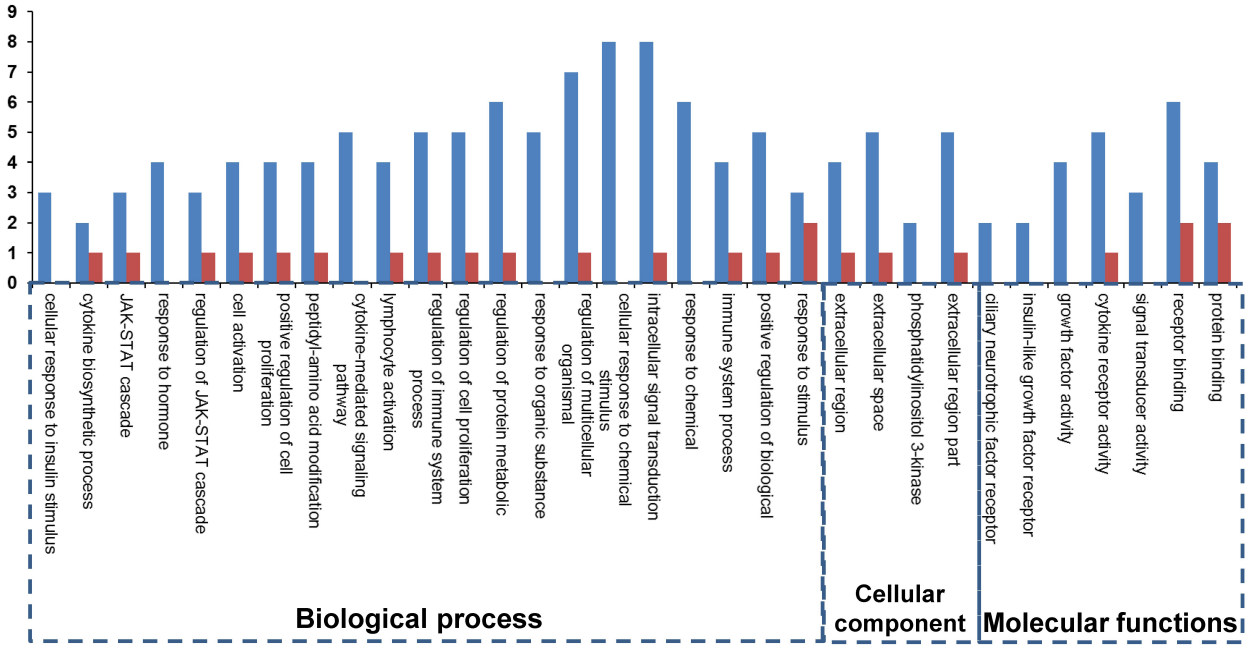

Figure 5

References

Long, J.R. Necrotic enteritis in broiler chickens. I. A review of the literature and the prevalence of the disease in Ontario. Can. J. Comp. Med. 1973, 37, 302-308.

Timbermont, L.; Haesebrouck, F.; Ducatelle, R.; Van Immerseel, F. Necrotic enteritis in broilers: An updated review on the pathogenesis. Avian Pathol. 2011, 40, 341-347.

Broussard, C.T.; Hofacre, C.L.; Page, R.K.; Fletcher, O.J. Necrotic enteritis in cage-reared commercial layer pullets. Avian Dis. 1986, 30, 617-619.

Shojadoost, B.; Vince, A.R.; Prescott, J.F. The successful experimental induction of necrotic enteritis in chickens by Clostridium perfringens: A critical review. Vet. Res. 2012, 43, 74.

Jang, S.I.; Lillehoj, H.S.; Lee, S.H.; Lee, K.W.; Lillehoj, E.P.; Hong, Y.H.; An, D.J.; Jeong, W.; Chun, J.E.; Bertrand, F., et al. Vaccination with clostridium perfringens recombinant proteins in combination with montanide ISA 71 VG adjuvant increases protection against experimental necrotic enteritis in commercial broiler chickens. Vaccine 2012, 30, 5401-5406.

Dahiya, J.P.; Wilkie, D.C.; Van Kessel, A.G.; Drew, M.D. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Feed Sci. Tech. 2006, 129, 60-88.

Hong, Y.H.; Song, W.; Lee, S.H.; Lillehoj, H.S. Differential gene expression profiles of beta-defensins in the crop, intestine, and spleen using a necrotic enteritis model in 2 commercial broiler chicken lines. Poult. Sci. 2012, 91, 1081-1088.

Kiu, H., Nicholson, S.E., 2012. Biology and significance of the JAK/STAT signalling pathways. Growth Factors 30, 88-106.

Schindler, C., Strehlow, I., 2000. Cytokines and STAT signaling. Adv Pharmacol 47, 113-174.

Zhang, M., Liu, X., Zhang, X., Song, Z., Han, L., He, Y., Xu, Z., 2014. MicroRNA-30b is a multifunctional regulator of aortic valve interstitial cells. The Journal of thoracic and cardiovascular surgery 147, 1073-1080.e1072.

Kim, D.K., Lillehoj, H.S., Jang, S.I., Lee, S.H., Hong, Y.H., Lamont, S.J., 2015. Genetically Disparate Fayoumi Chicken Lines Show Different Response to Avian Necrotic Enteritis. The Journal of Poultry Science 52, 245-252.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., Pachter, L., 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7, 562-578.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., Pachter, L., 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28, 511-515.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-140.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., Wold, B., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods 5, 621-628.

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Gene expression of GCLC and GCLM to Response to Exercise Stress in Horse.

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Pusan National Univeristiy

Introduction

Aerobic respiration of muscle tissue is an important process in animals. In steady state, as part of the ATP generation mechanism in the aerobic respiration of muscle tissue, cytochrome c, an essential component of the electron transport system, stabilizes cells that react with oxygen (Couto et al., 2013). During rapid exercise, an oversupply of oxygen in the blood reacts with electrons that are generated from the electron transport system, and generates reactive oxygen species (ROS) (Zhuo, 2012). These ROS, which represent oxidative stress, destroy DNA through muscle fatigue, damage, and apoptosis (Scott, 2007). Protection of cell organelles from ROS occurs in part via the oxidation of glutathione by ROS. Both the glutamate-cysteine ligase, modifier subunit (*GCLM*) gene and the glutamate-cysteine ligase, catalytic subunit (*GCLC*) gene, are coded for glutathione, and the *GCLC* gene is known to produce γ-glutamyl cysteine, which is a glutathione precursor (Gerardo, 2009). During strenuous exercise, and other situations that induce oxidative stress, the *GCLC* gene is expressed (Cecile, 2010; Vasanthi, 2012). In the case of *GCLM,* the expression pattern also increased during strenuous exercise (Vasanthi, 2012), again due to oxidative stress (Solis et al., 2002). Previous study showed that expression of GCLC and GCLM genes were up-regulated in muscle of Thoroughbred horses in response to exercise (Park et al., 2012). These data indicate that glutathione-synthesis related genes play important role in alleviating stress response by exercise. Despite various experiments with *GCLC* and *GCLM*, research concerning exercise, *GCLC,* and *GCLM* in horses is still insufficient.

In this study, we performed bioinformatics analyses of horse GCLC and GCLM, which are encoding glutathione synthesis-related proteins, and investigated their expression in muscle and blood in response to exercise stress to gain insights on their potential application as biomarkers for ROS responses in horses.

Materials and methodS

Total RNA isolation

Total RNA samples for investigation of *GCLC* and *GCLM* were extracted from three Thoroughbred and three Jeju horses. Tissues (from skeletal muscle, kidney, heart, liver, lung, colon, and spinal cord) were extracted for polymerase chain reaction (PCR) analysis. The various tissues sampled from the horses were crushed in a mortar and pestle by using 50–100 mg, or 3 mL in the case of blood, and mixed with 9 mL of red blood cell (RBC) lysis buffer (Solgent Co., Ltd., Daejeon, Korea) to remove red blood cells. The cells were then dissolved using 1 mL of TRIzol (Invitrogen, Karlsruhe, Germany) and 200 μL of chloroform was added to remove cells from the organic solvent. The mixture was then shaken for 10 s and left at 4 °C for 5 min. Centrifugal separation was carried out at 4 °C for 15 min, and the supernatant removed to a new test tube and mixed with the same amount of isopropanol. The test tube was left at 4 °C for 15 min to produce RNA pellets. Isopropanol was removed by carrying out centrifugal separation at 4 °C for 15 min and the sample was then sterilized with 85% ethanol and dissolved with RNase-free water. The purity of extracted RNA was confirmed by measuring the absorbance at 230 nm and 260 nm using a spectrophotometer (ND-100, Nano Drop Technologies Inc., Wilmington, DE, USA) and only the extracted RNA with purity (OD value of 230 nm/260 nm) over 1.8 (found via quantitative analysis) was used. The selected RNA was stored at –70 °C until the experiment occurred.

cDNA synthesis

In order to synthesize cDNA, 2 μg of RNA, 1 μL of oligo-dT (Invitrogen), and 1 μL of RNase-free water were added, denatured at 80 °C for 3 min, and cDNA was synthesized using 4 μL of 5 x RT buffer, 5 μL of 2 mM deoxynucleotide (dNTP), 0.5 μL of RNase inhibitor (Promega Corporation, Madison, USA), and 1 μL of moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, USA).

Polymerase chain reaction

The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and Ensembl Genome Browser

(www.ensembl.org) were utilized for the desired gene sequence information, and the primer used for checking the*single nucleotide polymorphism* was synthesized using PRIMER3 software (http://bioinfo.ut.ee/primer3-0.4.0/). The information of synthesized primer included *GCLC* Primer F (5′- ACCAGGGTGATCCTGTCGTA-3′), R (5′-ATCCCGTTTCTGTG CGACTT-3′), *GCLM* Primer F (5′-GTG TGA TGC CAC CTG ATT TG-3′), and R (5′-GCT TTT CAC GAT GAC CGA GT-3′). The PCR was carried out using cDNA under the following conditions: to amplify target genes on cDNA, 1.8 μL dNTP, 2 μL 10 X buffer, 0.2 μL HS-Taq, and 12 μL distilled water were added to 2 μL 50 ng/μL diluted DNA, 5 pmol/μL diluted forward primer and reverse primer, and PCR was carried out with a total of 20 μL. The PCR conditions were denaturation carried out at 94 °C for 10 min, and the second denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. This was repeated for 40 cycles, and then the extension occurred at 72 °C for 10 min. The band was confirmed on UV using a 1.5% SeaKem LE agarose gel (Lonza, Rockland, USA).

Real time qPCR amplification

The RT-qPCR was carried out using the C1000 Thermal Cycler (Bio Rad, Hercules, CA) in order to measure the relevant expression of target genes. 25 μL of reaction solution was used and the solution was produced as follows: 2 μL and 5 μL of distilled water, and 2 μL of diluted cDNA (50 ng/μL) were added to 14 μL of SYBR green master mix (Bio Rad, Hercules, CA), and 5 pmol/μL each of diluted forward primer and reverse primer. The RT-qPCR conditions were as follows: the first denaturation was carried out at 94 °C for 10 min, and then the second denaturation was at 94 °C for 10 s, the annealing occurred at 60 °C for 10 s and the extension at 72 °C for 30 s. This was carried out repeatedly 40 times. All measurements were performed in triplicate for all specimens, and the comparative method used was the 2- ΔΔ Ct method (Livak et al., 2001). The relevant expression of target genes was calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalizer.

Results and Discussion

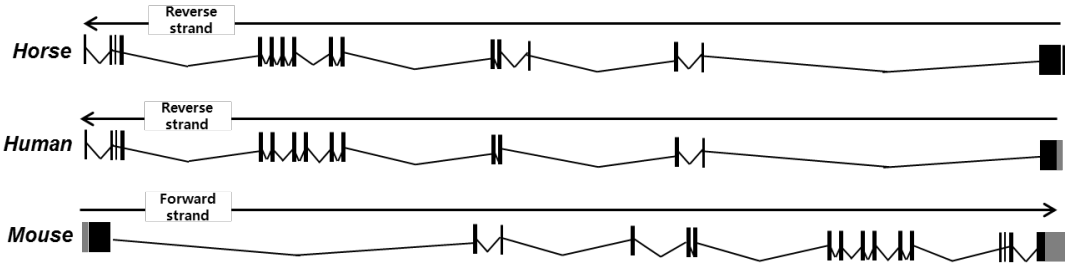
Bioinformatics analyses of horse GCLC and GCLM genes

Basic genetic structures for horse *GCLC* and *GCLM* is shown in Table 1. *GCLC* and *GCLM* gene both has single nucleotide polymorphism (SNPs). *GCLM* has two SNPs in genic region. Each of SNPs is located in Chromosome 5: 71,110,620- 71,110,620, Chromosome 20: 71,110,653- 71,110,653, and *GCLC* also has two SNPs in exon. Each of SNPs is located in Chromosome 20: 50,986,557- 50,986,557, Chromosome 20: 51,000,696- 51,000,696. All the SNPs are non-synonymous SNP which can affect to amino acids.To investigate the evolutionary distance of *GCLC* and *GCLM* in horses, we obtained gene sequences from nine species of mammals (human, chimpanzee, macaque, orangutan, cow, pig, horse, rat, and mouse) from Ensembl 62 and conducted phylogenetic analysis (Figure 2). We determined that there are two genes in macaques, humans, and chimpanzees arising from one internal node. Interestingly, the evolutionary distance of the horse is closest to the chimpanzee, orangutan, and macaque compared to other livestock. However, the human is further from horses than other primate species. mRNA similarity is high at 70~80% (Figure 3). This means that the mRNA sequence is conserved across the gene variants. Thus, the results of this study indicate that *GCLC* and *GCLM* genes play important roles in cell preserving gene function and structure and gene regulation mechanisms in various species.To investigate the expression pattern of these genes in various horse tissues, we conducted RT-qPCR for Jeju horse tissue (Figure 4). From the results of the RT-qPCR, it was found that *GCLC* and *GCLM* are expressed in various tissues, which means that their expression is ubiquitous.From studies of PCR and RT-qPCR with skeletal muscle and blood in Jeju horses and Thoroughbreds after 30 min exercise *GCLM* gene expression in both Jeju and Thoroughbred horses was up-regulated. These results show that *GCLC* in thoroughbred and Jeju horses is related to exercise stress, but *GCLC* is more affected by exercise stress in tissues in thoroughbreds than in Jeju horses.*GCLC* and *GCLM* are subunits of glutamate cysteine ligase (*GCL*), which is normally combined with γ-glutamate and γ-cysteine, and produces γ-glutamylcysteine. *GCLC* and *GCLM* are involved in the glutathione synthesis process as oxidative stress reducing mechanisms**,** and induced in response to stress in the body with strenuous exercise (Vasanthi, 2012).

Gene symbol	Location	Length (bp)	Length (aa)	Number of exon
GCLC	Chr 20:50,985,591-51,029,180	1,935	637	16
GCLM	Chr 5: 71,102,814-71,115,917	1,425	233	6

Table. 1. Genetic information for the glutamate-cysteine ligase, catalytic subunit (*GCLC*) and glutamate-cysteine ligase, modifier subunit (*GCLM*) genes. Avg fold is average fold change value of FPKM value following exercise though RNA sequencing. bp - base pair; aa - amino acid.

GCLC



GCLM

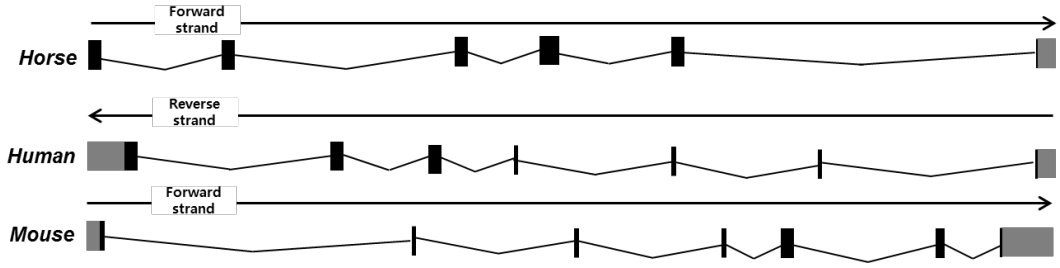
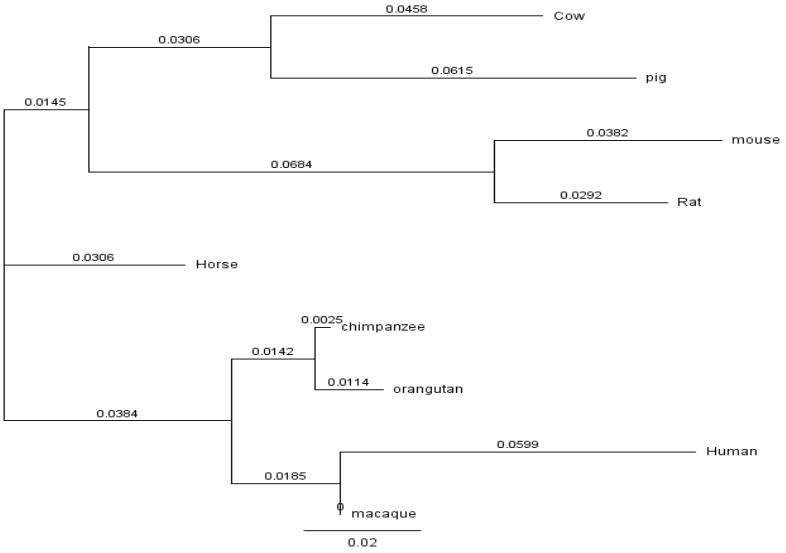


Figure 1. Gene structure of glutamate-cysteine ligase, catalytic subunit (*GCLC*) and glutamate-cysteine ligase, modifier subunit (*GCLM*) in horses. Gene structure of *GCLC*. GCLC has 16 exons. (Gene structure of *GCLM*. GCLM has 6 exons. Black boxes indicate exons, white boxes indicate untranslated regions (UTR), and black lines indicate introns.)

GCLC



GCLM

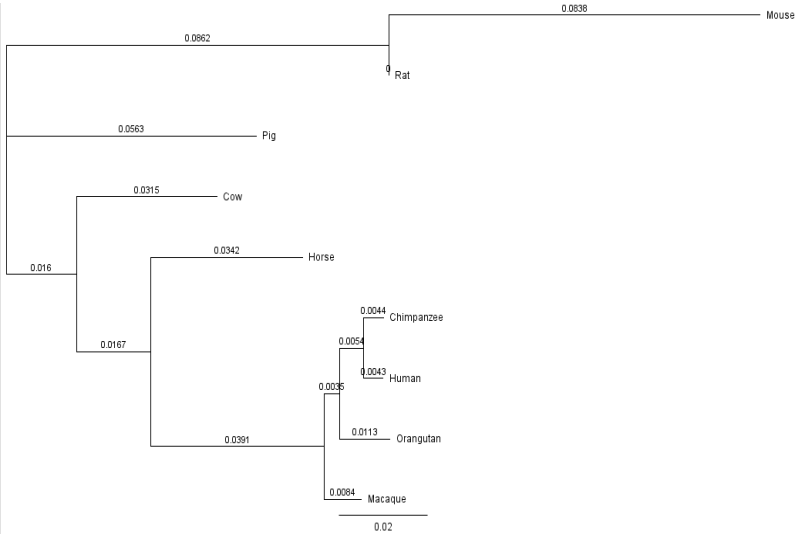
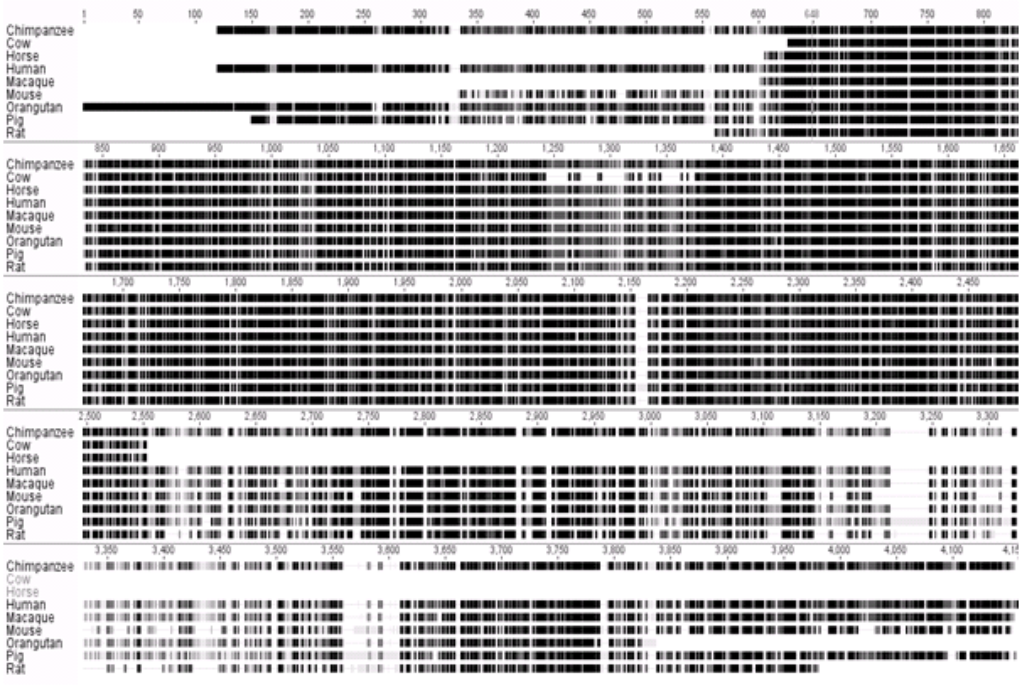


Figure 2. Unrooted phylogenetic trees for horse glutamate-cysteine ligase, catalytic subunit (*GCLC*) genes and glutamate-cysteine ligase, modifier subunit (*GCLM*) based on mRNA sequence.
*Values on branch mean substitution per site

GCLC



GCLM

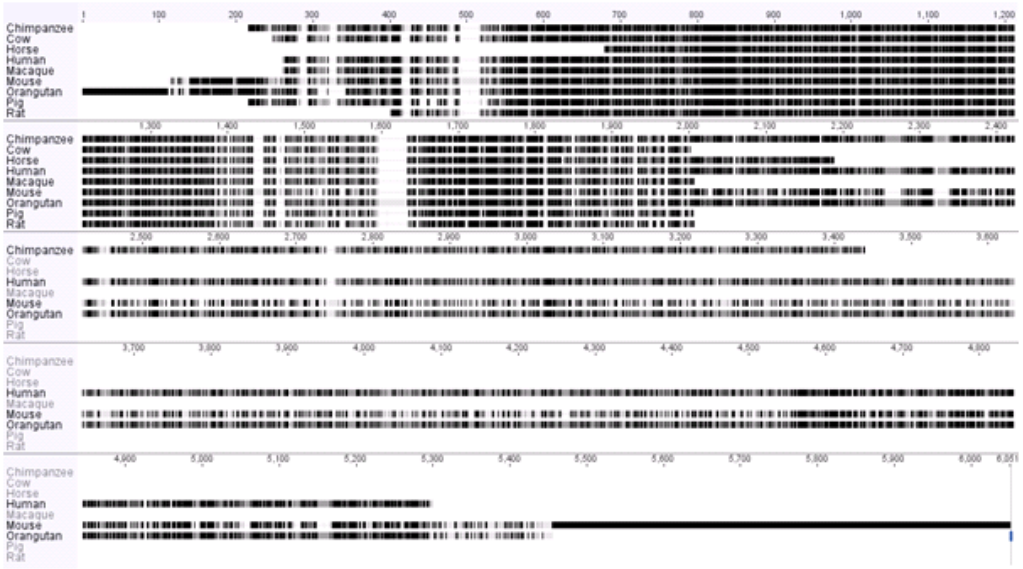
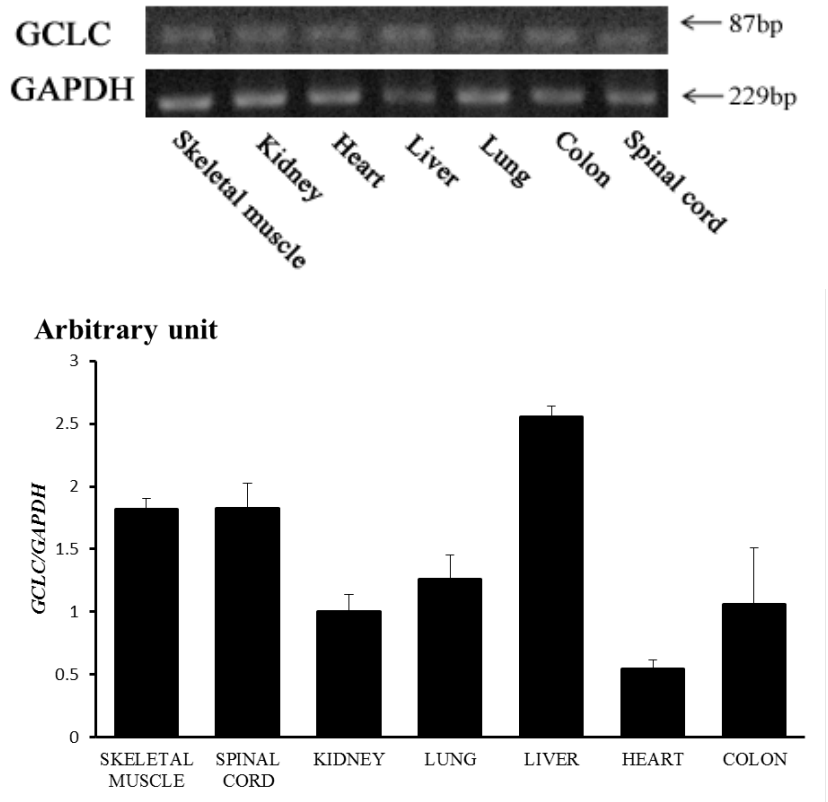


Figure 3. Similarity of mRNA sequences of various species.

(A)



(B)

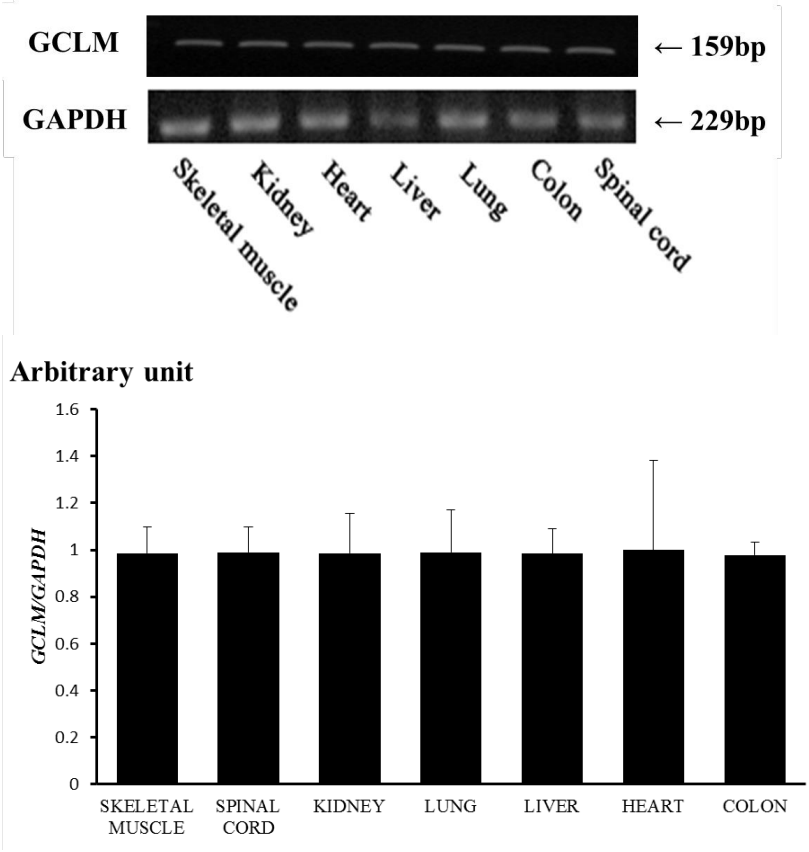
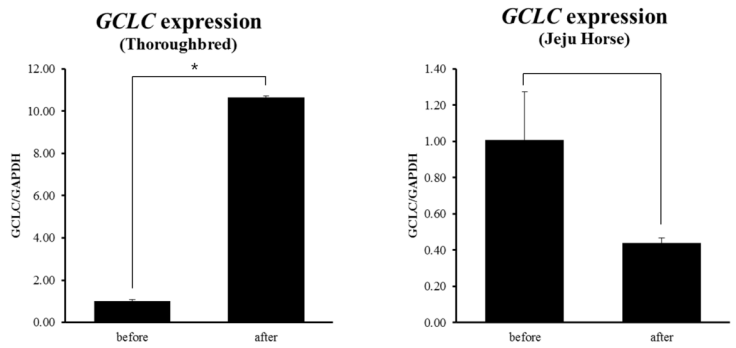
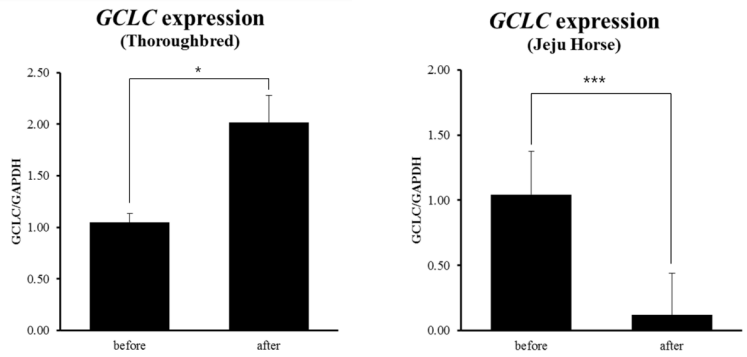


Figure 4. Expression of the horse glutamate-cysteine ligase, catalytic subunit (*GCLC*) gene determined by real-time quantitative polymerase chain reaction (RT-qPCR) in a variety of tissues from the Jeju horse (*Equus caballus*). (A) expression pattern of GCLC (B) Expression pattern of GCLM. The mean fold values are presented by mean±standard error. The difference value of gene expression was verified by Tukey's t-test. The GAPDH gene was used as control. GAPDH, glyceraldehyde 3-phosphate dehydrogenase. a, $p<0.05$.

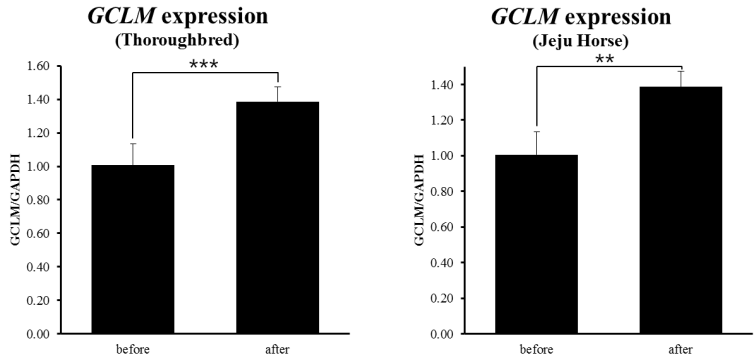
(A)



(B)



(C)



(D)

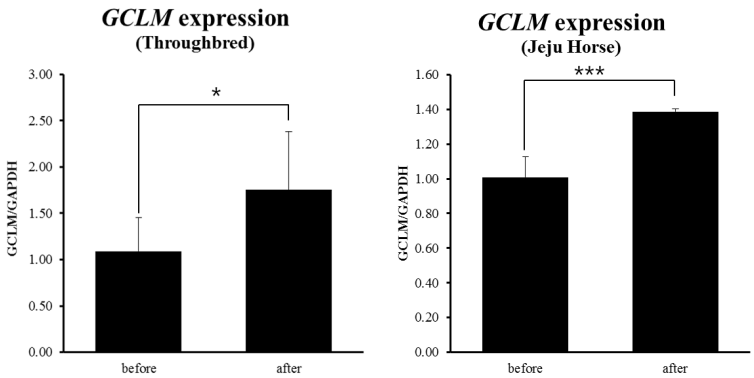


Figure 5. Expression of glutamate-cysteine ligase, catalytic subunit (*GCLC*) genes determined by real-time quantitative polymerase chain reaction (RT-qPCR) in horses before and after exercise (30 min). (A) Expression of the *GCLC* gene in horse tissue. (B) Expression of *GCLC* gene in horse blood. (C) Expression of *GCLM* gene in horse tissue. (D) Expression of *GCLM* gene in horse blood. Quantitative analysis was calculated using the 2-ΔΔCt method. GAPDH was used for normalization.

REFERENCES

KREJSA, Cecile M., et al. Rapid activation of glutamate cysteine ligase following oxidative stress. Journal of Biological Chemistry, 2010, 285.21: 16116-16124.

Lee, Kyoung-Young, et al. "Effect of exercise on hepatic gene expression in an obese mouse model using cDNA microarrays." Obesity 14.8 (2006): 1294-1302.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(Delta Delta C(T)) method. Methods 25:402-408.
Muthusamy, Vasanthi R., et al. "Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium." Free Radical Biology and Medicine 52.2 (2012): 366-376.

Nava, Gerardo M., et al. "Genomic analyses reveal a conserved glutathione homeostasis pathway in the invertebrate chordate Ciona intestinalis." Physiological genomics 39.3 (2009): 183-194.

PARK, Woncheoul, et al. 2014. Investigation of De Novo Unique Differentially Expressed Genes Related to Evolution in Exercise Response during Domestication in Thoroughbred Race Horses. PloS one 9.3:e91418.

Powers, Scott K., Andreas N. Kavazis, and Joseph M. McClung. "Oxidative stress and disuse muscle atrophy." Journal of applied physiology 102.6 (2007): 2389-2397.

SOLIS, Willy A., et al. Glutamate–cysteine ligase modifier subunit: mouse Gclm gene structure and regulation by agents that cause oxidative stress. Biochemical pharmacology, 2002, 63.9: 1739-1754.

Zhuo, Yehong, Hongrong Luo, and Kang Zhang. "Leber hereditary optic neuropathy and oxidative stress." Proceedings of the National Academy of Sciences 109.49 (2012): 19882-19883.

PO-01-40 EFFECTIVENESS OF TWO BREEDING METHODS WITH ESTRUS SYNCHRONIZATION IN ETTAWA GRADE GOATS USING CONTROLLED INTERNAL DRUG RELEASE

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Introduction

Dairy goats is a new commodity for most people in Indonesia and has a good development prospects. Dairy goats were developed in Indonesia is Peranakan Ettawa (Ettawa Grade Goat) and generally still more dominant as a source of meat compared to the milk source. This goats are very adaptive topography in Indonesia. This goat is widespread in rural areas and is usually maintained for the purpose of saving as well as livestock. Within two years, Ettawa Grade Goat can give birth three times with each time average of two kid.

Traditional farming business are generally only used as a sideline so that maintenance management was not too much attention. This has led to economic losses and aggravate subsequent descendants. The failure of the process of reproduction is often a problem on the farm people that led to the collapse lamb birth three times in two years, so the kidding interval will be long. This problem is very detrimental to farmer. Reproductive ability is influenced by many factors, including the reproductive management that are not mapped, the high incidence of *silent heat* (estrus quiet), the quality of the frozen sperm is used and interference with the function of the reproductive organs.

Good reproductive management accompanied by the application of reproductive technology is a shortcut to accelerate the achievement of objectives. For farmers who have applied the principles of agribusiness, especially for dairy goat farmers will feel comfortable if they can produce throughout the year. Businesses that are used to cope with the failure of the process of reproduction such as increasing intake of feed nutrients and synchronize estrus. Estrus synchronization is a method for manipulating and uniformity of the estrous cycle. Goat can be expected estrus at the same time, so that will facilitate the management and maintenance of breeding.

Implementation of estrus synchronization can be done by giving luteolitik agents (prostaglandin and estrogen) or progesterone either single or in combination with other hormones. The progesterone which has been used among other intravaginal progesterone intravaginal device (PIRD) and Controlled Internal Drug Release (CIDR). Controlled Internal Drug Release can be widely used to control the estrous cycle in cattle, buffaloes, goats and sheep. This treatment has been used for years as a method for controlling the estrus cycle in domestic ruminants.

One attempt to improve reproductive efficiency is by improving the quality of breeding. Improved quality of marriage includes recording, time of mating and mating method. Methods of mating there are two ways, the natural mating and artificial insemination (AI). The AI program is very profitable for farmers because without maintaining superior male can result superior lamb.

Utilization of reproductive technologies that synchronize estrus in goats has not been done in Indonesia. Utilization of estrus synchronization was limited to research, so the use CIDR should be considered to support the development of goats in Indonesia.

Objective

This research aims to determine the effectiveness of the mating between the two methods of natural mating and artificial insemination to the female goats were synchronized estrus using CIDR in Sardonoharjo Village, Yogyakarta Indonesia.

Material and Method

The experiment was conducted at Sardonoharjo Village, Yogyakarta. Eight goats were divided randomly into two groups, Group I (4 goats mated naturally) and group II (4 goats mated using AI). Implementation of estrus synchronization using CIDR, preceded by pregnancy check on all animals using data from the breeder to make sure there are no animals that were pregnant. The tool used in this research were a Eazi-Breed® CIDR-G, vaselin, fresh sperm, NaCl fisiologis, DEEA Gestdect® and insemination gun.

Controlled Internal Drug Release (CIDR-G) for goat was conducted after pregnancy check, after 12 days the installation of CIDR can be detached and observed signs of estrus up to 3 days after detached with an interval of

3 hours. After signs of estrus seen. Group I mated naturally using superior male who had been prepared, while group II mated with artificial insemination method using sperm from the male superior liquid that has collected and diluted using physiological saline. Pregnancy check conducted by using DEEA Gestdect®.
Data pregnancy rate of two mating method were analyzed using Chi-square analysis.

Results

CIDR Installation

Based on the research that has been conducted shows that the installation of CIDR for 12 days can make all the goat estrus. Similar results were presented by Suharto et al. (2008), based on research conducted goats estrous responses obtained by 100% on the installation of CIDR for 10 days. Installation CIDR containing progesterone resulted in negative feedback on the secretion of gonadotropin hormone, namely FSH and LH. After CIDR detached there will be a release of FSH and LH and follicle stimulating folliculogenesis and to form de Graaf which secretes estrogen, causing estrus. During the installation of CIDR, there is no female goat show estrus, it is in line with research Romano (1998) that CIDR capable of suppressing the activity of progestagen estrus and also has the ability to block the estrus in goats through a negative feedback mechanism.
Macmillan and Peterson (1993) states that there are two main objectives in synchronizing estrus, namely: 1) Getting all animals were given treatment within reach of estrus is known with certainty so that each animal can be in the AI at the same time; 2) To produce a pregnancy rate comparable to or better than the untreated group mated with AI or by male. Hafez (1993) states that, synchronizing estrus and ovulation is an alternative to improve the efficiency of livestock production.

Mating

In this research used two methods of mating, which is mated naturally group I and group II using AI. Group I mating on November 7, 2012 at 17:00 pm by using a locally owned stud herd. This natural mating time been based on the estimated time of ovulation is known based on the data that is above the onset of estrus 30 hours after CIDR detached. Group II using AI was held on 6 November 2012 at 18.00, this is due to the time of onset of estrus in group II 12 hours faster than group I.
Suharto et al. (2008) states that the prediction of ovulation time goats have been installed CIDR for 10 days occurred shortly after the LH surge, which is between 51 hours to 62 hours of detached of CIDR or soon after 25 hours to 36 hours from the beginning of estrus. Results of research Ngangi (2002), states that the treatment time of insemination with the time range of 14 to 23 hours after onset of estrus found 7 of 15 head is expected pregnant, while Budiarsana and Sutama (2012) stated that the delay time of AI from 20 to 25 hours to 35 to 40 hours after onset of estrus can improve pregnancy rate obtained from 37% to 41%, but still lower than the result of natural mating (82.4%).
The most decisive factor in the implementation of AI in goats is choosing the right time to do the AI, given the signs of estrus in goats is not as clear as other livestock, the time of ovulation is very long and time capacitation of spermatozoa goat relatively faster than other livestock, even so through technology estrus synchronization will be able to overcome some of these problems, so hopefully the success of AI in goats is higher (Budiarsana and Sutama, 2012).

Pregnancy

The result of the acquisition of pregnancy rate in this study was 75% in group I (natural mating) and 100% in group II (AI mating). Based on calculations *Chi-square* indicates that there is no real influence among mounting CIDR with mating method ($P < 0.05$). Pregnancy rate is one of the benchmarks that determine the success of a mating, because closer to the truth and give an exact picture of the level in fertility of a herd.
According Frandson (1993) pregnancy is a period of gestation from fertilization until birth occurred. Every individual has a varied pregnant time, this is caused by several factors: genetic factors, maternal factors, fetal and environment. Normal pregnant time for goat is 149 days (Bearden et al., 2004).
Early and accurate detection of pregnancy is an important factor in cattle reproductive management. Detection of early pregnancy, which will more quickly provide information about the success of mating so that it can immediately be evaluated if failure. Evaluation faster will be able to improve the efficiency of reproduction (Karen et al., 2004). However, these efforts require pregnancy detection methods that have high accuracy, easy to use, inexpensive and harmless to livestock.

Conclusion

Method of natural mating or artificial insemination does not significantly affect pregnancy rate of goats were synchronized estrus using Controlled Internal Drug Release and need to do a similar research that examines up to parturition, the number of litter size until weaning.

Reference

Bearden, H.J., J.W. Fuquay dan S.T. Williard, 2004. Applied Animal Reproduction sixth edition. Prentice Hall, Upper Saddle River. New Jersey. pp: 133-177.

Budiarsana, I. G. M., I. K. Sutama. 2012. Fertility Goat Peranakan Etawah on Natural Mating and Artificial Insemination. Livestock Research Institute Bogor.

Frandson, R. D. 1993. Anatomi dan Fisiologi Ternak. Edisi ke-4. Penerjemah: B. Srigandono dan Koen Praseno. Gadjah Mada University Press. Yogyakarta. Hal: 210-250.

Hafez, E.S.E. 1993. Reproduction in Farm Animals. 6th Ed. Lea and Febiger. Philadelphia.

Karen, A., K. Szabadoz, J. Reiczigel, J.F.Beckers and O. Szenci. 2004. Accuracy of transrectal ultrasonography for determination of pregnancy in sheep : effect of fasting and handling of the animals.

Macmillan K.L. and A.J. Peterson. 1993. A New Intravaginal Progesterone Releasing Aid For Cattle (CIDR-B) For Oestrous Synchronization, Increasing Pregnancy Rates and The Treatment of Post-Partum Anoestrus. J. Anim Reprod Sci. 33: 1-25.

Ngangi, L.R. 2002. Effectiveness of Progesterone implants giving duration and time of intravaginal insemination on reproductive performance of Etawah Grade Goat. Institut Pertanian Bogor.

Romano, JE. 1998. The effect of continuous presence of bucks on hastening the onset of estrus in synchronized does during the breeding season. Small Rumin. Res. 30: 99–103.

Suharto, K., A. Junaidi., A. Kusumawati., D.T. Widayati. 2008. Fertility Comparison Between Ettawa Grade Goat Body Condition Score (BCS) thin versus ideal after estrus synchronization and artificial insemination methode. Veterinary Media. p: 49-53.

PO-01-47

Changes in the morphological status of cumulus cells surrounding canine oocytes and their effects upon in vitro maturation.

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Objective

In many mammalian species, in vitro maturation (IVM) of oocytes consistently achieve 80~90% nuclear maturation in human (Coticchio et al., 2012), mouse (Fulka et al., 1995), bovine (Paramio and Izquierdo, 2016) and pig (Marchal et al., 2002). The IVM technique has brought about various new assisted reproductive technologies (ART), such as in vitro fertilization, intracytoplasmic sperm injection, embryo cryopreservation and production of clones (Smorag et al., 2008).

It has been studied on IVM of canine oocytes about the influence of co-culture with oviductal epithelial cells (Bogliolo et al., 2002), culture medium (Bolamba et al., 2002; Rota and Cabianca, 2004; Songsasen et al., 2002) and supplementation of protein (Lopes et al., 2011), energy substrate (Songsasen et al., 2007) and hormone (Evecen et al., 2011; Kim et al., 2010; Kim et al., 2005; Lee et al., 2007; Vannucchi et al., 2009; Willingham-Rocky et al., 2003). However, the efficiency of in vitro maturation (IVM) for canine oocytes is still very low, compared to that for other species. In many species, cumulus cells (CCs) surrounding oocytes play an important role in oocyte maturation (Anchordoquy et al., 2014; Nandi et al., 2008). Here, we investigated the role of CCs surrounding canine oocytes upon the efficacy of IVM.

Materials & methods

Ovaries were obtained from bitches by ovariohysterectomy and were sliced repeatedly in order to collect immature cumulus-oocyte complexes (COCs).

In experiment 1, oocytes were cultured in the maturation medium (IVMD101; Research Institute for the Functional Peptides) at 38.8℃ in humidified atmosphere of 5% CO_2 in air for 48h. We evaluated the spontaneous removal of CCs surrounding oocytes after 24, 36, 48, 60 and 72 h of IVM. Then, to evaluate the role of CCs during maturation, we evaluated the meiotic status of oocytes with or without CCs at a point 48h after IVM was commenced.

In experiment 2, in order to examine the maturational progress of canine oocytes with CCs, COCs were cultured in the maturation medium (IVMD101) at 38.8℃ in a humidified atmosphere of 5% CO_2 for 0, 12, 24, 36, 48 and 72 h, and meiotic status was determined.

At the end of the maturation period, the CCs were removed by gentle pipetting with a fine-bore glass pipette from oocytes, and the oocytes were transferred to Carnoy's solution, and mounting on a slide with an overlay of Hoechst 33342. Nuclear stage of the oocytes was assessed with a confocal laser scanning microscope. The meiotic status were classified as either, as germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI) stage or metaphase II (MII). Data were compared by Chi-squared test using the statistical analysis software StatView. Differences of $P < 0.05$ were considered significant.

Results & Discussion

In experiment 1, CCs were shed spontaneously from oocytes with culture time and the removal rate peaked at 31.5% following 60 h of IVM (Fig. 1). These results are not inconsistent with previous report, which showed that the cumulus-oocyte communications remained permeable for the first 24 h of maturation in many oocytes, whereas no communication was observed at 48 h of maturation (Luvoni et al., 2001).

In addition, the proportion of oocytes reached MII after 48 h of IVM with CCs was higher than that without CCs (Table1). Accordingly, these results indicate that CCs surrounding oocytes in mature probably play own important

role on IVM until 48h of maturation. However, the reason why CCs were shed spontaneously from oocytes has not been clarified. Further studies will be needed to determine the cause of removing CCs from oocytes.
In the second experiment, the meiotic resumption rates of oocytes with CCs after 48 and 72 h of culture were higher than that after 12 h of culture (Table2). Some reports suggest that culture time for 17-96 h was required for nuclear maturation to MII (Bolamba et al., 1998; De los Reyes et al., 2005; Fujii et al., 2000; Hewitt et al., 1998; Luvoni et al., 2003; Mahi and Yanagimachi, 1976; Nickson et al., 1993; Otoi et al., 2000; Reynaud et al., 2005; Saint-Dizier et al., 2001; Yamada et al., 1992).

Conclusion

In conclusion, these results indicate that CCs appear to play an important role on the maturation of canine oocytes and that the optimal culture period for canine oocytes with CCs may be around 48 h.

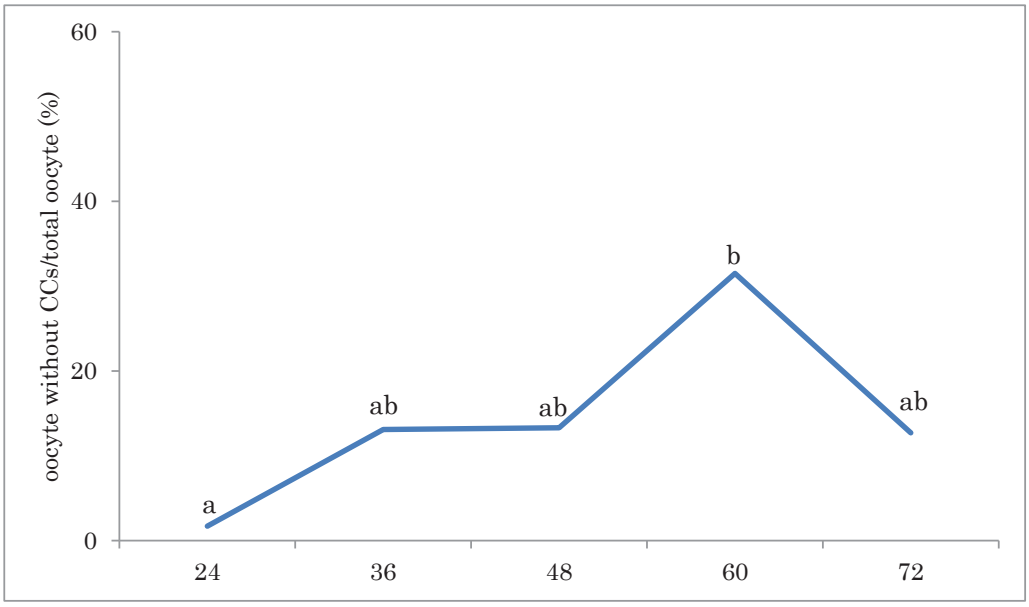

Fig.1. The removal rate of CCs from oocyte in IVM

Different superscripts within the same column indicate a significant difference a vs b $P < 0.05$.

Table1. Effect of CCs status on nuclear maturation of canine oocytes at a point 48h after IVM

	Oocytes (n)	Stage of meiotic progression (%)				Meiotic resumption (%)	Degeneration (%)	Unidentified (%)
		GV	GVBD	MI	MII			
Without CCs	42	10 (24.4)	14 (34.1)	17 (41.5)	0 (0.0)a	31/41 (75.6)	1 (2.4)	0 (0)
With CCs	70	4 (6.2)	26 (40.0)	19 (29.2)	16 (24.6)b	61/65 (93.8)	5 (7.1)	0 (0)

Meiotic resumption of oocytes: total number of oocytes between GVBD, MI and MII.

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase 1; MII, metaphase 2.

Different superscripts within the same column indicate a significant difference a vs b $P < 0.05$.

Table2. The maturational progress of canine oocytes with CCs

Culture Time(h)	Oocytes (n)	Stage of meiotic progression (%)				Meiotic resumption (%)	Degeneration (%)	Unidentified(%)
		GV	GVBD	MI	MII			
0	13	13 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/13 (0.0)	0 (0.0)	0 (0.0)
12	49	11 (26.2)	11 (26.2)	18 (42.9)	2 (4.8)	31/42 (73.8)a	4 (8.2)	3 (7.1)
24	30	4 (15.4)	10 (38.5)	9 (34.6)	3 (11.5)	22/26 (84.6)	3 (10.0)	1 (3.3)
36	42	7 (18.4)	13 (34.2)	16 (42.1)	2 (5.3)	31/38 (81.6)	3 (7.1)	1 (2.4)
48	118	9 (8.6)	35 (33.3)	39 (37.1)	22 (21.0)	96/105 (91.4)b	12 (10.2)	1 (0.8)
72	72	6 (8.8)	36 (52.9)	16 (23.5)	10 (14.7)	62/68 (91.2)b	4 (5.6)	0 (0.0)

Meiotic resumption of oocytes: total number of oocytes between GVBD, MI and MII.

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase 1; MII, metaphase 2.

Different superscripts within the same column indicate a significant difference a vs b $P < 0.05$.

REFERENCES

Bogliolo, L., Zedda, M.T., Ledda, S., Leoni, G., Naitana, S., and Pau, S. (2002). Influence of co-culture with oviductal epithelial cells on in vitro maturation of canine oocytes. Reproduction, nutrition, development *42*, 265-273.

Bolamba, D., Borden-Russ, K.D., and Durrant, B.S. (1998). In vitro maturation of domestic dog oocytes cultured in advanced preantral and early antral follicles. Theriogenology *49*, 933-942.

Bolamba, D., Russ, K.D., Olson, M.A., Sandler, J.L., and Durrant, B.S. (2002). In vitro maturation of bitch oocytes from advanced preantral follicles in synthetic oviduct fluid medium: serum is not essential. Theriogenology *58*, 1689-1703.

Coticchio, G., Dal-Canto, M., Guglielmo, M.C., Mignini-Renzini, M., and Fadini, R. (2012). Human oocyte maturation in vitro. The International journal of developmental biology *56*, 909-918.

De los Reyes, M., de Lange, J., Miranda, P., Palominos, J., and Barros, C. (2005). Effect of human chorionic gonadotrophin supplementation during different culture periods on in vitro maturation of canine oocytes. Theriogenology *64*, 1-11.

Evecen, M., Cirit, U., Demir, K., Hamzaoglu, A.I., Bakirer, G., Pabuccuoglu, S., and Birler, S. (2011). Adding hormones sequentially could be an effective approach for IVM of dog oocytes. Theriogenology *75*, 1647-1651.

Fujii, M., Otoi, T., Murakami, M., Tanaka, M., Une, S., and Suzuki, T. (2000). The Quality and Maturation of Bitch Oocytes Recovered from Ovaries by the Slicing Method. Journal of Veterinary Medical Science *62*, 305-307.

Fulka, J., Jr., Moor, R.M., and Fulka, J. (1995). Mouse oocyte maturation: meiotic checkpoints. Experimental cell research *219*, 414-419.

Hewitt, D.A., Watson, P.F., and England, G.C.W. (1998). Nuclear staining and culture requirements for in vitro maturation of domestic bitch oocytes. Theriogenology *49*, 1083-1101.

Kim, B.S., Lee, S.R., Hyun, B.H., Shin, M.J., Yoo, D.H., Lee, S., Park, Y.S., Ha, J.H., and Ryoo, Z.Y. (2010). Effects of gonadotropins on in vitro maturation and of electrical stimulation on parthenogenesis of canine oocytes. Reproduction in domestic animals = Zuchthygiene *45*, 13-18.

Kim, M.K., Fibrianto, Y.H., Oh, H.J., Jang, G., Kim, H.J., Lee, K.S., Kang, S.K., Lee, B.C., and Hwang, W.S. (2005). Effects of estradiol-17beta and progesterone supplementation on in vitro nuclear maturation of canine oocytes. Theriogenology *63*, 1342-1353.

Lee, H.S., Seo, Y.I., Yin, X.J., Cho, S.G., Lee, S.S., Kim, N.H., Cho, S.K., and Kong, I.K.

(2007). Effect of follicle stimulation hormone and luteinizing hormone on cumulus cell expansion and in vitro nuclear maturation of canine oocytes. Reproduction in domestic animals = Zuchthygiene *42*, 561-565.

Lopes, G., Alves, M.G., Carvalho, R.A., Luvoni, G.C., and Rocha, A. (2011). DNA fragmentation in canine oocytes after in vitro maturation in TCM-199 medium supplemented with different proteins. Theriogenology *76*, 1304-1312.

Luvoni, G.C., Chigioni, S., Allievi, E., and Macis, D. (2003). Meiosis resumption of canine oocytes cultured in the isolated oviduct. Reproduction in domestic animals = Zuchthygiene *38*, 410-414.

Luvoni, G.C., Luciano, A.M., Modina, S., and Gandolfi, F. (2001). Influence of different stages of the oestrous cycle on cumulus-oocyte communications in canine oocytes: effects on the efficiency of in vitro maturation. Journal of reproduction and fertility Supplement *57*, 141-146.

Mahi, C.A., and Yanagimachi, R. (1976). Maturation and sperm penetration of canine ovarian oocytes in vitro. The Journal of experimental zoology *196*, 189-196.

Marchal, R., Vigneron, C., Perreau, C., Bali-Papp, A., and Mermillod, P. (2002). Effect of follicular size on meiotic and developmental competence of porcine oocytes. Theriogenology *57*, 1523-1532.

Nickson, D.A., Boyd, J.S., Eckersall, P.D., Ferguson, J.M., Harvey, M.J., and Renton, J.P. (1993). Molecular biological methods for monitoring oocyte maturation and in vitro fertilization in bitches. Journal of reproduction and fertility Supplement *47*, 231-240.

Otoi, T., Murakami, M., Fujii, M., Tanaka, M., Ooka, A., Une, S., and Suzuki, T. (2000). Development of canine oocytes matured and fertilised in vitro. Veterinary Record *146*, 52-53.

Paramio, M.T., and Izquierdo, D. (2016). Recent advances in in vitro embryo production in small ruminants. Theriogenology *86*, 152-159.

Reynaud, K., Fontbonne, A., Marseloo, N., Thoumire, S., Chebrout, M., de Lesegno, C.V., and Chastant-Maillard, S. (2005). In vivo meiotic resumption, fertilization and early embryonic development in the bitch. Reproduction (Cambridge, England) *130*, 193-201.

Rota, A., and Cabianca, G. (2004). In vitro maturation rates of canine oocytes from anoestrous bitches in simple media. Reproduction, nutrition, development *44*, 105-109.

Saint-Dizier, M., Renard, J.P., and Chastant-Maillard, S. (2001). Induction of final maturation by sperm penetration in canine oocytes. Reproduction (Cambridge, England) *121*, 97-105.

Smorag, Z., Katska-Ksiazkiewicz, L., Skrzyszowska, M., Jura, J., Gajda, B., and Bochenek, M. (2008). Animal reproduction biotechnology in Poland. The International journal of developmental biology *52*, 151-155.

Songsasen, N., Spindler, R.E., and Wildt, D.E. (2007). Requirement for, and patterns of, pyruvate and glutamine metabolism in the domestic dog oocyte in vitro. Molecular reproduction and development *74*, 870-877.

Songsasen, N., Yu, I., and Leibo, S.P. (2002). Nuclear maturation of canine oocytes cultured in protein-free media. Molecular reproduction and development *62*, 407-415.

Vannucchi, C.I., Faustino, M., Marques, M.G., Nichi, M., Assumpcao, M.E., and Visintin, J.A. (2009). Effects of gonadotropin-exposed medium with high concentrations of progesterone and estradiol-17beta on in vitro maturation of canine oocytes. In vitro cellular & developmental biology Animal *45*, 328-333.

Willingham-Rocky, L.A., Hinrichs, K., Westhusin, M.E., and Kraemer, D.C. (2003). Effects of stage of oestrous cycle and progesterone supplementation during culture on maturation of canine oocytes in vitro. Reproduction (Cambridge, England) *126*, 501-508.

Yamada, S., Shimazu, Y., Kawaji, H., Nakazawa, M., Naito, K., and Toyoda, Y. (1992). Maturation, fertilization, and development of dog oocytes in vitro. Biology of reproduction *46*, 853-858.

PO-01-54

Effects of Dietary Energy and Crude Protein Levels on Growth Performance, Blood Profiles and Pork quality in Growing-finishing Pigs

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INTRODUCTION

Feed accounts for about two thirds of the cost of producing market-weight swine (Noblet and Perez, 1993). Using suitable nutrition concentration in feed is one of the ways to reduce production cost. Growth models of pigs are based on the assessment of whole body protein and lipid deposition (Nieto et al., 2012). In growing period, pig shows the highest weight gain in lifetime for extensive muscle development. Fat deposition is much greater than protein deposition in finishing period (Whittemore, 1993). Therefore energy and crude protein contents in growing-finishing pig diets are important. NRC 1998 showed higher CP requirement and lower energy and amino acid in growing-finishing pig diet than NRC 2012 (The value of CP is calculated from N by multiplying by 6.25; Swine Nutrition 2nd Edition, 2001). Also, growing-finishing pig has a long feeding period and high feed intake. Using suitable nutrition concentration in feed can improve growth performance and reduce cost of production. Therefore, the objective of this study was to evaluate the dietary energy and crude protein levels on growth performance, blood profiles, pork quality and economic analysis in growing-finishing pigs.

MATERIALS AND METHODS

For the feeding trial, a total of 180 growing pigs ([Yorkshire × Landrace] × Duroc), averaging 30.96 ± 3.07 kg body weight, were allotted to 2 × 3 factorial arrangement in a randomized complete block design (RCBD) by body weight and sex. The first factor was two levels of dietary energy density (3,200 kcal of ME/kg or 3,300 kcal of ME/kg), and the second factor was three levels of protein concentration (NRC1998 req., NRC1998 req.-1% or NRC1998 req.-2%). Body weight and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio). Blood samples were taken from the jugular vein of randomly selected six pigs in each treatment for measuring blood urea nitrogen (BUN). After feeding trial, the samples of longissimus muscle (10th rib) were collected from 24 pigs (4 pigs per each treatment) to determine pH, meat color, water holding capacity, cooking loss, shear force, proximate analysis and TBARS analysis. Collected data was analyzed using the PROC GLM procedures of SAS.

RESULTS AND DISCUSSION

During the whole experimental period, ADG and G:F ratio in early growing phase and G:F ratio in growing period were decreased as dietary CP level decreased (P=0.04, P=0.03, P=0.02, respectively, Table 1). During the whole experimental period, G:F ratio was improved with dietary energy levels increased (P=0.01, Table 1). According to Kil et al. (2011), decreased 500 kcal/kg in growing-finishing pig diet, G:F ratio was decreased. In blood profiles, blood urea nitrogen (BUN) concentration was decreased as dietary CP level decreased in early growing and early finishing phase (P=0.01 and P<0.01, respectively) and increased as dietary energy level increased in late growing phase (P<0.01). It indicated that efficiency of crude protein utilization can be improved by reduced dietary crude protein level or increased energy level. In addition, there was no significant difference in water holding capacity, shear force, cooking loss and TBARS of longissimus muscle (LM).

Table 1. Effect of energy and CP level on growth performance in growing-finishing pig diet

Item	ME 3,200 kcal/kg			ME 3,300 kcal/kg			SEM	P- value		
	NRC98	CP-1%	CP-2%	NRC98	CP-1%	CP-2%		ME	CP	M×C
Body weight, (kg)										
Initial	30.97	30.95	30.97	30.95	30.95	30.95	0.854	-	-	-
3 wk	42.86	40.59	41.17	43.48	42.73	40.37	1.012	0.77	0.66	0.86
6 wk	63.03	62.26	62.64	66.92	63.66	59.77	1.369	0.78	0.57	0.63
9 wk	80.78	83.03	82.56	87.06	84.65	78.87	1.553	0.67	0.66	0.47
12 wk	100.19	104.10	102.78	108.17	106.05	99.62	1.602	0.50	0.61	0.40
ADG, (g)										
0-3 wk	566.51	459.05	485.87	596.83	561.11	448.41	19.403	0.38	0.04	0.29
4-6 wk	960.14	1,031.74	1,022.61	1,116.38	996.52	924.06	32.240	0.91	0.73	0.28
7-9 wk	845.41	989.21	948.34	959.02	999.67	909.59	30.923	0.66	0.50	0.62
10-12 wk	924.13	1,003.17	962.86	1,005.24	1,019.05	987.94	16.944	0.26	0.53	0.71
0-6 wk	763.33	745.39	754.25	856.60	778.82	686.24	18.670	0.59	0.14	0.19
7-12 wk	884.77	996.19	955.60	982.13	1,009.36	948.76	16.099	0.27	0.19	0.37
0-12 wk	824.06	870.82	854.93	919.37	893.99	817.52	12.212	0.24	0.23	0.07
ADFI, (kg)										
0-3 wk	1.46	1.37	1.43	1.39	1.45	1.34	0.040	0.75	0.93	0.66
4-6 wk	2.28	2.31	2.49	2.35	2.38	2.17	0.046	0.51	0.97	0.15
7-9 wk	2.67	2.77	3.03	2.79	2.80	2.62	0.060	0.46	0.81	0.19
10-12 wk	3.52	3.58	3.75	3.51	3.66	3.21	0.073	0.29	0.70	0.18
0-6 wk	1.87	1.84	1.96	1.87	1.91	1.75	0.039	0.60	0.98	0.35
7-12 wk	3.10	3.18	3.39	3.15	3.23	2.91	0.064	0.33	0.88	0.17
0-12 wk	2.94	2.51	2.68	2.51	2.57	2.33	0.050	0.42	0.94	0.22
G:F ratio										
0-3 wk	0.39	0.34	0.34	0.43	0.39	0.34	0.012	0.18	0.03	0.58
4-6 wk	0.43	0.45	0.41	0.48	0.42	0.42	0.011	0.56	0.42	0.39
7-9 wk	0.31	0.36	0.31	0.35	0.36	0.35	0.009	0.31	0.38	0.67
10-12 wk	0.26	0.28	0.26	0.29	0.28	0.33	0.010	0.12	0.80	0.30
0-6 wk	0.41	0.39	0.38	0.45	0.40	0.38	0.008	0.18	0.02	0.52
7-12 wk	0.29	0.31	0.28	0.31	0.31	0.34	0.007	0.09	0.71	0.33
0-12 wk	0.33	0.35	0.32	0.37	0.35	0.36	0.005	0.01	0.43	0.13

Table 2. Effect of energy and CP level on BUN concentration in growing-finishing pig diet

Item	ME 3,200 kcal/kg			ME 3,300 kcal/kg			SEM	P-value		
	NRC98	CP-1%	CP-2%	NRC98	CP-1%	CP-2%		ME	CP	M×C
Blood urea nitrogen (BUN), mg/dL										
3 wk	12.33	10.75	9.13	10.95	10.48	8.52	0.396	0.31	0.01	0.81
6 wk	12.93	11.68	11.05	9.42	9.22	10.30	0.403	<.01	0.71	0.31
9 wk	12.65	12.35	8.43	16.22	10.68	11.10	0.584	0.11	<.01	0.06
12 wk	13.78	11.28	10.72	12.35	13.48	9.68	0.525	0.93	0.07	0.28

References

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2011. Net energy of soybean oil and choice white grease in diets fed to growing and finishing pigs. J. Anim. Sci. 89:448 - 459.

Nieto. R., L. Lara, R. Barea, R. García-Valverde, M. A. Aguinaga, J. A. Conde-Aguilera, and J. F. Aguilera. 2012. Response analysis of the Iberian pig growing from birth to 150 kg body weight to changes in protein and energy supply. J. Anim. Sci. 90:3809 - 3820.

Noblet. J and J. M. Perez. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci. vol. 71, no. 12, pp. 3389 - 3398.

NRC. 1998. Nutrient requirement of pigs. National Research Council, Academy Press.

NRC. 2012. Nutrient requirement of pigs. National Research Council, Academy Press.

Whittemore, C. T. 1993. The science and practice of pig production. Longman Ltd, London.

PO-01-55

Temporal changes of body conformation in Holstein-Friesian heifers

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Abstract

Modern animal breeding program enabled to modify not only production characteristics but also body conformation of domestic animals through intensive genetic selection. In fact, Holstein-Friesian, a typical dairy cow, average milk production per cow in Japan has increased from approximately 5,000 Kg/head/year in 1980th to 9,000 Kg/head/year in 2010th, whereas the proportion of cows with low udder has been decreased during the same period of time. However, objective data on the body conformations of Holstein-Friesian heifers have rarely been measured (Heinrichs and Losinge, 1998).

In the present study, therefore, body conformation [(body weight (BW), body height (BH), hip height (HH), chest girth (CG), chest depth (CD), body length (BL), hip width (HW), of 30 Holstein-Friesian heifers at the Agricultural and Forestry Research Center, University of Tsukuba between May 2005 through December 2014, was monitored monthly from 0 to 24 months old. Results from the present study revealed a continuous temporal increase of the frame size of heifers, particularly BH, HH, BL were observed during the observation period. These temporal increase of the frame size Holstein-Friesian heifers could influence dairy management including animal handling and designing of the dairy barn.

Introduction

An intensive genetic selection program in dairy cattle enabled to improve the milk production significantly for the past several decades. In fact, Holstein-Friesian, a typical dairy cow, average milk production per registered cow in Japan has increased from approximately 5,000 Kg/head/year in 1980th to 9,000 Kg/head/year in 2010th.

During the same period, it has been suggested that the body conformation of dairy cattle has been changed particularly after 2000. However, long-term monitoring of the body conformation of dairy cattle has been rarely monitored.

In the present study, therefore, body conformation of 30 Holstein-Friesian heifers born at the Agricultural and Forestry Research Center, University of Tsukuba, monitored monthly until reached 24 months after birth.

Materials and Methods

A total of 30 Holstein-Friesian heifers born and raised at the Agricultural and Forestry Research Center, University of Tsukuba, between May 2005 and October 2013, were used in the present study.

Three to five female calves were born during the testing period, except 2 and 1 cow were born in 2012 and 2013, respectively. A serial identification numbers (ID), sorted by the date of birth, is assigned to each calves. All animals are reared in a free barn and feed was prepared according to Japan feeding standards. Body conformation of cows, including body weight (BW), body height (BH), hip height (HH), chest girth (CG), chest depth (CD), body length (BL), hip width (HW) were measured monthly from 0 to 24 months old. The youngest calf reached 24 months of age at Oct 2015.

For each monthly data, regressing coefficient (RegC) and coefficient of determination (R^2) between calf ID and each of the previously listed items were calculated.

Results and Discussion

The compiled monthly RegC between calf ID and BW during the past 10-years of the observation period is shown in Fig.1.

Even through there was a fluctuation among months, the RegC between calf ID and BW tended to increase for the first 11 months after birth to reach a plateau. RegC remained at high value until 14 months old, but increment of RegC increase has started to diminish afterward. The second order regression for the monthly RegC during the observation period was calculated to be $y = -0.0009x2 + 0.0301x - 0.1314$, $R^2 = 0.8302$. This result strongly

indicates that the change of growth curve, in terms of BW, during the observation period has been quadratic.
It has to be noted, however, that the negative RegC for BW were observed during first 5 months after birth. This results indicate that birth weight has becoming smaller, and initial growth of calves had been slower during first 5 months after birth. This result may reflect that breeding program focused on calving ease limited the fetal growth *in utero* and also initial calf growth. However, a low R^2 values during the first 7 months after birth, indicates that growth rate of new born claves differ considerably.
A sharp increase of RegC, together with a sharp increase of R^2 values starting from 8 months old until 11 months old indicates that body growth has been promoted most likely associated with sexual maturation. RegC between calf ID and BW turned into positive at 6 months of age, and a sharp increase of RegC was observed between 9 and 11 months old to reach plateau until 14 months old. The increment of RegC increase has started to decrease after 15 months old. Considering a sharp decrease of R^2 values observed between 14 and 17 months old, could be attributed to the pregnancy, since the artificial insemination start in this period. The reasons for the second increase of R^2 values between 17 and 22 months old need to be elucidated in the future study. Almost the same tendency were observed for BH (Fig. 2) and GL (Fig. 3), which indicate that the increase of the body weight can be attributed to the increase of the body height and body length.
Studies on optimum body size of Holstein replacement heifers (Hoffman, 1997) and relationships between body measurements and milk productivity (Sieber *et al*. 1988) have been studied. With the increase of milk production, optimal body size for dairy heifers needs to be revised periodically. The increase of the growth rate most likely influences the digestive ability of feed and also metabolic pathways to develop body. In this respect, long-term modification of metabolism needs to be monitored in order to optimize the dairy calf management program. Also, influences of initial body growth on the life-long production performance needs to be evaluated in the future study. From the practical point of view, development of a reliable means of estimating body conformation is necessary for the proper management of dairy herd (Heinrichs *et al*. 1992, Dingwell *et al*, 2006).
In conclusion, the present results indicates that the growth of the new born calves have been accelerated during 10 years of the observation period.

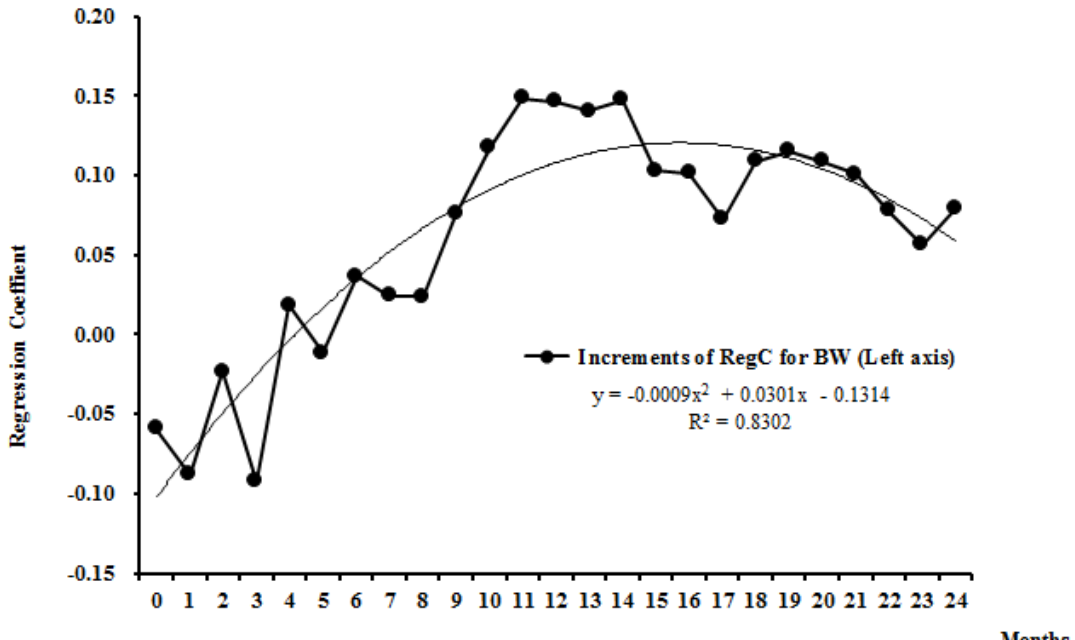

Fig. 1 Monthly Growth Rates of Body Weight

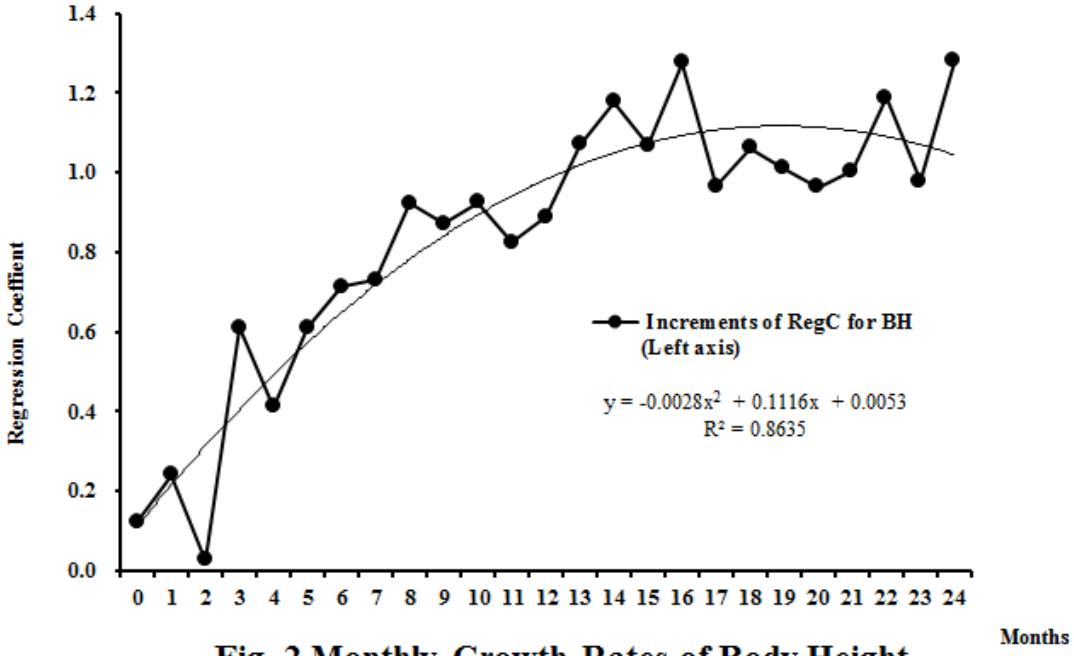

Fig. 2 Monthly Growth Rates of Body Height

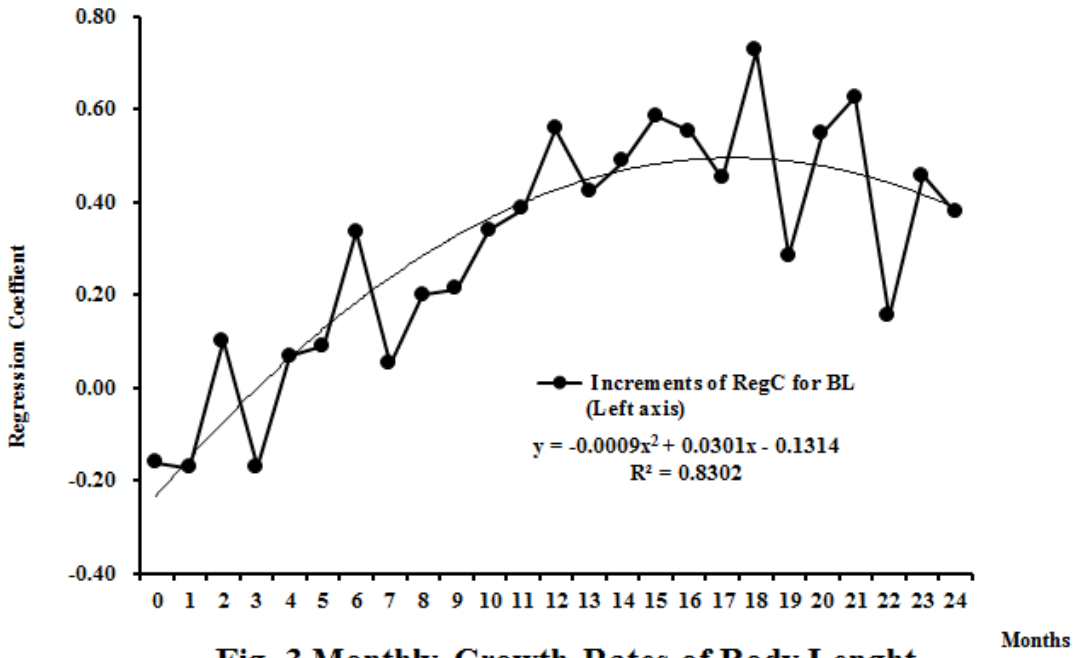

Fig. 3 Monthly Growth Rates of Body Lenght

References

Dingwell RT, Wallace MM, McLaren CJ, Leslie CF and Leslie KE.
An Evaluation of Two Indirect Methods of Estimating Body Weight in Holstein Calves and Heifers
J. Dairy Sci. 89:3992–3998, 2006.

Heinrichs AJ, Rogers GW and Cooper JB.
Predicting Body Weight and Wither Height in Holstein Heifers Using Body Measurements. J. Dairy Sci. 75:3576-3581, 1992.

Heinrichs AJ and Losinger WC
Growth of Holstein Dairy Heifers in the United States
Journal of Animal Science 76:1254–12, 1998.

Hoffman PC.
Optimum body size of Holstein replacement heifers.
Journal of Animal Science 75:836-845, 1997.

Sieber M, Freeman AE and Kelley H,
Relationships between Body Measurements, Body Weight, and Productivity in Holstein Dairy Cows. Journal of Dairy Science 71:3437-3445, 1988.

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Effect of Welfare Housing Types (Floor vs. Aviary) on Egg Production Performance and Egg Lutein in Brown Laying Hens

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ABSTRACT

Employment of the animal welfare housing system has been increased, especially since European Union banned the conventional cage as of 2012. Both floor and aviary systems have been generally accepted as the welfare bestowing system. However, not much information is available how those two systems affect on the laying hen performance and egg quality. In this study, two welfare housings (Floor vs. Aviary) were compared in terms of egg production and egg lutein concentration. In addition, we also compared two laying stages (early vs. mid stage). The aim of this study was to evaluate the effect of welfare housing types on egg production performance and egg lutein. Two housing types were designated as two treatments with 4 replications per treatment. Two feeding trials (early laying stage and mid laying stage) executed with 800 brown laying hens. Hen day egg production (HDEP) was higher ($P<0.05$) in floor group than that in aviary group. In both stage, however, the HDEP was not different between floor and aviary. Regardless of housing type, egg lutein concentration was increased ($P<0.05$) with the increasing duration of feeding. This study found that the floor system could be more suitable for early stage egg production than aviary housing system. However, once the hens accustomed to the housing, the egg production were not affected by the type of housing.

INTRODUCTION

The housing system is an external factor that influences both the performance of hens and the egg quality characteristics. (Englmaierová et al., 2014). Both floor and aviary systems have been generally accepted as the welfare housing system. This means that the effect of functional feed supplementations in the welfare housing system could be different from the effect in the conventional cage system.

Lutein is a carotenoid pigment that is found in green and yellow plants. Also, lutein plays an important role in eye by preventing age-related macular degeneration. Marigold flower contains high lutein and its extract is mostly used for functional feed supplement. There was not enough information what could be the mode of action for transferring dietary lutein to egg lutein. Therefore, this study was to evaluate the effect of welfare housing types on egg production performance and egg lutein.

MATERIAL AND METHODS

The experiment was approved by Institutional Animal Care and Use Committee (IACUC), Kangwon National University (KNU), Republic of Korea. This experiment was conducted in the research farm and analysis laboratory at KNU.

Totally 800 blown laying hens (Lohman brown lite) were used in two feeding trials (early laying stage and mid laying stage). Floor and aviary systems were chosen as the welfare housing system. Those housing systems provided birds with slat area, perches, nesting box, feeder and water-supply-line. 14L-10D cycle was used in this experiment. Commercial feed was used as basal diets with dosage marigold extract (MG) 0%, 0.3%, 0.5% and 1% of feed. Marigold extract contained 10,000 ppm lutein. Diets and water provide with *ad libitum*. All eggs were collected, weighed and recorded daily. HDEP, egg weight and egg mass were calculated and used the average value of a week. Haugh unit was calculated by using tripod micrometer to measure albumen height and followed the equation proposed by Haugh (Stadelman, 1995). Yolk color was measured by using Roche fan. Shell thickness was measured using thickness meter. Shell hardness was measured using hardness tester. Lutein from the egg yolk was analyzed using HPLC. Statistical analysis was conducted using T-test and general linear model procedures of IBM SPSS statistics software v. 22.

RESULTS AND DISCUSSION

HDEP was similar between floor and aviary housing systems (Table 1). However, HDEP tended to be higher (P=0.066) in floor than aviary. Egg weight and egg mass were not significantly differed among housing types. Feed intake and FCR in aviary were higher (P<0.05) than that in floor. Laying stage did not affected on HDEP and feed intake, but feed intake had tendency to be increased (P=0.074) with increasing age of the birds. Egg weight at mid laying stage was higher (P<0.05) than that at early laying stage. Egg mass was increased (P<0.05) as laying stage increased. FCR was improve (P<0.05) at mid laying stage than early laying stage.

Haugh unit, shell strength and shell thickness were not affected by housing systems (Table 2). However, significant differences between early and mid stage in haugh unit and shell thickness were observed. Haugh unit at early laying stage was higher (P<0.05) than at mid laying stage. Shell thickness was the thickest (P<0.05) at mid laying stage than early laying stage.

Floor and aviary housing systems did not affected on both yolk color score and lutein concentration of egg yolk (Table 3). Also yolk color score was not differed by laying stages. However, lutein concentration decreased (P<0.05) as laying stages increased. When lutein supplement levels were increased, yolk color score and lutein concentration of egg yolk were increased (P<0.05).

CONCLUSION AND IMPLICATION

This study showed that hen day egg production, egg weight and egg mass were not affected by housing types. However, feed intake in floor was lower than that in aviary and FCR was better in floor than aviary. This result recommended that nutrients recommendation should be differentiated between floor and aviary. Egg quality parameters were not differed between floor and aviary. Lutein concentration of egg yolk was highly affected by laying stages which means that marigold extract supplementation would be optimized according the age of the bird in the welfare housing system.

Keywords: Floor, Aviary, Egg, Lutein

Table 1. Effect of housing types and laying stages on egg performance parameters

Parameters	Floor		Aviary		SEM	P-value		
	Early	Mid	Early	Mid		Floor vs. Aviary	Early vs. Mid	Housing types x Laying stages
HDEP (%)	88.23	89.81	86.31	86.76	0.658	0.066	0.449	0.672
Egg Weight (g)	58.15	64.71	58.17	65.10	0.407	0.561	0.000	0.601
Egg Mass (g/day/bird)	50.83	58.14	50.13	56.48	0.365	0.009	0.227	0.001
Feed Intake (g/day/bird)	116.34	118.16	119.33	119.38	0.384	0.001	0.074	0.088
FCR	2.29	2.06	2.40	2.15	0.026	0.035	0.000	0.840

Table 2. Effect of housing types and laying stages on egg quality

Parameters	Floor		Aviary		SEM	P-value		
	Early	Mid	Early	Mid		Floor *vs* Aviary	Early *vs* Mid	Housing types x Laying stages
Haugh unit	97.43	86.45	97.88	87.77	1.215	0.129	0.000	0.448
Shell strength (kg/cm^3)	4.84	4.69	4.71	5.09	0.089	0.458	0.544	0.168
Shell thickness (mm)	0.37	0.39	0.37	0.40	0.003	0.730	0.000	0.530
Yolk color	10.08	9.95	9.84	10.06	0.056	0.564	0.702	0.156

Table3. Effect of housing types, laying stages and MG supplement levels on yolk color and lutein concentration of egg yolk

Housing types	Laying stages	MG supplement levels	Yolk color	Lutein concentration (μg/g yolk)
Floor	Early	0%	5.89	10.71
		0.3%	9.7	33.71
		0.5%	11.08	41.68
		1%	12.7	47.09
		Average	9.84	33.30
	Mid	0%	7.14	12.22
		0.3%	9.81	18.44
		0.5%	11.36	21.51
		1%	11.5	24.17
		Average	9.95	19.09
Aviary	Early	0%	5.85	10.83
		0.3%	9.5	34.39
		0.5%	11	43.72
		1%	12.6	48.68
		Average	9.74	34.41
	Mid	0%	7.19	12.57
		0.3%	9.81	17.43
		0.5%	11.53	21.78
		1%	11.69	24.94
		Average	10.06	19.18
SEM			0.26	0.925

P-Value	Floor *vs* aviary	Early *vs* mid	MG supplement levels	Housing types x Laying stages	Housing types x MG supplement levels	Housing types x laying stages x MG supplement levels
Yolk Color	0.994	0.109	0.000	0.421	0.974	0.994
Lutein concentration (μg/g yolk)	0.295	0.000	0.000	0.383	0.794	0.919

REFERENCES

Stadelman, W. J., Newkirk, D., & Newby, L. (1995).Egg science and technology. CRC Press.

Englmaierova, M., T?mová, E., Charvatova, V., & Sk?ivan, M. (2014). Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination.Yeast,15, 0.

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Effect of Feed Ingredients and Feeding Method on Growth Performance, Nutrient Digestibility, Fecal Microbial Populations in Weanling Pigs

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Objective

Feeding is the most important aspect of swine production that contributes for their performance and health status. The results of previous studies have indicated that pigs subjected to early dietary AA restrictions or low quality protein sources can exhibit compensatory growth (Chiba et al., 2002; Fabian et al., 2004), utilize nutrients more efficiently (Chiba et al., 2002; Fabian et al., 2004), have better carcass traits and reduce N excretion (Fabian et al., 2004).

In the normal course, pig receives dry feed and water is provided separately. Previous studies reported that wet-dry feeder can increase the ADG and ADFI of finishing pigs compared with a conventional dry feeder (Brumm et al., 2000; Gonyou and Lou, 2000). Reduction in the waste water and manure production issue can also be answered using such type of feeding that can help in lowering the environmental impact of intensive pig farming (Shaw et al., 2006).

The present experiment was designed to study the effect of wet feeding and liquid feeding with different feed quality on the performance, digestibility of nutrients and intestine healthy in pigs.

Methodology

A total of 200 weaned piglets (Landrace × Yorkshire × Duroc; initial body weight (BW): 8.12 ± 0.85 kg; 25 ± 2 days of age) of mixed sex were randomly allotted to 4 treatments on the basis of BW and sex. There were five replicates pens in each treatment with 10 pigs per pen. Dietary treatments included 2 levels of feed cost (high and low) and 2 types of feeding (liquid and wet) in a factorial arrangement experiment. Treatment diets were fed in a meal form in 4 phases (d 0 to 7, phase I; d 8 to 21, phase II, d 22 to 35, phase III and growing). All diets (Table 1) met or exceeded the nutrient requirements as suggested by NRC (1998).

The project underwent proper ethical standards and the experiments were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. These experiments were conducted at the facility of Kangwon National University farm and the piglets were housed in partially slotted and concrete floor pens with a pen size of 1.90 m × 3.0 m. All pens were equipped with a self-feeder and nipple drinker to allow ad libitum access to feed and water.

Individual weanling piglets weight and feed disappearance from each pen were recorded at the beginning of the experiment and at the end of every phase to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F). To evaluate the effects of dietary treatments on the apparent total tract digestibility (ATTD) of nutrients, 0.25% chromic oxide (an inert indigestible indicator) was added to all four phases (Phase I, 0 to 7 days; phase II, 8 to 21 days, phase III, 22 to 35 days and grower phase) diets of each experiments. Pigs were fed diets mixed with chromic oxide from d 0 to 7, 14 to 21, 28 to 35 and 55 to 61 days and fecal grab samples were collected from each pen on the last 3 d of each experiment to determine the ATTD of dry matter (DM), gross energy (GE) and crude protein (CP). The fecal samples were pooled within pen and dried in a forced air oven at 60 °C for 72 h, and ground in a Wiley mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) using a 1-mm screen and used for chemical analysis.

To study the effects of dietary treatments on small intestinal morphology and microbiota of ileal and cecal digesta, representative piglets from each group (2 per pen) reflecting the average BW of the pen were selected and sacrificed by electrocution at d 35 of each experiment. The digesta from the ileum and caecum were collected in sterile plastic bottles for microbial analysis. The samples collected for microbial analysis were immediately placed on ice until analyses were conducted. The samples of the intestinal segment from the region of duodenum, jejunum and ileum were collected after removing the content and flushing with physiological saline. The samples were then submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2%

paraformaldehyde and 1.5% acrolein and then brought to the laboratory to study the morphological changes.
Experimental diets and excreta samples were analyzed in triplicate for DM (Method 930.15) and CP (Method 990.03) using AOAC (2007) methods. Gross energy of diets and feces were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL), and chromium concentration was determined with an automated spectrophotometer (Jasco V-650; Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979).
The microbiological assay of ileum and cecum digesta was carried out by culturing in different media as suggested by Choi et al. (2011).
One gram of the composite cecum or ileum sample was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in the samples were then conducted by plating serial 10-fold dilutions. For the determination of *Lactobacillus* spp. (using MRS agar + 0.200 g/l NaN3 + 0.500 g/l l-cystine hydrochloride monohydrate), *Clostridium* spp. (TSC agar) and coliforms (violet red bile agar) were used.
Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Yoon et al., 2012). A total of ten intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip of the villi to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi. All morphological measurements (villus height and crypt depth) were made in 10-μm increments by using an image processing and analysis system (Optimus version 6.5 software, Media Cybergenetics, North Reading, MA).
Data generated in the experiment was analyzed as a 2 × 2 factorial arrangement in a completely randomized design. Pens were considered the experimental unit for growth performance, and piglets were experimental units for measuring the digestibility of nutrients and all intestinal sampling. The main effects of feed form and feed cost, and their interaction were determined by mixed procedure of SAS statistical program (SAS Inst., Inc., Cary, NC). P-values ≤0.05 were considered statistically significant.

Results

Growth performances of weanling pigs are shown in Table 2. There was no feed ingredient × feeding method interaction for any of the measured variables. There were no effects of feed ingredient and feeding methods on average daily gain, average daily feed intake and feed conversion ratio of weanling pigs ($p>0.05$) during the first 7 days. Feed ingredient and feeding method had significant effects on average daily feed intake ($p<0.05$) on day 21and comparatively greater feed consumed on liquid feeding method. Average daily gain and feed conversion ratio were not affected ($p>0.05$) hence, average daily gain tended to be higher on liquid feeding ($p=0.067$) on day 21. Higher feed consumed ($p<0.05$) on the liquid feeding method on day 35 and no effects on average daily gain and feed conversion ratio ($p>0.05$). The overall performances showed that there were greater average daily gain and fed intake on liquid feeding methods than dry feeding ($p<0.05$). Feeding method had significant effects ($p<0.05$) on feed conversion ratio and it was lower in liquid feeding.
There was significant effect ($p<0.05$) on final body weight in grower phase (36-61). Average daily gain, average daily feed intake and feed conversion ratio of growing pigs were not affected ($p>0.05$) by of feed ingredient or feeding methods.
Apparent total tract digestibility (%) of weanling pigs are shown in Table 3. There was no feed ingredient × feeding method interaction for any of the measured variables. Dry matter, gross energy and crude protein digestibility (day 0-7) in weanling pigs were greater in high and low cost liquid feeding ($p<0.05$) with the exception of gross energy digestibility for feed ingredient ($p=0.076$) and dry matter digestibility for feeding method ($p=0.055$). Feed ingredient and feeding method had significant effects on crude protein digestibility ($p<0.05$). However, dry matter ($p=0.090$) and gross energy ($p=0.064$) digestibility tended to be higher in liquid feeding (day 8-21). Feeding method had significant effect on dry matter, gross energy and crude protein digestibility of weanling pigs ($p<0.05$) on day 22-35.
Bacterial populations in feces of weanling pigs are shown in Table 4. There was no feed ingredient × feeding method interaction for any of the measured variables. There were no significant effects ($p>0.05$) on total anaerobic bacteria, *Lactobacillus spp*., *Clostridium spp*., *E. Coli* on day 0-7 and day 8-21. Total anaerobic bacterial populations were significantly higher ($p<0.05$) in high and low cost liquid feeding on day 22-35 and no effects on *Lactobacillus spp*., *Clostridium spp*., *E. Coli.* However *Lactobacillus spp*. tended to be higher on liquid feeding method ($p=0.068$).

Small intestinal morphology in weanling pigs is shown in Table 5. There were no significant effects and interaction (p>0.05) between feed ingredient and feeding method for any of the measured variables on small intestinal morphology in weanling pigs.

Conclusion

In conclusion, the results obtained in the present study indicate that the high cost diet and liquid feeding are shown to influence the performance of piglets in the first phases; however, the final weight was similar among the groups. there were no interactive effects between bacteriophages and probiotics.

Table 1. Ingredient and chemical composition of experimental diets.

Items	0-7 day		7-21 day		21-35 day		Growing
	40% whey	14% whey	20% whey	-	Fishmeal: HP300 3:05	-	
Ingredients, %							
Corn	19.44	46.35	42.3	62.69	61.72	63.94	51.31
Wheat	-	-	-	-	-	-	10
Fish meal, 60%	5	5	5	5	3	-	-
Whey powder	40	14	20	-	-	-	-
HP300	-	10	-	10	5	-	-
SBM, dehulled	28	17.85	27.1	17.4	24.55	29.78	28.45
Soy oil	4.3	3.4	2.65	1.85	2.4	-	
Animal fat	-	-	-	-	-	2.8	4.05
Molasses	-	-	-	-	-	-	3
$_{L}$-lysine, 78%	0.26	0.41	0.32	0.41	0.3	0.14	0.17
$_{DL}$-Methionine, 98%	0.2	0.19	0.16	0.15	0.1	0.04	0.08
MCP	1	1	0.77	0.8	0.95	-	-
MDCP	-	-	-	-	-	1	0.93
Limestone	0.5	0.5	0.4	0.4	0.38	1	1.11
Salt	-	-	-	-	0.3	0.3	0.3
Mineral premix[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.2
Vitamin premix[2]	0.3	0.3	0.3	0.3	0.3	0.3	0.3
ZnO	0.3	0.3	0.3	0.3	0.3	-	-
Choline 50%	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Organic acid	0.3	0.3	0.3	0.3	0.3	0.3	-
Phytase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100	100
Calculated composition, %							
ME, kcal/kg	3,400	3,400	3,350	3,350	3,350	3,300	3,300
CP	22.6	22.6	21.9	21.9	21	18	18
Ca	0.81	0.81	0.75	0.75	0.71	0.66	0.66
Av. P	0.41	0.41	0.36	0.36	0.33	0.31	0.31
SID Lys	1.51	1.51	1.41	1.41	1.26	0.98	0.98
SID Met + Cys	0.83	0.83	0.78	0.78	0.7	0.55	0.55
Lactose	26.95	9.52	13.53	-	-	-	-

[1]Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

[2]Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin D_3, 40 IU vitamin E, 5.0 mg vitamin K_3, 5.0 mg vitamin B_1, 20 mg vitamin B_2, 4 mg vitamin B_6, 0.08 mg vitamin B_{12}, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid, 12 mg antioxidant.

Table 2. Effect of feed ingredient and feeding method on growth performance of weanling and growing pigs[1]

Feed ingredient	H		L		SEM[2]	P-value[3]		
Feeding method	HD	HL	LD	LL		FI	FM	FI×FM
D 7[4]								
ADG (g)	247	258	237	245	5.76	0.344	0.450	0.862
ADFI (g)	391	399	383	391	4.74	0.445	0.418	0.990
F:G	1.59	1.57	1.61	1.60	0.02	0.609	0.749	0.949
D 21[5]								
ADG (g)	429	455	413	435	6.55	0.167	0.067	0.861
ADFI (g)	694	709	684	697	2.68	0.013	0.003	0.865
F:G	1.63	1.57	1.66	1.61	0.01	0.386	0.238	0.913
D 35[6]								
ADG (g)	563	584	553	570	6.09	0.328	0.133	0.840
ADFI (g)	1,041	1,053	1,032	1,046	3.26	0.366	0.048	0.818
F:G	1.85	1.80	1.87	1.84	0.01	0.602	0.415	0.940
Overall								
ADG (g)	455	490	433	467	6.58	0.040	0.003	0.933
ADFI (g)	772	784	763	775	2.50	0.029	0.006	0.947
F:G	1.70	1.60	1.76	1.67	0.01	0.161	0.011	0.939
D 36-61								
ADG (g)	720	731	717	724	11.42	0.874	0.774	0.960
ADFI (g)	1736	1750	1725	1741	9.25	0.755	0.843	0.994
F:G	2.41	2.39	2.41	2.40	0.01	0.937	0.946	0.865

HD: D: high feed cost/dry feeding, HL: high feed cost/liquid feeding, LD: low feed cost/dry feeding, LL: low feed cost/liquid feeding.

[2]SEM: Standard error mean.

[3]FI: Feed ingredient, FM: Feeding method, FI*FM: Feed ingredient*feeding method.

[4]Whey:^{HP300} (%); high=40:0, low=14:10.

[5]Whey:HP300 (%); high=20:10, low=0:10.

[6]Fish meal:HP300 (%); high=3:5, low=0:0.

Table 3. Effect of feed ingredient and feeding method on apparent total tract digestibility (%) in weanling pigs[1]

Feed ingredient	H		L		SEM[2]	P-value[3]		
Feeding method	HD	HL	LD	LL		FI	FM	FI×FM
D 0~7[4]								
Dry matter	82.82	83.33	80.70	82.96	0.61	0.025	0.055	0.101
Gross energy	82.63	83.20	81.02	82.94	0.57	0.076	0.022	0.187
Crude protein	75.41	76.75	74.22	75.64	0.60	0.047	0.020	0.933
D 8~21[5]								
Dry matter	83.77	84.08	82.37	83.91	0.55	0.144	0.090	0.248
Gross energy	83.83	84.44	82.44	83.97	0.58	0.103	0.064	0.407
Crude protein	77.10	78.48	76.40	77.44	0.43	0.021	0.003	0.628
D 22~35[6]								
Dry matter	84.08	85.20	83.80	84.19	0.38	0.079	0.042	0.304
Gross energy	84.30	85.10	83.86	84.48	0.35	0.117	0.040	0.777
Crude protein	77.66	78.44	76.91	77.93	0.42	0.120	0.033	0.754

[1]HD: D: high feed cost/dry feeding, HL: high feed cost/liquid feeding, LD: low feed cost/dry feeding, LL: low feed cost/liquid feeding.
[2]SEM: Standard error mean.
[3]FI: Feed ingredient, FM: Feeding method, FI*FM: Feed ingredient*feeding method.
[4]Whey:HP300 (%); high=40:0, low=14:10.
[5]Whey:HP300 (%); high=20:10, low=0:10.
[6]Fish meal:HP300 (%); high=3:5, low=0:0.

Table 4. Effect of feed ingredient and feeding method on bacterial populations (Log_{10} CFU/g) in feces of weanling pigs[1]

Feed ingredient	H		L		SEM[2]	P-value[3]		
Feeding method	HD	HL	LD	LL		FI	FM	FI*FM
D 0~7[4]								
Total anaerobic bacteria	8.28	8.33	8.29	8.27	0.02	0.294	0.994	0.799
Lactobacillus spp.	8.20	8.34	8.10	8.23	0.04	0.441	0.150	0.903
Clostridium spp.	5.23	5.15	5.35	5.19	0.04	0.825	0.230	0.965
E. Coli	5.18	5.13	5.27	5.23	0.04	0.411	0.654	0.929
D 8~21[5]								
Total anaerobic bacteria	8.43	8.49	8.44	8.46	0.02	0.732	0.221	0.686
Lactobacillus spp.	8.38	8.52	8.30	8.44	0.03	0.467	0.150	0.960
Clostridium spp.	5.27	5.19	5.39	5.23	0.04	0.586	0.268	0.925
E. Coli	5.24	5.18	5.35	5.28	0.04	0.279	0.496	0.925
D 22~35[6]								
Total anaerobic bacteria	9.24	9.32	8.92	9.28	0.04	0.015	0.006	0.504
Lactobacillus spp.	8.52	8.98	8.33	8.67	0.06	0.123	0.068	0.760
Clostridium spp.	5.30	5.24	5.42	5.27	0.05	0.722	0.722	0.951
E. coli	5.27	5.22	5.40	5.33	0.04	0.212	0.531	0.948

[1]HD: D: high feed cost/dry feeding, HL: high feed cost/liquid feeding, LD: low feed cost/dry feeding, LL: low feed cost/liquid feeding.
[2]SEM: Standard error mean.
[3]FI: Feed ingredient, FM: Feeding method, FI*FM: Feed ingredient*feeding method.
[4]Whey:HP300 (%); high=40:0, low=14:10.
[5]Whey:HP300 (%); high=20:10, low=0:10.
[6]Fish meal:HP300 (%); high=3:5, low=0:0.4Whey:HP300 (%); high=40:0, low=14:10.

Table 5. Effect of feed ingredient and feeding method on small intestinal morphology in weanling pigs[1]

Feed ingredient	H		L		SEM[2]	P-value[3]		
Feeding method	HD	HL	LD	LL		FI	FM	FI*FM
Villus height, μm								
Duodenum	505	485	503	487	12.22	0.993	0.173	0.855
Jejunum	422	403	416	406	17.81	0.920	0.790	0.974
Ileum	367	358	364	356	10.71	0.816	0.478	0.997
Crypt depth, μm								
Duodenum	317	330	318	326	12.86	0.942	0.463	0.841
Jejunum	247	263	251	257	10.21	0.930	0.339	0.652
Ileum	228	237	230	241	10.65	0.811	0.358	0.943
VH/CD[4]								
Duodenum	1.60	1.49	1.59	1.51	0.09	0.998	0.321	0.872
Jejunum	1.73	1.54	1.66	1.58	0.10	0.872	0.208	0.569
Ileum	1.62	1.53	1.60	1.48	0.09	0.723	0.287	0.875

[1]HD: D: high feed cost/dry feeding, HL: high feed cost/liquid feeding, LD: low feed cost/dry feeding, LL: low feed cost/liquid feeding.
[2]SEM: Standard error mean.
[3]FI: Feed ingredient, FM: Feeding method, FI*FM: Feed ingredient*feeding method.
[4]Villus height:crypt depth ratio.

References

AOAC., 2007: Official Methods of Analysis. 18th edn Association of Official Analytical Chemists. Gaithersburg, MD, USA.

Brumm, M. C., J. M. Dahlquist., and J. M. Heemstra. 2000. Impact of feeders and drinker devices on pig performance, water use, and manure volume. Swine Health Prod. 8:51–57.

Chiba, L. I., A. J. Lewis, and E. R. Peo. 1991. Amino acid and energy interrelationships in pigs weighing 20 to 50 kilograms: I. Rate and efficiency of weight gain. J. Anim. Sci. 69:694–707.

Choi, J. Y., J. S. Kim., S. L. Ingale., K. H. Kim., P. L. Shinde., I. K. Kwon., B. J. Chae. 2011. Effect of potential multimicrobe probiotic product processed by high drying temperature and antibiotic on performance of weanling pigs. J. Anim. Sci. 89:1795–1804.

Fabian, J., L. I. Chiba., L. T. Frobish., W. H. McElhenny., D. L. Kuhlers., and K. Nadarajah. 2004. Compensatory growth and nitrogen balance in grower-finisher pigs. J. Anim. Sci. 82:2579–2587.

Fenton T. W., and M. Fenton. 1979. An improved method for chromic oxide determination in feed and feces. Can. J. Anim. Sci.59:63–634.

Gonyou, H. W., and Z. Lou. 2000. Effects of eating space and availability of water in feeders on productivity and eating behavior of grower/finisher pigs. J. Anim. Sci. 78:865–870.

National Research Council. 1998. Nutrient Requirements of Swine, 10th edn. National Academy Press, Washington, DC.

Shaw, M. I., A. D. Beaulieu., and J. F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. J. Anim. Sci. 84:3123-3132.

Yoon J. H., S. L Ingale., J. S Kim., K. H Kim., S. H Lee., Y. K Park., I. K Kwon., and B. J Chae. 2012. Effects of dietary supplementation of antimicrobial peptide-A3 on growth performance, nutrient digestibility, intestinal and fecal microflora and intestinal morphology in weanling pigs. Anim. Feed. Sci. Tech. 177:98–107.

PO-01-70

Goblet cell population modulated by Schizosaccharomyce pombe particle

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Introduction

In animal production, selective breeding and improvement of feed have been used to produce high quality animal protein. Improvement in the chickens has been remarkable, especially, chicken carcass weight and egg weight in the past decade. Producers profit is greatly affected by the carcass weight and egg quality, improved chicken mortality and health.

The intestine has an important role in the absorption of nutrition and in defense from the pathogens and viruses. This role is mainly performed by intestinal epithelial cells (IECs). IECs are divided into 4 groups according to function: goblet cells, enteroendocrine cells, paneth cells, and enterocytes (van der Flier, *et al.*, 2009). Goblet cells secrete mucin which is an intestinal barrier protecting mucosal surfaces from physical and chemical injury and prevents the entry of enteric pathogens (Bansil and Turner, 2006).

Recently, use of beta-galactomannan has become a promising strategy to control and prevent intestinal infections (Lowry, *et al.*, 2005). *Schizosaccharomyce pombe* (*S. pombe*) was focused on because this yeast is used as a brewery yeast and is used to produce proteins, such as 1,6-phytase, a key feed additive (EFSA, 2012). The wall of *S. pombe* has a polysaccharide rich structure (Bush, *et al.*, 1973). Our preliminary data showed heat killed *S. pombe* (HK-*S. pombe*) cells grain feed supplement (< 1,000 um) had a tendency to improve body weight gain. However, the effect of particle size was unknown. HK-*S. pombe* powder feed (S. pombe serum.

Method

Animals

This experiment was performed on six 1day male Sanuki Cochin broiler chickens, three for both the control group (Group C), and the experimental group (Group S). Group C and Group S were reared on basal diets with 0% HK- *S. pombe* cell and 1.0% HK-*S. pombe* cells: 2.2×10^9 cells/kg, respectively. Feed and water were *ad libitum*. This experiment was conducted in accordance with regulations of the Kagawa University Animal Care and Use Committee.

Measurement of body weight

Body weight was measured weekly until the chickens were 105 days old.

Sample preparation

On 105 days, the broilers were slaughtered and duodenum jejunum and ileum were collected. A part of the duodenum, jejunum and ileum were collected and treated with 4% PFA in a PBS (pH 7.4) solution. Each part was embedded in paraffin.

Measuring the number of goblet cells by alcian blue staining

5μm sections were made from the each block and put on slide glasses with aminosilane coating. Then, the sections were stained with the alcian blue after deparaffinized treatment for 1.5 hours. After washing, each section was stained with nuclear fast red (Polysciences, Inc., U.S.) for nuclear staining. After washing, they were mounted with Entellan (Merck Millipore Corp., U.S.). An Olympus BX51 microscope (Olympus Corp., Japan) was used for observation. 8 villi was selected and photographed. The villi were divided approximately in half, into the upper part of the villi and the lower part from the half line and the crypt. The number of goblet cells from each part were counted by manual measurement with Win-Roof V.7.4 (Mitani Corp., Japan).

Localization of HK-*S. pombe* cells in intestine by immunohistochemical staining

5μm sections were made from each block. After deparaffinization, blocking of endogenous peroxidase was be performed by Histo VT One (Nacalai Tesque Inc., Japan). Primary antiserum, rabbit polyclonal anti-serum *S. pombe* (Applied Biological Science Laboratory, Kagawa University) was diluted in 1/1000 and put 100μl on sections. After overnight incubation in 4 ºC, a secondary, antibody Alexa Flour 488 goat anti-rabbit IgG (Thermo Fisher Scientific Inc., U.S.), was diluted in 1/1000 and 100μl of solution was added to the sections. The sections were then washed, mounted with Vecta Shield (Vector Laboratories, U.S.), and observed with a Fluoiview FV1000

(Olympus Corp., Japan) to observe the localization of HK-*S. pombe* cells.

Statistics

Statistical analysis on the number of goblet cells was performed using one-way ANOVA, and significant differences among the groups were determined with the Tukey Method and SPSS Statistics 19 Software (IBM Corp., U.S.).

Results

Body weight

HK-*S. pombe* supplementation did not cause any statistically significant effect on body weight (Fig.1).

Measuring the number of goblet cells by alcian blue staining

The number of the goblet cells stained by alcian blue was 66% higher in the crypts in duodenum in Group S compared Group C. Furthermore, in the villus top in ileum, number of the goblet cells was 60% higher Group S compared Group C. (Fig.2). In particular, a higher number of goblet cells were located and in the top part of the villus and the ileum (Fig.3).

Localization of HK-*S. pombe* cells in intestine by immunohistochemical staining

HK-*S. pombe* cells were observed on the surface of villi in duodenum by immunohistochemical staining (Fig.4).

Discussion

The aim of this study was to investigate the effect of body weight and intrinsic localization in intestine of male Sanuki Cochin broiler chickens treated HK-*S.pombe* cells. Powdered HK-*S. pombe* cells treatment had no effect on body weight (Fig.1). The number of the goblet cells increased in the crypts in duodenum and in the top of villi in ileum by the HK-*S. pombe* cells treatment (Fig.2, Fig.3). HK-*S. pombe* cells were observed on the surface of the villi (Fig.4).

Our preliminary data suggested HK-*S. pombe* cells grain feed supplement (< 1,000 um) had a tendency to improve body weight gain, as described previously. Alternatively powdered HK-*S. pombe* cells treatment had no effect to body weight (Fig.1). It was reported that a bigger size of feed particle increased body weight in broilers (Amerah, *et al.*, 2008). However, in this experiment, refined HK-*S. pombe* cells size caused no significant gain in body weight.

HK-*S. pombe* cells could not be mashed nor absorbed in neither the gizzard nor the cecum and reached the intestine with the remaining HK-*S. pombe* forms as indicated by the immunohistological data. *S. pombe* had carbohydrate chains and protein on their cell walls. Interaction between goblet cells and proteins affected mucin production (Claustre, *et al.*, 2002) so that IECs may have gotten some stimulation from carbohydrate chains or protein which were expressed on the surface of *S. pombe* cells. As a result, it can be concluded that the amount of mucin increased. Since, mucin secreted by goblet cells are first defense against injury and pathogen (Chadee, *et al.*, 1987), a protective function occurs when using feed with HK-*S. pombe* cells.

Acknowledgements

The authors would like to thank Biomaterial in Tokyo Co., Ltd. for providing HK-*S. pombe* cells.

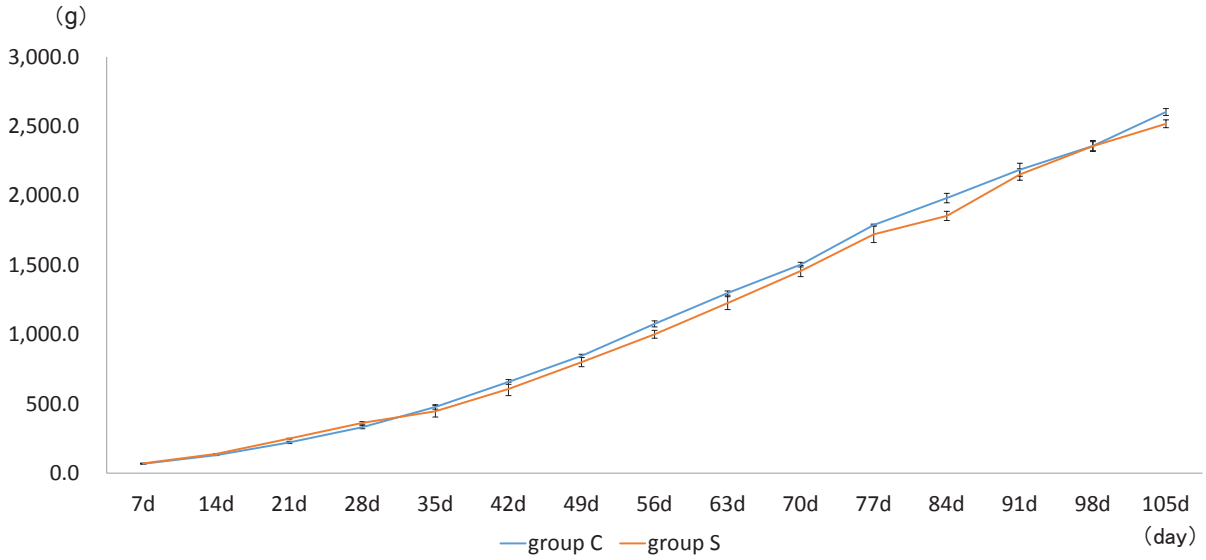

Figure 1. Body weight change during experiment.

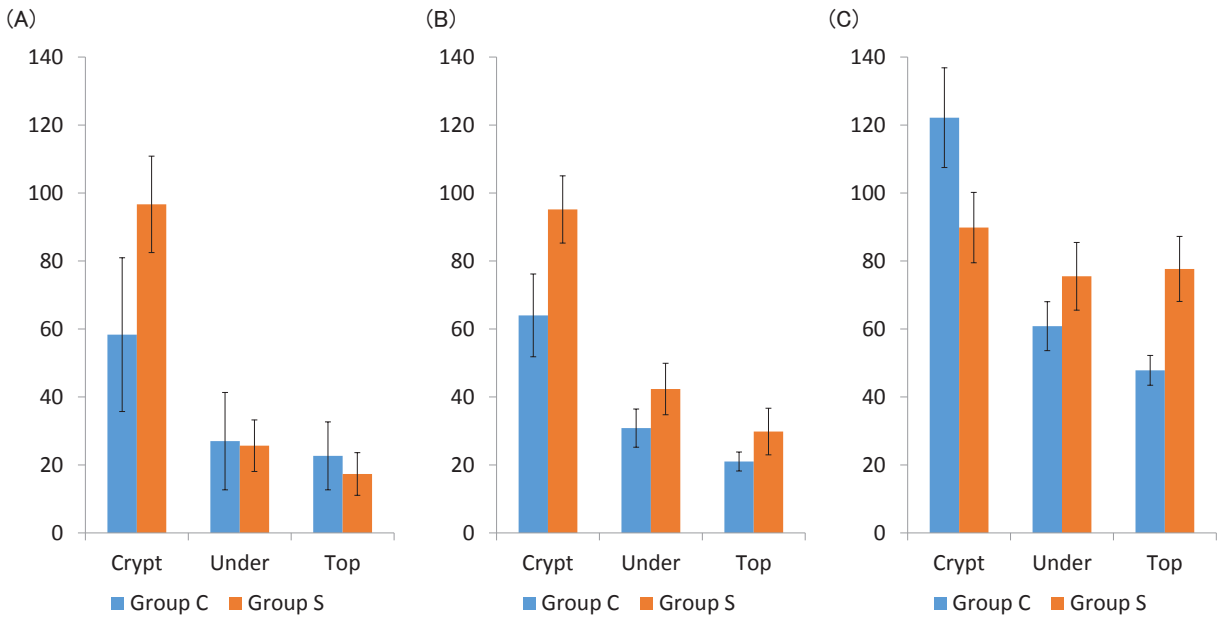

Figure 2. The number of goblet cells in duodenum (A) , jejunum (B) , ileum (C).

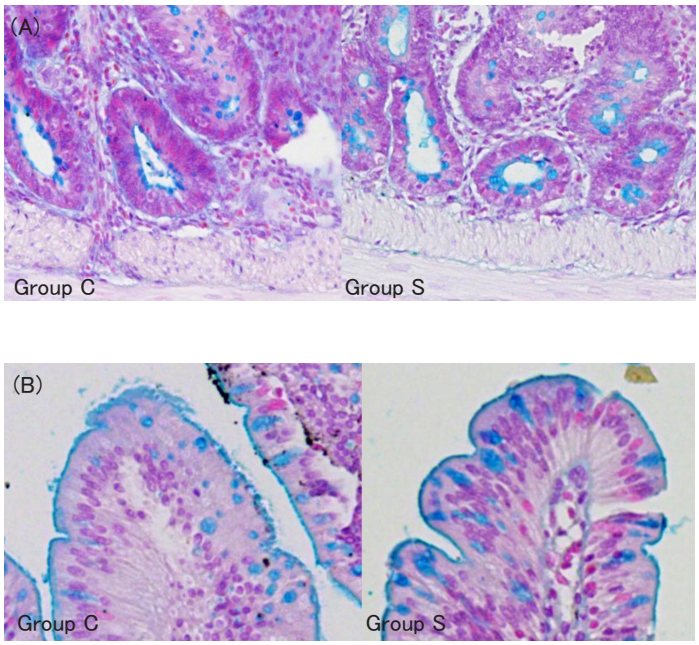

Figure 3. The crypt in duodenum (A) and the top of villi in ileum (B) of alcian blue staining.

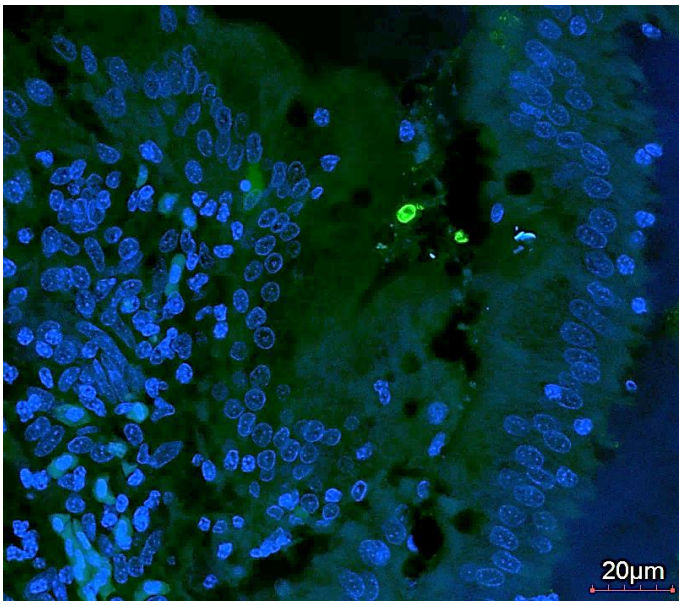

Figure 4. Localization of HK-S.pombe cells in intestine by immunohistochemical staining

REFERENCES

Amerah A. M., Ravindran V, Lentle R. G. and Thomas D. G.. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poultry Science. 2008; 87(11): 2320-2328

Bansil R, Turner B. Mucin structure, aggregation, physiological functions and biomedical applications. Current Opinion in Colloid & Interface Science. 2006; 11(2-3): 164- 170

Bush D. A., Horisberger M, Horman I and Wursch P. The wall structure of *Schizosaccharomyces pombe*. Journal of General Microbiology. 1974; 81(1): 199-206

Chadee K., Petri W. A., Jr., Innes D. J. and Ravdin J. I. Rat and human colonic mucins bind to and inhibit adherence lectin of *Entamoeba histolytica*. Journal of Clinical Ivestigation. 1987; 80(5): 1245-1254

Claustre J, Toumi F, Trompette A, Jourdan G, Guignard H, Chayvialle J. A. and Plaisancie P. Effects of peptides derived from dietary proteins on mucus secretion in rat jejunum. American Journal of Physiology. 2002; 283(3): 521–528

EFSA. Opinion of the Scientific panel on Additives and Products or Substances used in Animal Feed and the Scientific panel on Genetically Modified Organisms on the safety and efficacy of the enzymatic preparation Phyzyme XP (6-Phytase) for use as feed additive for chickens for fattening. EFSA Journal. 2006; 350: 1-14

Lowry V. K., Farnell M. B., Ferro P., Ferro P. J., Swaggerty C. L., Bahl A and Kogut M. H.. Purified ß-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis*. International Journal of Food Microbiology. 2005; 98(3): 309-318

van der Flier L.G., Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annual Review of Physiology. 2009; 71: 241-260

PO-01-73

Aptitudes of Ashitaba (Angelica Keiskei Koidzumi) Supplementation on the physiological responses in lactating Holstein-Friesian cows

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Introduction

Domestic animals experience different level of stresses during their production lives, which will influence their production performance or animal's health condition. Lactating Holstein-Friesian cow is not an exception and they are susceptible to the stresses that occurs by various environmental or management factors including climate, nutrition, housing, inflammatory, ultra violet irradiation and heat stress that have ability to provoke a physiological, biochemical, immunological and behavioral responses (Offiong *et al*., 2013). As a consequence, animals display various physiological responses such as reduction of immunological resistance, loss of appetite and changes in the time required for blood coagulation as fight and flight body response (Etim *et. al*., 2013). Metabolic changes with stress come by oxidation-reduction to mentation the electrochemical gradients for proper cell function (Mobreg and Mench, 2001) which assist with oxidative stress and change the balance reactive oxygen species (ROS) in the body of animal. Hence, elimination of free radicals may improve the physiological status of the animals. Therefore, the current experiment conducted to evaluate the effect of feeding Ashitaba *(Angelica keiskei* Koidzumi*)* supplement to the Holstein Friesian cows. *Angelica keiskei* Koidzumi is a perennial Japanese plant from Umbelliferae family (Akihisa *et. al*., 2003) that growing in the Izu islands and the Izu, Bouso and Miura peninsulas of Japan (Nakamura *et, al*., 2012) and introduced as a potent anti-oxidant due to containing 4-hydroxyderricin and xanthoangelol as two main chanlcones, and coumarins (Akihisa *et.al*., 2003).

Materials and Methods

The experiment was conducted at the Agriculture Forestry Research Center, University of Tsukuba, Japan for 6 weeks between July to August 2015. Four multiparous Holstein Friesian cows (BW =671 kg ± 0.5) were divided into two groups and randomly allocated into either Ashitaba supplementation (AS) group or control (C) group.

In AS group, 0.3 % of DM requirement was supplied by Ashitaba, mixed with 1 Kg of concentrate and 1Kg of corn silage prior to evening feeding after milking for 12 days. In C group, supplemental feed without containing Ashitaba was fed to the animals during the same period. The experiment was repeated 3 times after switching AS group and C group taking two days interval between replications.

Physiological status, including respiration rate, hearth beat rate were recorded four times a day, whereas the body weight was measured every other day. The milk production, barn and ambient temperature and humidity were recorded every day. Milk sample and blood samples were collected three days at the beginning of experimental period and five days at the end of each cycle. Milk components including fat %, protein %, lactose %, solid not fat (SNF) %, somatic cell count (SCC), milk urea nitrogen (MUN), freezing point (°C) and Osmotic point were examined in the milk production of cows under experiment. The superoxide dismutase (SOD) activity and glutathione (GSH: γ-L glutamyl-L-cysteinylglycine) activity were measured in the blood plasma and blood cell, respectively.

Result

Environment condition

As shown in Figure 1, the average ambient temperature in first, second and third cycle were 28.66 °C ± 0.28, 26.14 °C ± 0.34 and 22.59 °C ± 0.59, and Temperature-Humidity Index (THI) were 79.86 ± 0.35, 77.24 ± 0.42 and 71.36 ± 0.91, respectively.

Body weight

At the end of first period, the body weight (BW) decreased rapidly by 6 ± 1.5% and 6 ± 1.4 % in AS group and C

group, respectively.

Milk production

The data shows an increase of 8.8% milk yield in low milk production cow and decrease of 6.5 % milk yield in high milk production cow in period 1 during feeding Ashitaba supplement and the same result observed in period 2 by increase in performance of low milk production cow by 1.8 % and 5.6% respectively in cycle 1 and 3 during feeding Ashitaba and decrease of milk yield in high milk production cow by 17.7 % and 3.8 % respectively in period 1 and 3.

Milk composition

The fat percentage data shows a decrease by 42% ± 0.5 and 12.8% ± 0.9 in AS group and C group after receiving Ashitaba supplement respectively which synchronous with increase of temperature and THI in period 1 and period 2. There was an increase of 4.7% ± 1.4 solids not fat (SNF) ratio during Ashitaba feeding in period 1 but The change of solids not fat ratio is not significantly appearing in period 2 during feeding supplement. The analyzed data shows that protein percentage increased 4.7% ± 0.9 in AS group during feeding Ashitaba supplement coincidence with decrease of ambient temperature but the protein percentage decreased by 3.1% ± 1.8 in period 2 during feeding Ashitaba. Lactose percentage got increase 6.8 % ± 3.7 in period 1 but it decreased 2.7 % ± 2.8 in period 2 after feeding Ashitaba supplement. The somatic cell count (SCC) significantly decreased by 72.3% ± 0.5 and 24.6 % ± 0.6 in period 1 and period 2 respectively during feeding Ashitaba supplement. There was a cow with mastitis as an inflammatory problem under current experiment. The data of milk component on SCC of the mastitis teats and non-mastitis teats of this cow shows a 59.3% and 88.2% decrease during receiving Ashitaba supplement in second cycle of experiment (Figure 2).

The level of milk urea nitrogen (MUN) presented a decrease by 14.9 % ± 0.5 in third cycle in period 2 after receiving Ashitaba supplement which is coincidence with decrease of ambient temperature but the level of MUN showed an increase by 21.8 % ± 0.5 during receiving Ashitaba supplement in second cycle.

Superoxide dismutase (SOD) activity in blood plasma

In first cycle, the SOD activity ratio of both groups decreased by increasing THI. In second cycle, the ratio of SOD activity shows a bit increase in group which received Ashitaba but this increase of SOD activity is coincidence with decreasing the THI from average of 77.5 to 74.5.

Glutathione GSSG/GSH Quantification

The ratio of glutathione quantity is different by the cows within the group after receiving the Ashitaba supplement. In both groups, the high milk production cows presented an increase of glutathione ration at the begging of feeding Ashitaba supplement period and it followed by dropped at the end of feeding Ashitaba supplement period but the glutathione ratio in low milk production cows did not change by feeding Ashitaba in both groups.

Discussion

Many of the reported stresses which display physiological dysfunction is associated with the increase of ROS in the body (Lobo et. al., 2010). Furthermore, an increase of free radical due to stresses is reported to suppress the anti-oxidant activities (Ellah, 2013). Present study, therefore, was conducted to examine the possibility of diminishing stresses in lactating Holstein cows by feeding Ashitaba which is known to contains anti-oxidative agents such as xanthoangelol and 4-hydroxyderricin.

Fidler and VanDevender (2014) reported that moving of cows from 26.6 °C, 50%RH to 32.2 °C, 90 % RH, a moderate sign of heat stress may occur. The sign of moderate heat stress will be coincidence with rapid shallow breathing, plentiful sweating and an approximately 10 percent decrease in milk production. In the present experiment, the milk production has decreased at the first period of experiment, reduction of milk production was observed in both AS group and C group by 11.6% ± 1.4 and 8% ± 0.5 respectively. Therefore, the cows during the period 1 was presumed to be under a mild heat stressed condition. Even through the SOD concentration in the blood of AS group was not significantly higher compared with control group ($P > 0.5$), a decrease of SCC has been observed for a cow suspected to be in mastitis during experimental period. The results in the present study do not agree with Atroshi *et. al.* (1986) who reported that the blood SOD in the cow under mastitis is lower compared with normal cows. The reason for the disagreement needs to be revealed in the future study.

The present result may indicate that SCC in milk can be reduced by feeding anti-oxidant such as Ashitaba. Since mastitis paralyzes the milk production in dairy animals, further research need to be conducted to further examine the possibility of alleviating animals under various stresses by supplementing anti-oxidative agents such as Ashitaba.

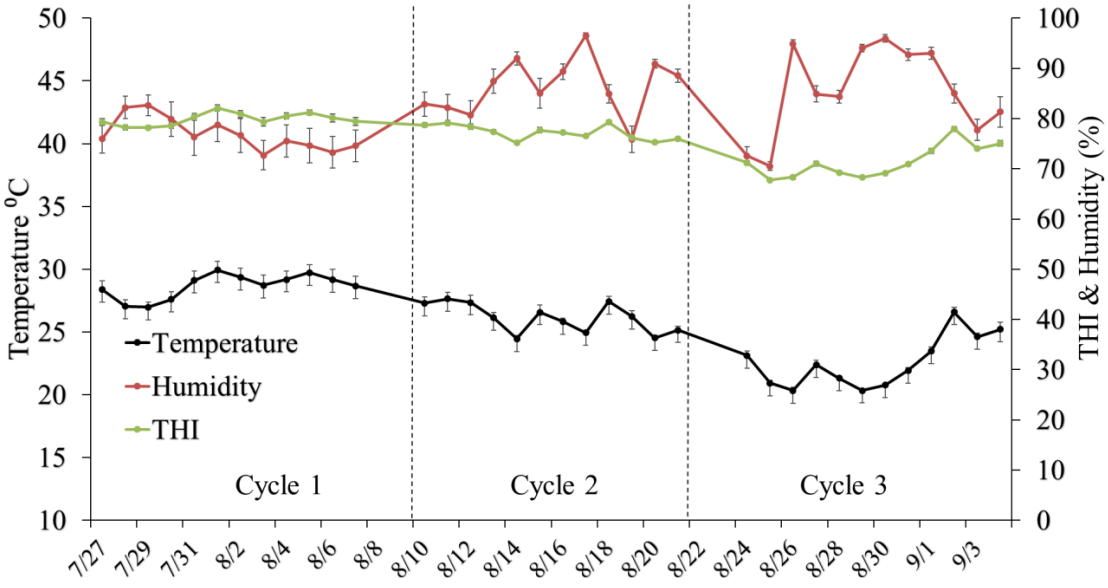

Figure 1 Average of ambient temperature, humidity and THI of Tsukuba city during Ashitaba feeding experiment (July 27th to September 4th, 2015)

References

Akihisa, T., Tokuda, H., Ukiya, M., Iizuka, M., Schneider, S., Ogasawara, K., Mukainaka, T., Iwatsuki, K., Suzuki, T., Nishino, H., 2003, Chalcones, coumarins, and flavanones from the exudate of Angelica keiskei and their chemopreventive effects. Cancer Letters 201:133-137

Atroshi, F., Parantained, J., Sankari, S., Osterman, T., 1986, Prostaglandin and glutathione peroxidase in bovine mastitis, Research in Veterinary Science, 40: 361-366

Ellah abd, M.R., 2013, Role of free radicals and antioxidant in mastitis. Journal of Advanced Veterinary Research, 3:1-7

Etim, N.N., Williams, E.M., Evans, I. E. and Offiong, E.A.E., 2013, Physiological and behavioural responses of farm animal to stress: Implications to animal productivity. American Journal of Advanced Agricultural Research, 1:53-61

Fidler, A.P., VanDevender, K., 2014, Heat stress in dairy cow, Arkansas Cooperative Extension Service, FSA3040

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010, Free radicals, antioxidants and functional food: impact on human health. Pharmacognosy Review., 4(8):118-128

Moberg, G.P and Mench, J.A., 2001, The Biology of Animal stress: Basic principles and implications for animal welfare, CABI Publishing, 2:91-103

Nakamura, T., Tokushima, T., Kawabata, K., Yamamoto, N., Miyamoto, M. and Ashida, H., 2012 Absorption and metabolism of 4-hydroxyderricin and xanthoangelol after oral administration of Angelica keiskei (Ashitaba) extract in mice. Archives of Biochemistry and biophysics, 521: 71-76

Offiong, E.A.E., Etim, N.N., Eyoh, D.G. and Udo, M. D., 2013, Stress and animal welfare: an uneasy relationship. European Journal of Advanced Research in Biological and Life Science, 1:9-16

PO-01-75

Rapid Identification of 4 Enterococcus Species using PCR Based on Genome Comparison.

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Introduction

Entercocci are gram-positive and catalase-negative bacteria, belonging to the lactic acid bacteria (LAB). They were frequently found in the various environments, such as human/animal gastrointestinal tract, livestock feces, fermented foods, water and soil. Certain *Enterococcus* strains are used as a probiotics for improvement of immune system, treatment of antibiotic-associated diarrhea, lowering serum cholesterol in human.

But, other *Enterococcus* strains are well-adapted to the hospital settings by, acquiring virulence factors, such as vancomycin resistant gene (vanA, vanB, vanC, vanD), *Enterococcus* surface protein, Hyaluronidase. So, in Europe, genetic verification of *E. faecium* strains are highly recommend for their uses by using a PCR method to secure the feed safety. For these reasons, simple cost-effective identification methods are required for practical purposes in clinical and industrial areas. 16S rRNA PCR was traditionally used to distinguish in species level, but *Enterococcus* sequences are too similar to distinguish species. Alternative identification methods were developed by using species-specific primers ddl, eda, and sodA genes, these methods are limited to determine single species. Thus, we developed novel rapid method for simultaneous identification of 4 *Enterococcus* species by using comparative genomics.

Materials and Methods

Bacterial Genomes and Ortholog Collection

Thirty eight genomes of 13 different *Enterococcus* species were obtained from the National Center for Biotechnology Information (NCBI) GenBank. Genome sequences were re-annotated by using an web-based annotation system, RAST under the default parameters for bacteria. All the protein coding sequences (CDS) obtained from the annotation were mutually aligned to similar CDS using GASSST under the parameters of ≥65% sequence identity. During the alignment, we obtained 25,623 ortholog groups. Only one CDS was randomly selected from each group as a representative ortholog and we finally defined 25,623 orthologs from 13 *Enterococcus* species (**Table 1.**).

Primer Design and PCR conditioning

For species-specific genes, we designed primers using Primer3Plus. We used different size options to obtain different sizes, respectively, from species-specific genes. The candidate primers were further check to see whether they are species-specific by using the NCBI Primer-BLAST. Finally selected primer sequences were prepared by Bioneer(Daejeon, South Korea). For gradient PCR, template DNA (genomic DNA or bacterial cells) was added to 18 μl PCR reaction master mixture containing sterile DW, dNTPs (200 μM each), 375 μM MgCl2, 0.6 unit i-Taq DNA polymerase (iNtRON Biotechnology, South Korea), 10X PCR buffer (2 μl, w/or w/o $MgCl_2$), and each species-specific primer set (forward and reverse, XX pmole each, Table 2. Gradient PCR was conducted to determine optimal annealing temperatures for each species-specific primer set with following steps: 94°C for 3 min; 40 cycles of 94°C for 60 sec, 50-64°C for 60 sec, and 72°C for 60 sec; and 72°C for 10 min.

Results

Comparison of 16S rRNA sequences

Previous studies mentioned 16S rRNA have already designed as a genus-specific primers and found useful for distinguishing of *Enterococcus*. But, some *Enterococcus* are difficult to distinguish in speceis levels by 16S rRNA gene sequencing. Thus, we verified their 16s rRNA sequences similarity in in silico levels. Available 12 *Enterococcus* species 16S rRNA gene sequences were downloaded from NCBI and aligned and compiled using MEGA 7 software(1). In **Figure 1.**, Several strains are distinguish clearly, but some strains show their sequence approximately similar and formed a one branch. For instance, *E. hirae* ATCC9790 and *E. durans* JCM 8725 are joining as a same tree. This result shows *Enterococcus* species are quite difficult to identify using 16s rRNA

sequences and needs to detect of species specific genes for certain verification but not 16s rRNA.

Selection of species-specific marker genes

We screened 38 genomes of 13 *Enterococcus* species to identify species-specific genes. Among 25,623 orthologs found from the genomes, several genes were identified to be species-specific genes (data not shown). We identified four genes that are specific to *E. durans*, *E. faecalis*, *E. faecium*, *E. hirae*, respectively (Table 1).

Optimization of individual primer sets and PCR condition

To optimize PCR conditions for identification of individual species, Gradient PCR method was used in this study. We examined annealing temperatures ranged from 50°C to 64°C and see the results by gel electrophoresis (**Figure 2**). In 3 *Enterococcus* species (*E. faecalis, E. hirae, E. durans),* all annealing temperature conditions showed clear single bands that we expected (1209bp, 442bp, and 231bp). In *E. faecium*, while 3 annealing temperature conditions (64°C, 62.9°C, and 61.2°C) showed clear single bands as expected (1032bp), others (58.5°C to 50°C) made unexpected double bands (1032bp and around 200bp). On the basis of this optimization, we determined a candidate optimal annealing temperature (64°C) for multiplex-PCR.

Optimization of multiplex primer sets and PCR condition

For detection of multiple species, we made individual PCR mastermix and input each species detection primers, and it's time and work inefficient. But multiplex PCR can overcome the disadvantage of cost and time efficiency then using individual PCR. So, we designed Multiplex PCR for identification of *Enterococcus* species in one condition simultaneously for the further research. Through the optimization of individual primer sets, we confirmed each species annealing temperature equally 64°C that showed clear and one band. PCR results are showed strains including 4 *Enterococcus* species for positive control and 5 *Enterococcus* species which their strains are similar in 16s rRNA phylogenetic condition for negative control and 2 *Lactobacillus* species (**Figure 3)**. Likewise in individual PCR results, multiplex PCR also showed clear one band for each 4 positive control samples. In contrast, other species are not react what we designed primer sets. So, both individual primer sets and multiplex primer sets can identified these 4 *Enterococcus* species clearly.

Conclusions

The aim of this study is to develop a novel PCR method that was specific for *Enterococcus* 4 species. Novel species-specific multiplex PCR that we designed by using novel computer analysis programming tool, showed clear one band to each species. This method can detect various *Enterococcus* spp. just PCR and not need to sequencing for species identification. It can also use colony samples as a template so it can reduce time and cost efficiency. This method can adapt various condition to detect *Enterococcus* spp. on commercials and clinicals for their further study to reduce time, economically and growth work efficiency.

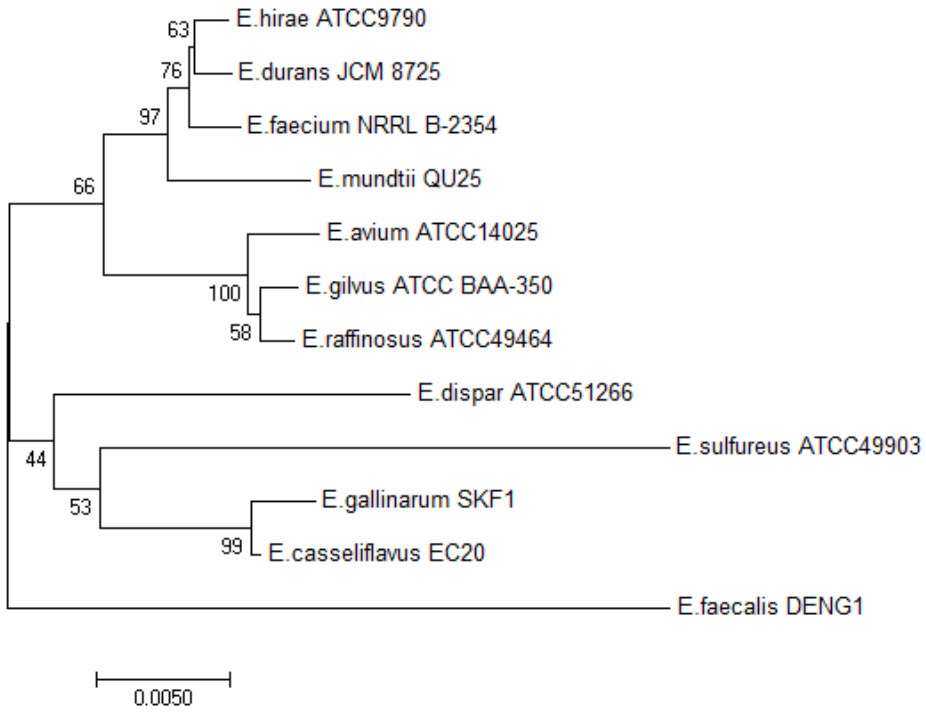

Figure 1. Phylogenetic tree of the *Enterococcus* 16s rRNA gene sequences. Each species genes were aligned and compiled using MEGA 7 software with a neighbor-joining method. Number of bootstrap replications are 1000 and the values are shown on the left branches.

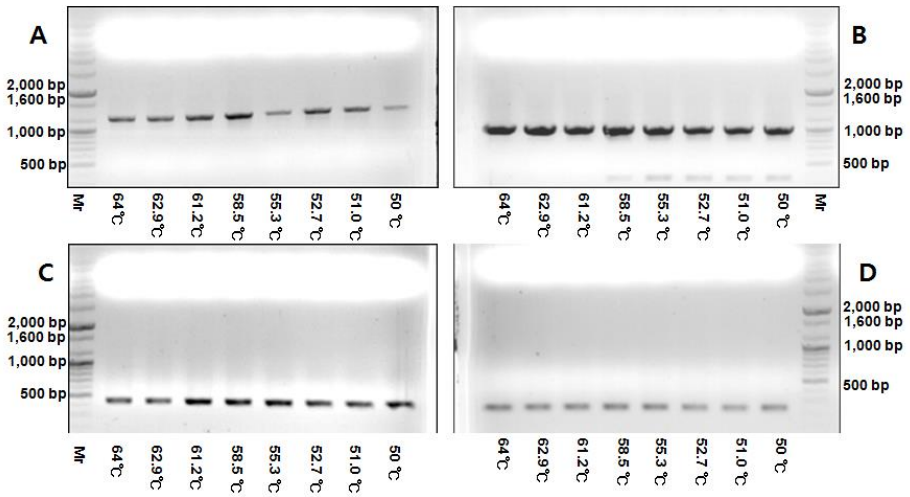

Figure 2. gradient PCR products gel electrophoresis results amplified with each 4 primer sets.

Enterococcus faecalis (A), *Enterococcus faecium* (B), *Enterococcus hirae* (C), *Enterococcus durans* (D) groups. Mr means size marker ; 100bp plus DNA ladder (Bioneer, Korea)

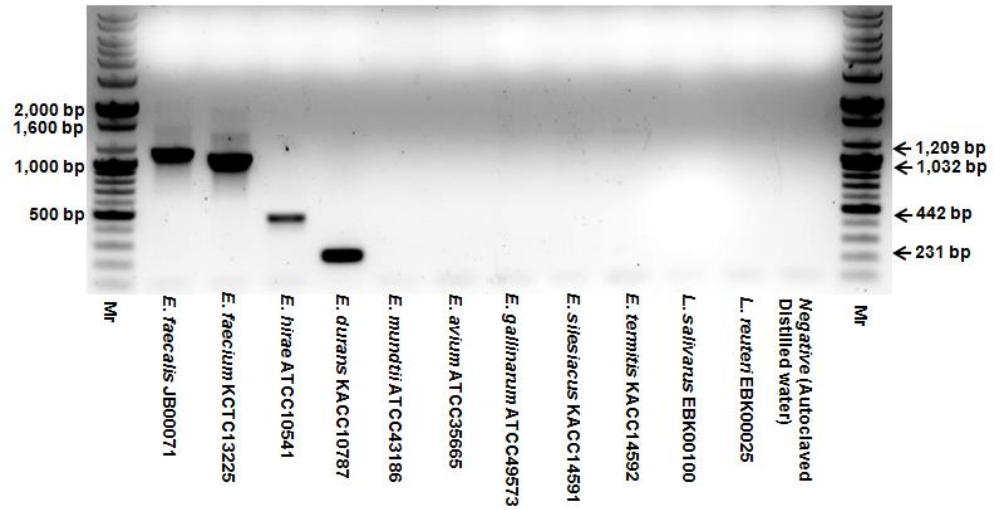

Figure 3. Comparison of *Enterococcus* species specific PCR with other species and genus.

Comparison of 4 Enterococcus species specific primer sets with other species and genus. All primer sets were pooled before PCR reaction. Mr means size marker ; 100bp plus DNA ladder (Bioneer, Korea). Negative shows that PCR mastermix didn't contaminate.

Table 1. Species-specific genes for 4 Enterococcus species.

Species	Ortholog_10209	Ortholog_14163	Ortholog_21771	Ortholog_3492
E. avium	N.D.	N.D.	N.D.	N.D.
E. casseliflavus	N.D.	N.D.	N.D.	N.D.
E. dispar	N.D.	N.D.	N.D.	N.D.
E. durans	2/2	N.D.	N.D.	N.D.
E. faecalis	N.D.	4/4	N.D.	N.D.
E. faecium	N.D.	N.D.	N.D.	7/7
E. gallinarum	N.D.	N.D.	N.D.	N.D.
E. gilvus	N.D.	N.D.	N.D.	N.D.
E. hirae	N.D.	N.D.	4/4	N.D.
E. italicus	N.D.	N.D.	N.D.	N.D.
E. mundtii	N.D.	N.D.	N.D.	N.D.
E. raffinosus	N.D.	N.D.	N.D.	N.D.
E. sulfureus	N.D.	N.D.	N.D.	N.D.

* N.D. = Not Detected in the species

* N/N = Gene-Detected Genomes per Target Genomes

Acknowledgments/References
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Azhahianambi, P., Ghosh, S., Kumar, C. A., & Suryanarayana, V. V. S. (2008). Cost effectiveness of colony lysis and colony PCR methods for screening of recombinant Escherichia coli colonies--a comparative study. *Indian journal of experimental biology*, *46*(10), 731.
Elnifro, E. M., Ashshi, A. M., Cooper, R. J., & Klapper, P. E. (2000). Multiplex PCR: optimization and application in diagnostic virology. *Clinical microbiology reviews*, *13*(4), 559-570.
Jackson, C. R., Fedorka-Cray, P. J., & Barrett, J. B. (2004). Use of a genus-and species-specific multiplex PCR for identification of enterococci. *Journal of Clinical Microbiology*, *42*(8), 3558-3565.
Nayak, B. S., Badgley, B., & Harwood, V. J. (2011). Comparison of genotypic and phylogenetic relationships of environmental Enterococcus isolates by BOX-PCR typing and 16S rRNA gene sequencing. *Applied and environmental microbiology*, *77*(14), 5050-5055.
Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, *44*(4), 846-849.
Van Tyne, D., & Gilmore, M. S. (2014). Friend turned foe: Evolution of enterococcal virulence and antibiotic resistance. Annual Review of Microbiology, 68, 337.
Fox, G. E., Wisotzkey, J. D., & Jurtshuk JR, P. (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. *International Journal of Systematic and Evolutionary Microbiology*,*42*(1), 166-170.

PO-01-79

Estimation of genetic parameters of swine population under selection

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Abstract

This study was carried out to investigate the genetic characteristics of swine population under selection through estimation of genetic parameters for production and reproduction traits. The production records of 7,048 pigs and the reproduction records of 3,041 litter from Duroc, Landarace and Yorkshire, collected from 2014 to 2016 were used for analysis. The production traits were days to 90 kg, average daily gain and backfat thickness (measured with ultrasound device piglog 105), and the reproductive traits were total litter size and number of born alive. The model for reproductive traits included breed, farrowing-year-month as fixed effects and random additive genetic effects. In the model for production traits included fixed effects of breed, birth-year-month, sex and maternal parity and random additive genetic effect of animal. The estimates of heritability for total litter size and number of born alive were 0.27 and 0.25, respectively, and the genetic correlation of both traits were 0.41. Heritability estimates for days to 90kg, backfat thickness and average daily gain were 0.38, 0.43 and 0.36, respectively. The genetic correlation between days to 90kg and backfat thickness was 0.17 and the genetic correlation between days to 90kg and average daily gain was -0.98. The genetic association of days to 90kg with backfat thickness was relatively low and high genetic variation has been preserved even within the population under selection.

INTRODUCTION

Productivity and reproductivity are recognized as a main factor in the pig industry. The breading goal for increasing profit is generally to increase the number of born alive piglets (NBA) and total liter size (TLS) in dam line. Also, the breading goal for increasing profit in sire line is generally to improve the backfat thickness (BF), days to 90kg (D90kg) and average days gain (ADG). Studies have been continuously conducted to estimate parameter for productive traits and reproductive traits and In the nucleus farm, selection has been based on a total merit index with multivariate BLUP-EBV for production traits and a separate repeatability model for reproduction traits. However, if genetic changes are too rapid, the population might not be able to adapt to the changes imposed by selection (Dunnington, E. A. 1990.). Therefore, the object of this study was that the genetic characteristics of swine population under selection investigate through estimation of genetic parameters.

MATERIALS AND METHODS

Data : All records for analysis collected by six nucleus herds from Duroc, Landarace and Yorkshire from 2014 to 2016. In this study, reproductive traits composed of total liter size (TLS) and number of piglet born alive (NBA) and productive traits composed of backfat thickness (BF), days to 90kg (D90kg) and average daily gain.
Productive correction equation was:

$$\mathrm{D90kg} = \mathrm{age} + \frac{(90 - WT)}{WT} \times (AGE - 38)$$

$$\mathrm{BF} = BF_m + \frac{(90 - WT) \times BF_m}{(WT - 11.34)}$$

$$\mathrm{ADG} = \frac{WT}{age}$$

where,
BFm: Real backfat thickness was measured by PIGLOG 105 A-mode
WT: body weight
In this populations, selection has been based on an index composed of ADG, BF and NBA.
Outliers over ± 3 SD for all traits were considered to be incorrect recordings and excluded from the analysis. The basic and descriptive statistics are provided in Table 1. The number of production records and reproduction

records were 7,048 pigs and 3,041 litter size, respectively, after editing.

Statistical analysis : Genetic parameters for productive traits, including genetic (co)variance components, were estimated by the restricted maximum likelihood (REML) procedure based on multivariate anlysis using the Wombat program (Meyer, 2015).
The linear model for productive traits analysis was as follows:

$$Y_{ijk} = u + B_i + Farm_j + FYM_k + a_{ijkl} + pe_{ijkl} + e_{ijkl}$$

where,

Y_{ijk}= observation of the trait,
B_i = breed effect
$Farm_j$ = farm effect
FYM_k = fallowing-year-month
a_{ijkl} = addtive genetic effect
pe_{ijkl}= permanent enviroment effect
e_{ijkl} = residuals

The linear model for reproductive traits analysis was as follows:

$$y_{ijklm} = u + B_i + BYM_j + SEX_k + MP_l + a_{ijklm} + e_{ijklm}$$

Y_{ijk} = observation of the trait
B_i = breed effect
BYM_j = birth-year-month effect
SEX_k = sex effect
MP_l = maternal parity effect
a_{ijklm} = addtive genetic effect
e_{ijklm} = residuals

Table 1. Number of record reproductive and productive traits by breed, farrowing-year- month, sex and maternal parity in a swine herd

Breed	Number	Farm	Number	Sex	Number	MP	Number
Reproductive traits							
LL	524	1	1653				
YY	1961	2	9				
DD	556	3	208				
		4	112				
		5	4				
		6	1055				
Productive traits							
LL	1387			Female	6264	1	2008
YY	4671			Male	784	2	2114
DD	990					3	1215
						4	1125
						5	586

FYM: farrowing-year-month, BYM: birth-year-month, MP: maternal parity
LL = Landrace, YY = Yorkshire, DD = Duroc

Table 2. simple static of reproductive and productive traits in a swine herd

Traits	N	MEAN	SD	MIN	MAX
Reproductive traits					
TLS	3041	9.84	3.27	1	22
NBA	2969	9.80	3.08	1	22
Productive traits					
D90kg	7048	148.7	12.44	102	239
ADG	6825	616.13	57.23	500	876
BF	7039	14.58	2.52	7.78	24.82

TLS : total litter size, NBA : number of born alive

D90kg : days to 90 kg, ADG : average daily gain, BF backfat thickness

RESULTS AND DISCUSSION

Basic statics of reproductive and productive are presented in table 2. Mean of NBA, TlS, D90kg, ADG and BF were 9.80 ± 3.08, 9.84 ± 3.27, 148.7 ± 12.44, 616.13 ± 57.23 and 14.58 ± 2.52, respectively.

Estimates for parameter of reproductive and productive are given in table 3and table 4, respectively.

The estimates of heritability for TLS, NBA, D90kg, BF and ADG were 0.27, 0.25, 0.38, 0.43 and 0.36, respectively. To compare other studies (Chungil Cho (2012), Hanenberg, E (2001) ChangHee Do (2007)), heritability of reproductive estimated relatively higher in this study because it was considered result of population under selection and heritability of productive estimate similar. High genetic variation has been preserved even within the population under selection.The genetic correlation between reproductive traits was 0.41. The genetic correlation in productive traits between D90kg and BF, D90kg and ADG were 0.17 and -0.98, respectively. For this reasons, although breeding scheme were continuously conducted, genetic variance components of productive and reproductive variate high. Therefore, population under selection can sustain breeding scheme.

Keywords : selection, genetic parameters, days to 90 kg, backfat thickness, number of born alive

REFERENCES

Holm, B., et al. “Genetic correlations between reproduction and production traits in swine.” Journal of animal science 82.12 (2004): 3458-3464.

Imboonta, N., L. Rydhmer, and S. Tumwasorn. “Genetic parameters for reproduction and production traits of Landrace sows in Thailand.” Journal of animal science 85.1 (2007): 53-59.

Hoque, M. A., et al. “Genetic parameters for measures of the efficiency of gain of boars and the genetic relationships with its component traits in Duroc pigs.”Journal of animal science 85.8 (2007): 1873-1879.

PO-01-80

Post-transcriptional Gene Silencing (PTGS) of PRRS Virus in MARC-145 Cells by Small Interfering RNA (siRNA) Targeting ORF7 gene Region

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus which is the etiologic agent of PRRS, a disease of epidemic proportions in swine (Albina 1997; Rossow 1998; Prieto et al., 2005) PRRSV replicates in primary pig macrophages *in vitro* (Wills et al 1997; Thanawongnuwech et. al., 2000 and Murtaugh et al., 2002). PRRSV infection has been studied in MARC-145 cells, a PRRSV-permissive monkey kidney cell line. PRRSV-infected MARC-145 cell cultures may include death of some cells by modified apoptosis (Kim et al., 2002). The PRRSV has a positive single stranded RNA genome, approximately 15 kb in length and contains ten open reading frames (ORFs) (Kroses et al., 2008; Yun and Lee 2013). ORF1a and ORF1b encode viral replicase polyproteins, while ORF2a, ORF2b, and ORFs 3–7 encode the viral structural proteins GP2, E, GP3, GP4, GP5, M, and N, respectively (Murtaugh et al., 1995; Wu et al., 2001). The ORF7 encodes the structural protein call "Nucleocapsid protein" (N protein), the most abundant viral protein in virus-infected cells (Snijder and Meulenberg 1998) and the most immunodominant antigen in the pig immune response to PRRSV (Murtaugh et al., 2002). RNA interference (RNAi) is a eukaryotic post-transcriptional control mechanism involving degradation of a target mRNA by double stranded RNA (dsRNA) to inhibit homologous gene expression at the RNA level. The specificity is sequence based and depends on the sequence of one strand of the dsRNA corresponding to part or all of a specific gene transcript (Fire 1999; Price and Gatehouse 2008; Borgio 2010). The degradation of dsRNA by dsRNA-specific endonucleases referred to as Dicers is mediated through the production of small interfering RNAs or short interfering RNAs (siRNAs) (Bernstein et al., 2001). The siRNAs are 21 bp dsRNA fragments carrying two base extensions at the 3´ end of each strand; one strand of the siRNA is assembled into an RNA-induced silencing complex (RISC) in conjunction with the argonaute protein domain, which responsible for mRNA target degradation (Price and Gatehouse,2008). The siRNA technique would be applied for interfering PRRSv replications. Thus, the objective of this study were to directly *in vitro* interfere the PRRSv targeting ORF7 gene region by siRNA and to measure efficiency of inhibition of viral replication in MARC-145 cultured cell.

Materials and Methods

siRNA sequence selection

Sequences from the ORF 7 gene of porcine respiratory and reproductive syndrome virus strain VR2332 (GenBank: EF536003.1) were designed based on the website siRNA designing tools and synthesized by Invitrogen (USA). The siRNA duplexes were resuspended in 0.1% DMPC treated water to obtain 20 μM solution.

Cells culture and PRRSv stock preparation

MARC-145 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) contained 5% heat-inactivated fetal calf serum (FBS; GIBCO, USA) and antibiotics (100 μg/ml streptomycin and 100 U/ml. penicillin). The maintenance medium was not contained FBS and antibiotic. MARC-145 cells were trypsinized and seeded in 24 well plates at $0.5x10^5$ cells/well and incubated 24 hours before being transfection. When the cell obtained 50-60% confluence, they were washed and replace with the maintenance medium. North America strain of PRRSv was propagated form a commercial modified live vaccine (Ingelvac PRRS®;Boehringer Ingelheim, USA) in MARC-145 cultured cell.

siRNA transfection and PRRSv infection

siRNA-transfection reagent complexes were contained 20 pmol of siRNA-ORF7-US in 100 μl serum free medium with 4 μl Lipofectamine® RNAIMAX reagent (Invitrogen, USA) following the according to the manufacturer's recommendations. After being incubated in a 5% CO_2 at 37°C for 4 hours, the transfection complex was removed and the medium was replaced by growth medium. The cells were inoculated with PRRSv at a multiplicity of infection (MOI) of 1. The cultures were then incubated in a 5% CO_2 at 37°C for 1 hour and then the culture medium was changed. The co-transfected MARC-145 were then incubated for 48 hour in a 5% CO_2 at 37°C and

determined by cytopathic effect and RT-PCR

Total RNA extraction and RT-PCR

Total RNA was extracted form co transfected cell with supernatant by High pure viral RNA kit (Roche, Germany) following the manufacturer's recommendations. A RT-PCR was conducted by using a Superscript™ III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogen, USA), according to manufacturer's instructions with primer sequence of ORF7_FT2: 5'-GTGAGCGGCAATTGTGTCTGTCG-3' and ORF7_N26: 5'-GCCCTAATTGAATAGGTGAC-3' (Trang et al, 2015). Following a cDNA synthesis step at 55°C for 30 min, 1 cycle, Initiation step of amplification were performed at 94°C for 2 min, Denaturation step at 94°C for 15 sec, Annealing step at 55°C for 30 sec, and Extension step at 70°C for 1 min. Gel electrophoresis were performed using 5 μl of samples into 2% agarose gel with Gel red™ Nucleic acid stain (Biotium, USA) and extracted positive band form agarose gel by QIAquick® Gel Extraction Kit (Qiagen, Germany) following the manufacturer's recommendations. The purified products were quantified using a Nano drop spectrophotometer by MaestroNano (Maestrogen, Taiwan), then calculated for DNA concentration as 260/280 absorbance ratio.

Results

After co-transfection experiment, RNA extraction and RT-PCR methods used in this study were successful in all transfected samples. DNA degradation was measured using agarose gel electrophoresis (Figure 1). The amount of DNA concentrations are displayed in Table 1.

Discussion

In this research, siRNA-US targeting PRRSv ORF7 coding sequences were designed via online tool. Then were co-transfected in MARC-145 cell with PRRSv that siRNA-US can potent to inducing gene silence. The experimental results demonstrated that the cells in the treatment group had a trend of lower DNA concentration of PRRSv when compared with those in the viral control group. This result might be explained that siRNA-US is one site specific targeting on ORF-7 region, resulting in incompletion of interfering PRRSv replication. This result would be in agreement with the report of Yang et al (2011) who demonstrated that three site specific targeting N-gene siRNA expressing plasmids in MARC-145 were resulted different efficacy of inhibition of PRRSv replication.

The concentrations of DNA in samples were usually estimated by running on an agarose gel and then estimated concentrations of semi-quantitative at confounded when numerous bands or a 'smear' of DNA were observed. For a more accurate determination of the concentration of DNA in a sample, a UV spectrophotometer (such as Nano drop spectrophotometer) is commonly used for DNA solutions. However, real time RT-PCR (TaqMan® or SYBR® Green) for measuring gene expression levels would give higher sensitive and specific (Sandy et al., 2005), accompanied with expensive costs.

Therefore, under current *in vitro* study, it could be concluded that there is possible to use the siRNA technique targeting on ORF7 gene region of PRRSv to inhibit post-transcription of PRRSv infected in MARC-145 cells.

Conclusion

The use of the siRNA technique targeting on ORF7 gene region of PRRSv is possibly inhibit post-transcription of PRRSv infected in MARC-145 cells.

Acknowledgement

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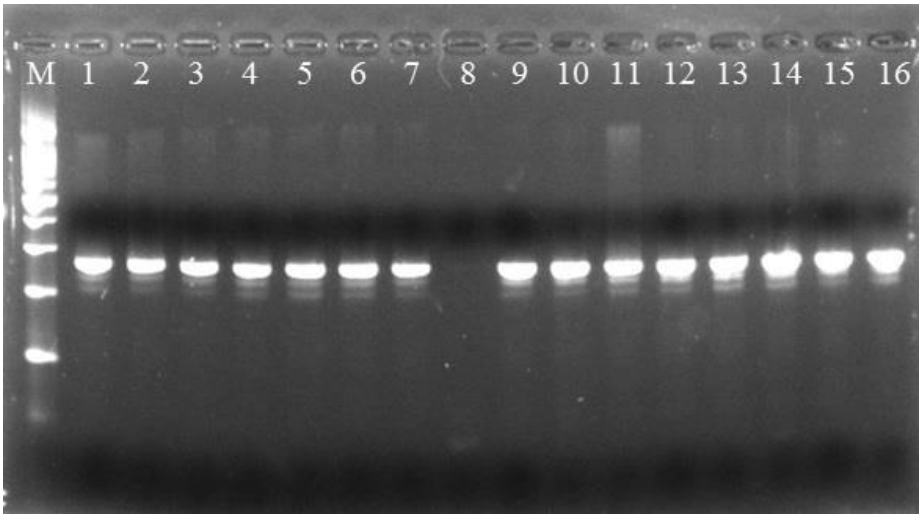

Figure 1. Gel red™ Nucleic acid stained agarose gel (2%) electrophoresis demonstrating RT-PCR amplified products of PRRSv N gene form co-transfected MARC-145 cell. Lanes are as follow: M=100 bp ladder; 1-7 = co-transfected siRNA and PRRSv in MARC-145 cell, 8 = negative control, 9 -15 = PRRSv infected MARC-145 cell (viral control group) and 16 = positive control.

Table 1. Mean ± SD of DNA concentration (ng/ul)

DNA concentration (ng/ul)		
PRRSv	siRNA	*p-value*
333.19 ± 31.37	293.15 ± 32.94	0.06

References

Albina E. 1997. Epidemiology of porcine reproductive and respiratory syndrome (PRRS): an overview. Vet Microbio. 55:309-16.

Bernstein E., Caudy A. A., Hammond S. M., Hannon G. J. 2001 Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature, 409:363–366.

Fire A., Xu S., Montgomery M.K., Kostas S.A., Diver S.E., Mello C.C. 1998. Potent and Specific Genetic Interference by Double-stranded RNA in Caenorhabditis elegans. Nature. 391:806-811.

Kim T.S., Benfield D.A., Rowland R.R.R. 2002. Porcine reproductive and respiratory syndrome virus-induced cell death exhibits features consistent with a non-typical form of apoptosis. Virus Res. 85:133-140.

Kroese M.V., Zevenhoven-Dobbe J.C., Bos-deRuijter J.N., Peeters B.P., Meulenberg J.J., Cornelissen L.A. 2008. Then sp1α and nsp1β papain-like autoproteinasesare essential for porcine reproductive and respiratory syndrome virus RNA synthesis. J. Gen. Virol. 89:494–499.

Meng X.J. 2000 Heterogeneity of porcine reproductive and respiratorysyndrome virus: implication for current vaccine efficacyand futurevaccine development. Vet Microbio. 74:309-329.

Murtaugh M.P., Elam M.R., Kakach L.T. 1995. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus. Arch. Virol. 140:1451–2160.

Murtaugh M.P., Xiao Z., Zuckermann F. 2002. Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. Viral Immunol. 15:533-47.

Price D. R. G., Gatehouse J. A. 2008. RNAi-mediated crop protection against insects. Trends in Bio technology, 26 (7):393 – 400.

Prieto C., Castro J.M. 2005. Porcine reproductive and respiratory syndrome virus infection in the boar: a review. Theriogenology. 63:1-16.

Rossow, K.D. 1998. Porcine reproductive and respiratory syndrome. Vet. Pathol. 35(1):1–20.

Sandy P., Ventura A., Jacks T. 2005. Mammalian RNAi: a practical guide. BioTechniques. 39(2):215-224

Snijder E.J., Meulenberg J.J. 1998. The molecular biology of arteriviruses. J Gen Virol. 79 (Pt 5):961-979.

Thanawongnuwech R., Halbur P.G., Thacker E.L. 2000. The role of pulmonary intravascular macrophages in porcine reproductive and respiratory syndrome virus infection. Anim Health Res Rev. 1:95-102.

Trang N.T.M.h., Makliang J., Kaenson W., Buapaichit K., Luengosluechakul S., Assavacheep p., Wattanaphansak S. 2015. Detection of PRRS and PCV2 Viral Antigen during the Severe Loss in Suckling and Nursery pigs. Proceedings of the 14th Chulalongkorn University Veterinary Conference. April 20-22, 2015, Bangkok, Thailand. 1:257-258.

Wills R.W., Zimmerman J.J., Yoon K.J., Swenson S.L., McGinley M.J., Hill H.T., Platt K.B., Christopher-Hennings J., Nelson E.A. 1997. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet Microbiol. 55:231-240.

Wu W.H., Fang Y., Farwell R., Steffen-Bien M., Rowland R.R.R., Christopher- Hennings J., Nelson E.A. 2001. A 10-kDa structural protein of porcine reproductive and respiratory syndrome virus encoded by ORF2b. Virology. 287:183–191.

Yang M., Xiang Q., Zhang X., Li X., Sylly S., Ding Z. 2011. RNA Interference Targeting Nucleocapsid Protein Inhibits Porcine Reproductive and Respiratory Syndrome Virus Replication in Marc-145 Cells. Virology J. 8:491.

Yun S.I., Lee Y.M. 2013. Overview: replication of porcine reproductive and respi-ratory syndrome virus. J. Microbiol. 51 (6):711–723.

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Availability of Lactobacillus salivarius strain isolated from piglet feces as probiotic uses

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Introduction

Lactobacillus salivarius is a gram positive bacteria that has been found in the human or animal. In recently Several of them used as probiotics, should exclude pathogenic bacteria in livestock gut and human gut. Thus *L. Salivarius* which have pontential of probiotics, not yet found in probiotics market in South Korea. *L. Salivarius* is very important source of substitute antibiotics because they secrete lactic acid or bacteriocin. In this study, we isolated *L. salivarius* strains from piglets feces in south korea and characterized their probiotic potentials.

Materials and Methods

Salivarius isolation.We isolated 500 lactic acid bacteria colonies from feces of 10 post-weaning piglets using MRS agar plates. piglet feces was incubated on MRS plate and modified multiplex PCR.

Antimicrobial Test

We search for available probiotics potentials among antimicrobial activity in Salmonella enterica Typhimurium. We used modified disk diffusion method. 50 L. Salivarius strains tested, 10^4 cfu of the Salmonella enterica Typhimurium spread into TSA agar. 50 L. salivarius strains supernant Samples(50ul) were pipetted into paper disk. The plates were then incubated at 37.5°C. Antimicrobial activity was recorded as growth free inhibition zones.

Acid tolerance test

We selected to antimicrobial activity good ability one *L. salivarius* strains, to test acid tolenrance of the *L. salivarius, Lactobacillus plantarum, Lactobacillus reuteri* and *Lactobacilus fermentum*. The cultures were grown in MRS culture. Then incubated *Lactobacillus spp.* 1ml input 10ml pH4 MRS broth. each 0min, 30min, 90min sampling after on MRS agar plate and survivable bacterial colony counted.

Bile salt tolerance test

We used modified bile salt tolerance test. Overnights incubated *Lactobacillus spp.* In MRS broth pipetted 1ml each 0min, 30min, 90min 0.5% bile salt MRS broth. After we survivable counted bacterial colony.

Bacterial Growth test

We used modified growth test to test *Lactobacillus spp.* Overnight incubated and each pipetted 1ml fresh broth. The sample were analyzed at 0h, 1h, 2h,3h 4h, 5h, 6h, 7h, 8h, 9h, 10h, 11h, 12h, 24h cultivation, and was twice by measuring optical density (O.D 600nm).

Result

Salivarius isolation.we isolated 500 LAB colony in 10post-weaning piglet incubated 37C and identified 120 *Lactabcillus* strains including *L. salivarius*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacilus fermentum* by using modified Multiplex-PCR, and 50 L. salivarius strains.

Antimicrobial Test

50 *L. salivarius* strains used disk diffusion test in Salmonella enterica Typhimurium. the results, observed to one strains high free inhibition zones and we repeatability test after we selected *L. salivarius* strains with high against Salmonella enterica Typhimurium.

Acid tolerance test, Bile salt tolerance test

All 4 Lactobacillus spp. were to grow in each MRS broth supplemented with pH4, 0.5% bile salt. Observed we seleted L. salvarius starains other Lactobacillus species similar pattern. we confirmed probiotics potentials.

Bacterial Growth test

As the result, selected *L. Salivarius* strains showed high growth better than other Latobacillus spp. and we compared our *L. Salivarius* strains with commercial probiotics growth data.

Conclusions

In this study, we confirmed *L. salivarius* strains probiotic potential. In pig industry, *L. salivarius* starins used as probiotics commercial products almost not found. Therefore if we develop *L. salivarius* strains used as probiotics, they should contribute piglet healths and human healths. theseresults indicate that *L. salivarius* LS.EBK strain have apotential characteristics for probiotic uses in livestock industries. In the future,we willverify its probiotic effects on piglets by animal trials.

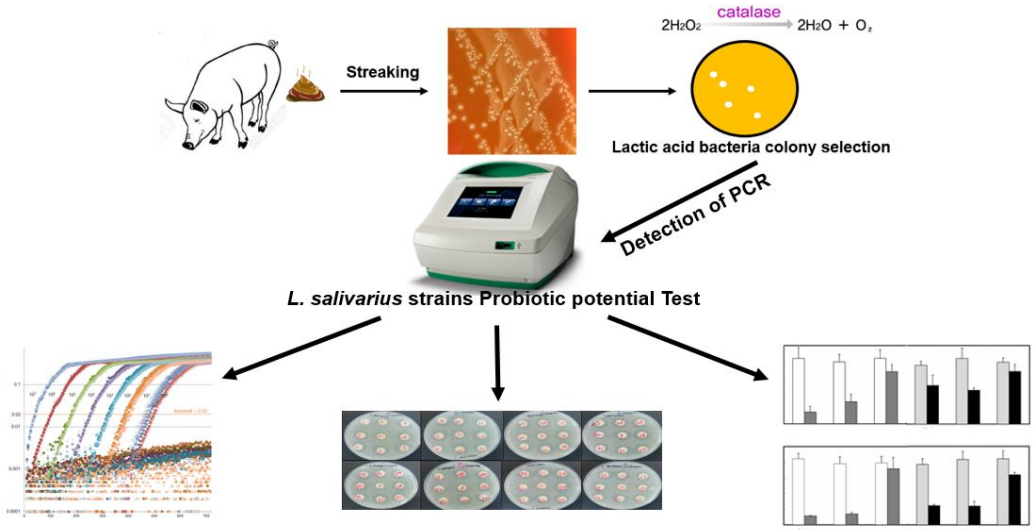

Figure 1. Experimental steps. For isolation *L. salivarius* strains, we were used piglet feces and modified Multiplex PCR and them, we characterized probiotic potential.

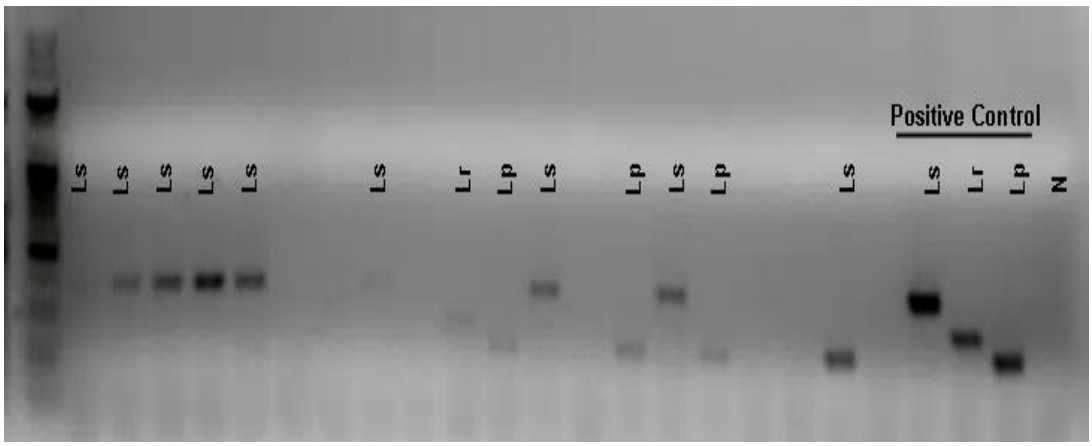

Figure 2. *L. salivarius* strains isolated using modified Multiplex PCR. Ls = *L. salivarius 144,* Lp = *L. plantarum 133,* Lr = *L. reuteri* F36, N=Negative

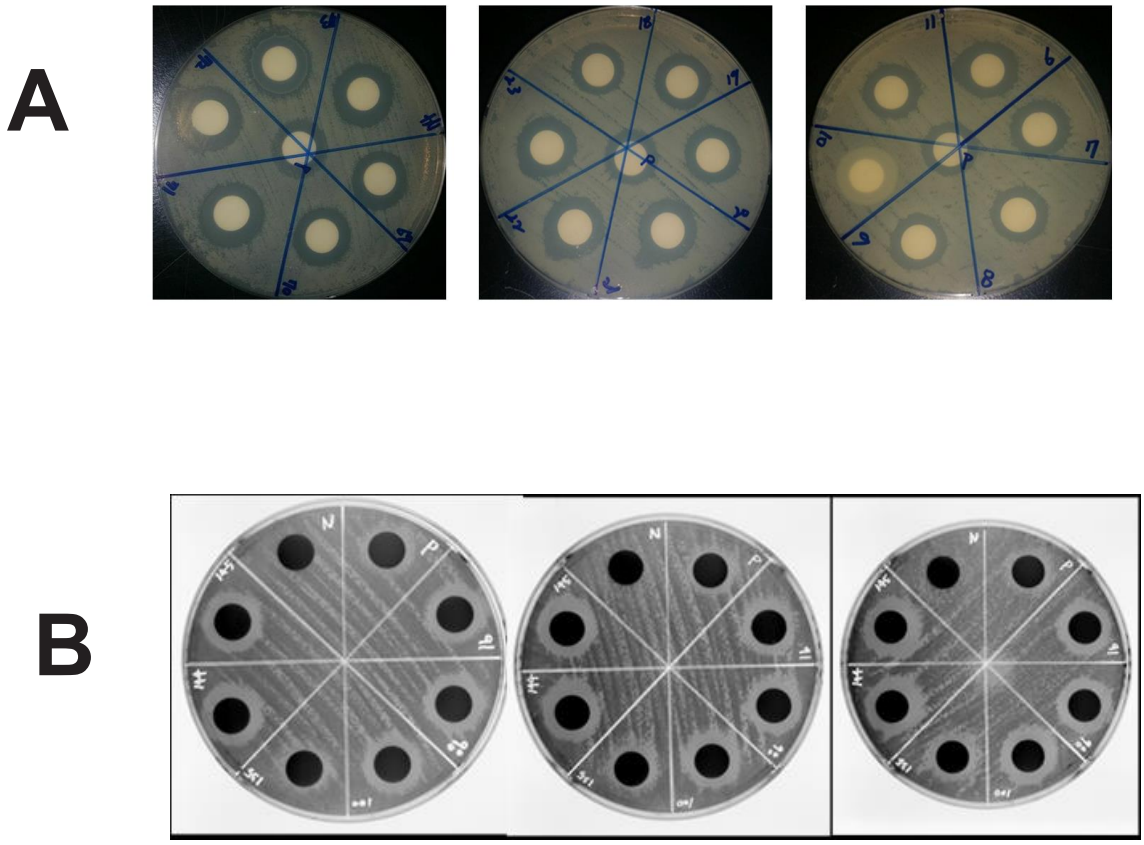

Figure 3. Antimicrobial activity against pathogenic *Salmonella enterica serovar* Typhimurium. A: first test and B:reproducibility test.

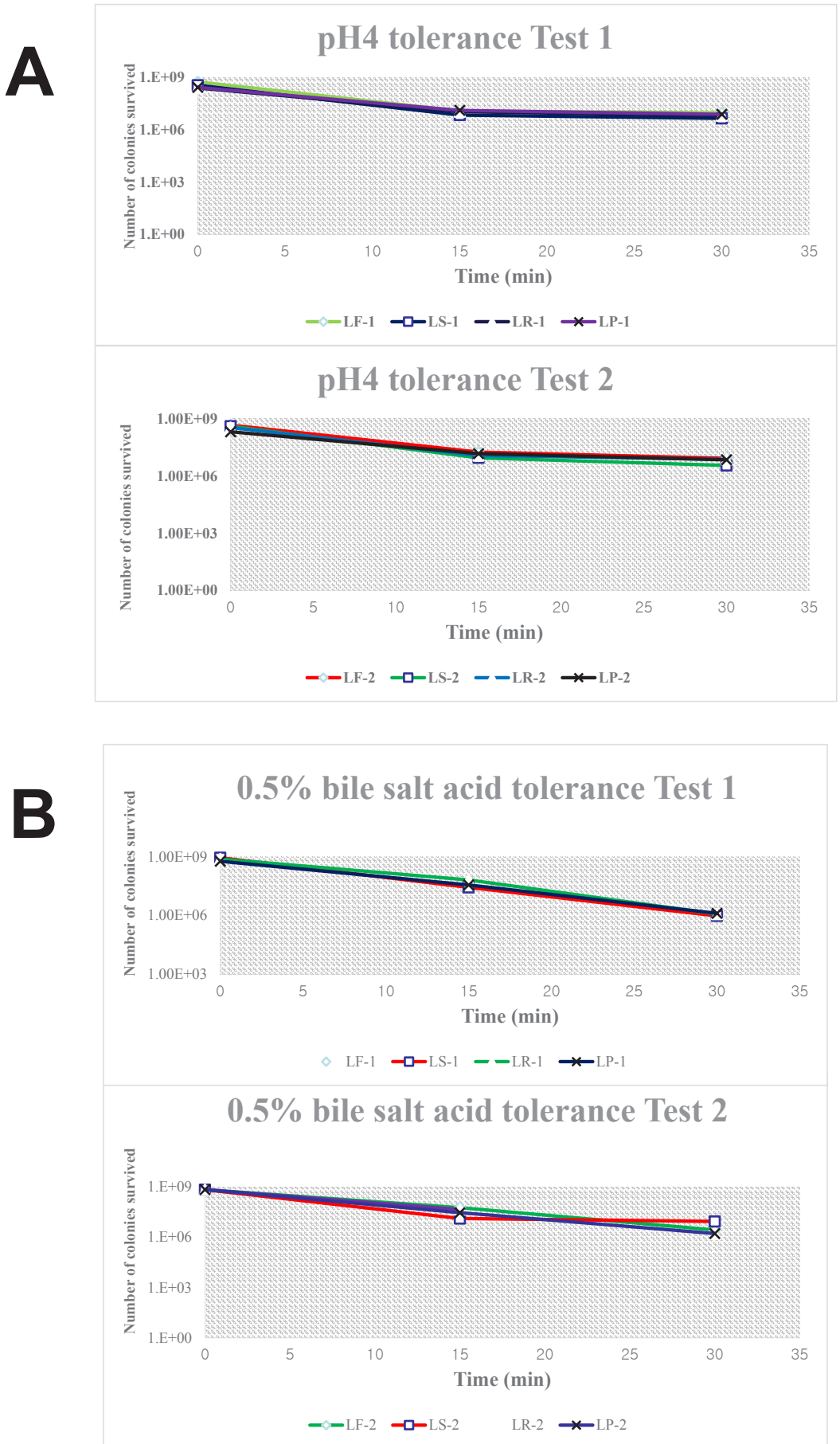

Figure 4. Survival test: pH4 resistance test, bile resistance test. A: pH4 resistance test result, B:0.5% bile resistance test result.

Reference

- Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., ... & Kiely, B. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *The American journal of clinical nutrition*, *73*(2), 386s-392s.
- Peran, L., Camuesco, D., Comalada, M., Nieto, A., Concha, A., Diaz-Ropero, M. P., ... & Galvez, J. (2005). Preventative effects of a probiotic, Lactobacillus salivarius ssp. salivarius, in the TNBS model of rat colitis. *World journal of gastroenterology: WJG*, *11*(33), 5185-5192.
- O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., ... & Quigley, E. M. (2005). Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles.*Gastroenterology*, *128*(3), 541-551.
- Ngoc Lan, P. T., Binh, L. T., & Benno, Y. (2003). Impact of two probiotic Lactobacillus strains feeding on fecal lactobacilli and weight gains in chicken.*The Journal of general and applied microbiology*, *49*(1), 29-36.
- O'Hara, A. M., O'Regan, P., Fanning, A., O'Mahony, C., MacSharry, J., Lyons, A., ... & Shanahan, F. (2006). Functional modulation of human intestinal epithelial cell responses by Bifidobacterium infantis and Lactobacillus salivarius. *Immunology*, *118*(2), 202-215.

PO-02-3

Effect of feeding soybean meal protected with mangosteen peel liquid on lactating dairy cows

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Abstract

The aim of this study was to determine the effect of soybean meal protected (SBM) with mangosteen peel liquid (SBMPMSL) and urea supplementation on feed intake and performance of lactating dairy cows. Four, multiparous Holstein crossbred dairy cows in mid-lactation with initial body weight (BW) of 410 ± 20 kg were randomly assigned according to a 4×4 Latin square design to receive four dietary treatments. The dietary treatments were as follows: T1 = unprotected SBM, T2 = (SBMPMSL), T3 and T4 = (SBMPMSL) with urea addition at 1 and 2 % in concentrate, respectively. Cows were fed with concentrate diets at a ratio of concentrate to milk yield of 1:2 and rice straw was fed*ad libitum*. SBMPMSL remarkably increased milk yield, fat and protein percentage but had no effect on milk lactose percentage. Based on this study, it could be concluded that supplementation of SBMPMSL in the concentrate supplement containing urea remarkably improved milk yield and milk composition in lactating dairy crossbreds fed on rice straw.

Introduction

Soybean meal (SBM) is the most commonly used protein supplement in beef and dairy diets. With excellent palatability and higher amino acid availability, soybean meal also contains a high quality post-ruminal essential amino acid index that is next to only ruminal microbial protein beating all other undegradable protein sources (Chandler, 1989). SBM are excellent sources of RDP and can be extensively degraded (≥57.4 to 69.6%) by ruminal microbes (NRC, 2001). Various methods of treating SBM have been studied to alter the rate and extent of protein degradation in the rumen. Various methods of treating proteins have been used to reduce their degradation in the rumen and they can be categorized into chemical and physical treatments. Some of the techniques, e.g., extrusion, roasting, expeller, lignosulfonate, formaldehyde have been used to protect SBM from ruminal degradation. Treating SBM by these methods increases its ruminal bypass protein content up to 70% (Waltz and Stern, 1989). The use of heat is more environmentally acceptable than the chemical treatment methods (Soltan, 2009; Giallongo et al., 2015).

Mangosteen (*Garcinia mangostana*) peel is a fruit by-product containing a high level of condensed tannins and saponins which can exert a specific effect against rumen protozoa, while the rest of the rumen biomass remains unaltered (Poungchompu et al.,2009).However, there is little available data about the method of the heat treatment and plant secondary compounds methods on ruminal fermentation in dairy cows. Therefore, the aim of the present study was to investigate the effect of feeding soybean meal protected with mangosteen peel liquid on feed intake, rumen fermentation, milk production and milk composition in lactating dairy cows.

Materials and method

Preparing of SBMPMSL

Mangosteen peel solution mixture was prepared by using ration of water: mangosteen peel at 20:4, mixed well, and then sprayed on soybean meal to 100 kg and then heated at 140°C for 1 hour, and 500g of SBMPMSL were sampled for chemical analysis.

Animals, experimental design and dietary treatments

Four, multiparous Holstein crossbred dairy cows in mid-lactation with initial body weight (BW) of 410 ± 20 kg were randomly assigned according to a 4×4 Latin square design to receive four dietary treatments. The dietary treatments were as follows: T1 = unprotected SBM, T2 = (SBMPMSL), T3 and T4 = (SBMPMSL) with urea addition at 1 and 2 % in concentrate, respectively. Cows were fed with concentrate diets at a ratio of concentrate to milk yield of 1:2 and rice straw was fed*ad libitum*. Animals were housed in individual pens and individually fed with dietary treatments twice daily at 06.00 and 16.00 after each milking time. Clean fresh water and mineral blocks were available at all times. Body weights of each cows were weighed at the first and last day of each period for feed offered calculation. Milk yield was recorded during the 21 day-period and samples were collected during the

last 7 days of each period.

Sampling procedure, data collection and analysis

Feed offered and refusals were recorded daily throughout the experimental period for dry matter (DM) intake measurement. Samples of concentrate, rice straw and SBMPMSL were collected daily during the collection period and composited by period for chemical analysis. At the end of each period, rumen fluid and jugular blood samples were collected at 0 and 4 h after feeding. Approximately 200 ml of rumen fluid was taken from the rumen by a stomach tube connected with a vacuum pump at each time at the end of each period. Rumen fluid was immediately measured for pH using a portable pH meter (Hanna instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were filtered through four layers of cheesecloth. Samples were used for ammonia- nitrogen (NH_3-N) analysis using the Kjeltech Auto 1030 analyzer. A blood sample (about 10 ml) was collected from a jugular vein (at the same time as rumen fluid sampling) into tubes containing 12 mg of EDTA, and plasma separated by centrifugation at 500x*g* for 10 minutes (Table Top Centrifuge PLC-02, U.S.A.) and stored at -20 °C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967).

Milk yield were recorded daily and milk samples were composited daily, according to yield, for both the morning and afternoon milking time, preserved with 2-bromo-2 nitropropane-1, 3- dial, and stored at 4°C until analysis for fat, protein, lactose, totals solids, and solids-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO).

Statistical analysis

All data were subjected to ANOVA according to a 4 × 4 Latin square design using the General Linear Models (GLM) procedures (SAS, 1998). The results were presented as mean values with the standard error of the means. Difference among means with $P<0.05$ was accepted as statistical differences while $0.05<P<0.10$ was accepted as a tendency. Treatment means were compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results and discussion

Rice straw and total feed intake were not affected by SBMPMSL and level of urea in concentrate (Table 1). Ruminal pH values did not differ among treatments and ranged from 6.7 to 6.8. These values were optimal for normal rumen fermentation, microbial growth and microbial activity (Wanapat, 2000). Moreover, BUN and MUN in present study were not affected by feed supplementation, the normal ranged as reported by Roseler et al. (1993) who found that balanced diets for lactating dairy cows were associated with average BUN concentration of 15 mg/dl and average MUN concentration of 5 to 16 mg/dl (Jonker et al., 1999). The ruminal NH_3-N concentration was reduced by SBMPMSL supplementation, this result similar with El-Waziry et al. (2007), who reported the concentration of ammonia nitrogen were reduced when SBM replaced by treated SBM. The reduction of ammonia nitrogen in the rumen may indicate that treated SBM lowered proteolysis, degradation of peptides and deamination of amino acids in the rumen (New bold et al., 1990). Supplementation of SBMPMSL and urea in concentrate remarkably increased milk yield and protein percentage but had no effect on milk lactose (Table 2). Soltan (2009) and Giallongo et al. (2015) reported protected SBM products in dairy cow diets has a positive effect on milk production in dairy cows.

Conclusions and recommendations

Based on this study, it could be concluded that supplementation of SBMPMSL in the concentrate supplement containing a high level of urea significantly enhanced milk yield and milk composition in lactating dairy crossbreds fed on rice straw. The SBMPMSL could be used as a high quality rumen protected protein source in improving performance of lactating dairy cows in terms of milk production and milk quality.

Keywords: Soybean meal, Milk production and composition, Dairy cows, Mangosteen peel

Acknowledgement

Authors would like to express their most sincere thanks to Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand and the Thailand Research Fund (TRF) through the International Research Network (IRN) program for their kindly support on research fund and facility.

Table 1. Effect of feeding soybean meal protected with mangosteen peel liquid on voluntary feed intake and ruminal fermentation in lactating dairy cows.

Items	Dietary treatments				SEM	P-value
	Control	SBMPMSL	SBMPMSL + 1%U	SBMPMSL + 2%U		
Rice straw DM intake						
kg/day	3.4	3.7	3.6	3.9	0.21	0.51
%BW daily	0.8	0.9	0.9	0.8	0.43	0.34
Concentrate DM intake						
kg/d	8.9	9.5	10.4	10.8	1.31	0.58
%BW daily	2.0	2.1	2.4	2.3	0.41	0.43
Total DM intake						
kg/d	12.3	13.2	13.6	14.7	0.97	0.88
%BW daily	2.8	3.0	3.3	3.1	0.32	0.09
Ruminal fermentation						
pH	6.7	6.8	6.8	6.8	0.47	0.44
Temperature °C	39.7	39.6	39.5	39.6	0.32	0.09
NH3-N	15.4 [b]	12.1 [a]	16.1 [c]	17.0 [d]	0.86	0.04
Blood urea nitrogen	14.7	12.6	16.1	16.8	0.73	0.14

[a,b,c,d] Means in the same row with different superscripts differ (P<0.05).
Control = Unprotected soybean meal
PSBM = Protected soybean meal products
PSBM +1%U = Protected soybean meal products + 1% urea
PSBM +2%U = Protected soybean meal products + 2% urea

Table 2. Effect of feeding soybean meal protected with mangosteen peel liquid on milk yield and composition in lactating dairy cows.

Items	Dietary treatments				SEM	P-value
	Control	SBMPMSL	SBMPMSL + 1%U	SBMPMSL + 2%U		
Production						
Milk yield, kg/d	17.8 [a]	19.2[b]	21.8 [c]	23.5 [d]	0.82	0.04
3.5% FCM, kg/d[1]	18.6 [a]	21.1 [b]	24.3 [c]	26.2 [d]	0.95	0.03
Milk composition, %						
Fat	3.8	4.1	4.2	4.2	0.58	0.47
Protein	3.1[a]	3.4 [b]	3.7 [c]	3.8 [c]	0.24	0.04
Lactose	4.6	4.7	4.7	4.7	0.17	0.35
Solids not fat	9.2	9.1	9.2	9.3	0.39	0.42
Total solids	13.8	14.1	14.3	14.0	0.47	0.33
Milk urea N, mg/dL	12.4	12.6	12.8	13.1	0.72	0.54

[a,b,c,d] Means in the same row with different superscripts differ (P<0.05). [1]3.5% FCM (fat collected milk) = 0.432 (kg of milk/d) + 16.23 (kg of fat).
Control = Unprotected soybean meal
PSBM = Protected soybean meal products
PSBM +1%U = Protected soybean meal products + 1% urea
PSBM +2%U = Protected soybean meal products + 2% urea

References

Chandler, P.T. 1989. Achievement of optimum amino acid balance possible. Feedstuffs 61 (26): 24.

Crocker, C.L.1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol. 33: 361-365.

El-Waziry, A.M., M.E.A., Nasser, A.M.A. Sallam, A.L. Abdallah and I.C.S. Bueno. 2007. Processing methods of soybean meal, 2. Effect of autoclaving and Qucbraho tannin treated soybean meal on gas production and rumen fermentation in vitro .J. Appl. Sci., 3: 17-24.

Jonker, J.S., R.A. Kohn, and R.A. Erdman. 1999. Milk urea nitrogen target concentrations for lactating dairy cows fed according to national research council recommendations. J. Dairy Sci. 82:1261-1273.

Giallongo, F., J. Oh, T. Frederick, B. Isenberg, D. M. Kniffen, R. A. Fabin, and A. N. Hristov. 2015. Extruded soybean meal increased feed intake and milk production in dairy cows. J. Dairy Sci. 98: 6471- 6485.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. 7th Rev. ed.Nat. Acad. Press, Washington, DC.

Newbold, C.J., R.J. Wallace and N.Mckain. 1990. Effects of the ionophore tetronasin on nitrogen metabolism by ruminal microorganisms in vitro. J. Anim. Sci., 68: 1103-1111.

Poungchompu, O., M. Wanapat, C. Wachirapakorn, S. Wanapat, and A. Cherdthong. 2009. Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangosteen peel and soapberry fruit. Arch Anim Nutr, 63: 389 - 400.

Roseler, D.K., J.D. Ferguson, C.J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk non protein in Holstein cow. J. Dairy Sci. 76: 525-534.

SAS, 1998. User's Guide: Statistic, Version 6, 12th ed. SAS Inst. Inc., Cary, NC.

Soltal, M.A., 2009.Rumen fermentation characteristics and lactation performance in dairy cows fed different rumen protected soybean meal product. Pak. J. Nutr. 8(5): 695-703.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill Book Company, New York, NY.

Waltz, D.M. and M.D. Stern, 1989. Evaluation of various methods for predicting soy-bean protein from degradation by rumen bacteria. Anim Feed Sci. Technol., 25: 111-122.

Wanapat, M. 2000. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. Asian-Aust. J. Anim. Sci. 13 (Suppl.): 59 - 67.

PO-02-4

Effect of the fast on leptin secretion of saliva in sheep

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Leptin secreted by white fat cells plays an important role in control of the fat mass and management of the weight by the inhibition of the appetite and hypermetabolism. In monogastric animals, leptin is secreted into saliva, and these affect oral cavity and gastrointestinal tract (Gröschl, 2009.; Randeva et al., 2003). In ruminants, volume and the contents of the diet affect blood leptin concentration (Blache et al., 2000; Marie et al., 2001). However, the secretion kinetics of the leptin to saliva and effect by the fast in ruminants are unclear. Therefore in this study we aimed to investigate effect of the fast on leptin secretion of saliva in sheep.

Five Suffolk female sheep were used in this study. Before the experiment, a cannula was inserted in the one side parotid gland opening of the sheep. Blood and saliva samples were collected hourly for 54 hours from 7:00 a.m. Sheep were fed for one hour from 8:30 a.m. of the first day and the third day. Sheep were fasted for a short term (24 hours) on the second day. The concentration of collected samples of blood and saliva were measured using Bovine Leptin ELISA Kit. The 3-day sample was divided into feeding (Fed), fast (Fast) and re-feeding (Re-Fed), respectively, between from 7:00 to 13:00.

The plasma and salivary leptin concentration did not change with time in all groups (Figure 1 and 2). In addition, the difference was not recognized between groups. However, the amount of salivation significantly increased by the feeding of the re-feeding and significantly decreased afterwards (Figure 3). Therefore the amount of salivation of the leptin significantly increased by the feeding of the re-feeding, too and significantly decreased afterwards (Figure 4). Neither of the amount of salivation and the amount of salivation of the leptin changed by the fast. Carr and Titchen (1978) shown the feeding increases the amount of salivation temporarily. Therefore, it is suggested that the amount of salivation increased in both the feeding and the re-feeding in this experiment, but did not increase by the fast. And, it is suggested that increase of the salivation increases amount of leptin secretion in saliva.

From these, in this study, it is suggested that the fast has leptin secretion increase to saliva disappear, but does not affect the later re-feeding.

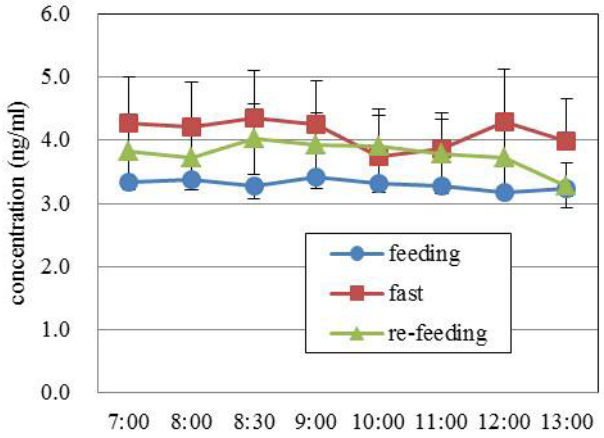

Figure 1. The plasma leptin concentration at the feeding, the fast and the re-feeding.

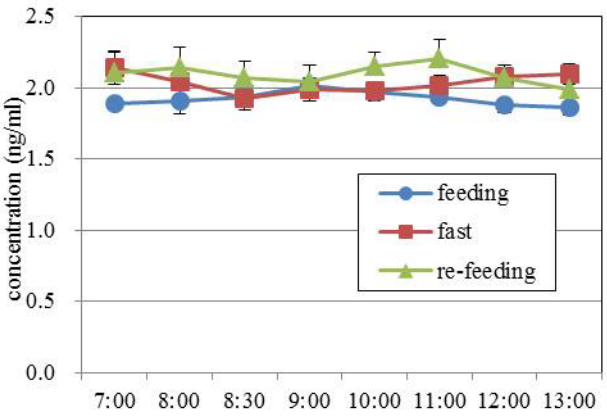

Figure 2. The salivary leptin concentration at the feeding, the fast and the re-feeding.

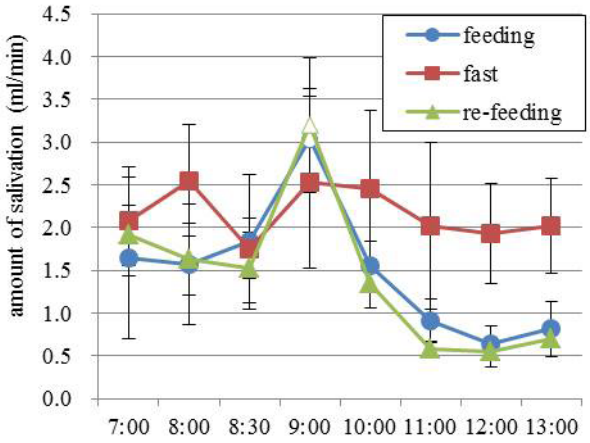

Figure 3. The amount of salivation at the feeding, the fast and the re-feeding.
○, △ significantly differ from 7:00 ($P < 0.05$, t-test)

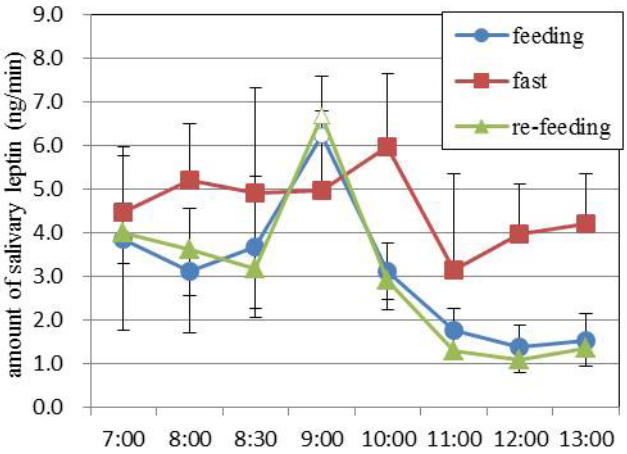

Figure 4. The amount of salivary leptin at the feeding, he fast and the re-feeding.
○, △ significantly differ from 7:00 ($P < 0.05$, t-test)

References

Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE and Martin GB. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. J Endocrinol. 165(3):625-37.

Carr DH and Titchen DA. 1978. Post prandial changes in parotid salivary secretion and plasma osmolality and the effects of intravenous of saline solutions. Q J Exp Physiol Cogn Med Sci. 63(1):1-21.

Gröschl M. 2009. The physiologicalrole of hormones in saliva. Bioessays. 31(8):843-52.

Marie M, Findlay PA, Thomas L and Adam CL. 2001. Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. J Endocrinol. 170(1):277-86.

Randeva HS, Karteris E, Lewandowski KC, Sailesh S, O'Hare P and Hillhouse EW. 2003. Circadian rhythmicity of salivary leptin in healthy subjects. Mol Genet Metab 78(3):229-35.

PO-02-7

Microbiota of dairy cow feces collected at various regions in the South of Vietnam

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Objectives

The dairy cow population in Vietnam has increased at 10% annual rate in the last decade, and is predicted to keep growing in the next 30 years. Although dairy production in hot tropical climate is a tough task, the population in the south is about half of the dairy cow heads in Vietnam. Fecal microbiota of cattle plays an important role for not only animal health and productivity but also environment including methane emission. It has been demonstrated that different management practices rather than geographical factors may affect the fecal bacterial community and metabolic potential.

In this study, we made a survey of fecal microbiota of dairy cows at 3 different regions in the south of Vietnam. Two major bacterial groups (*Bacteroides* spp. and *Clostridium* spp.) and *Lactobaciilus* spp. were examined by denaturing gradient gel electrophoresis (DGGE) and real-time qPCR. In addition, population of methanogenic bacteria were also quantified.

Methodology

Sample collection

A total of 74 fecal samples were collected from 15 dairy farms (4 to 5 individual cow samples per one farm) residing in 3 different geographical locations (Tay Ninh, Tien Giang, and Lam Dong) in the south of Vietnam. All animals were visibly healthy and no illnesses were reported when collecting feces samples. Dairy cows were divided in 3 groups based on sampling regions; 1) Tay Ninh where most feed consisted of elephant grass, wet brewers grains, concentrates, and corn silages (< 4 kg/cow/day), 2) Tien Giang where feed was mainly elephant grass, rice straw, concentrates, and fermented cassava and wet brewers grains, 3) Lam Dong where feed contained plenty of crop by-products such as corn stalk and sweet-potato. Questionnaire was carried out to understand the recent milk productivity of cows examined (Table 1).

Microbiota community analysis

DGGE was performed to determine bacterial communities by using *Bacteroides* spp., *Clostridium spp.*, and *Lactobacillus* spp. specific primers (Bartosch *et al.*, 2004; Hung *et al.*, 2008; Heilig *et al.*, 2002). The DGGE band patterns were converted to binary matrix and cluster analysis was carried out to demonstrate similarity and differences between farms and individual cows.

Real-time PCR was employed to determine total bacteria, *Bacteroides* spp., *Clostridium* spp., and *Lactobacillus* spp. In addition, methanogen population was examined by targeting methyl coenzyme-M reductase α -subunit (*mcr*A) gene (Stuart *et al.*, 2007).

Results and Discusion

According to DGGE band profiles and cluster analyses, *Bacteroides* spp. was shared at about 30% similarity index regardless of management, climate, and milk productivity. Cow-to-cow variation was seen greatly in this group even under the same management practice in the same farm. However, *Bacteroides* proportion to total 16S rRNA gene quantification appeared stable, suggesting that composition rather than population of *Bacteroides* spp. may vary probably due to interaction between host and the gut microbiota. Likewise, Guan *et al.* (2008) reported that influence of the host genetics may play an important role in the gut microbial structure. In addition to host genetics, the host body can create the abiotic environment, including temperature, pH, and moisture, while providing nutrients that would be used by microbiota (Sadowsky *et al.*, 2011).

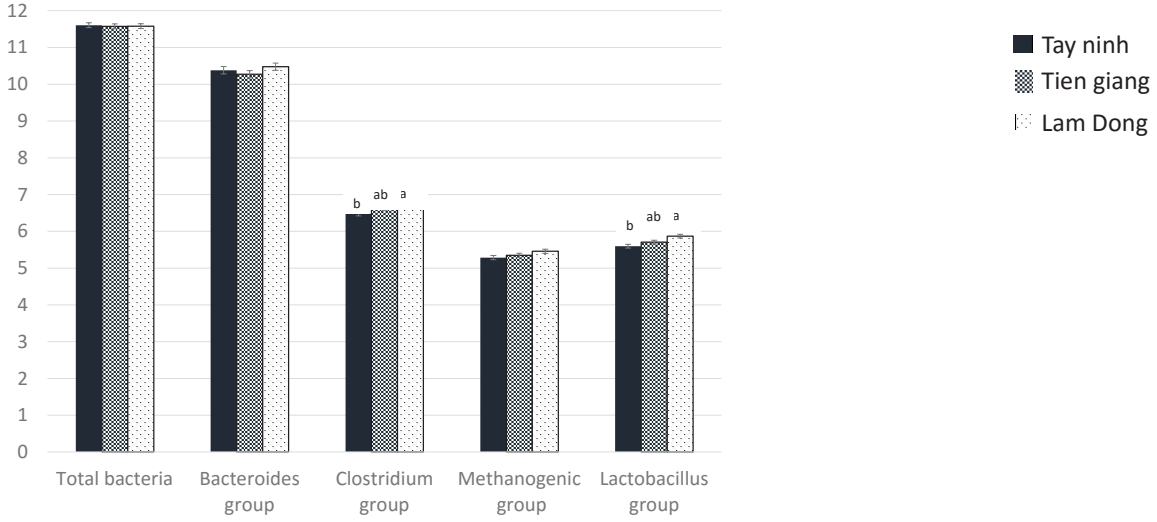
The population of *Clostridium* spp. was as small as 10^6 copies/g feces and demonstrated difference in the population from region to region. The dairy cows in Lam Dong, where highest milk productivity with plenty of by-products feeding was recorded, showed a highest *Clostridium* spp. population at $10^{6\text{-}7}$ copies/g feces. The microbiota structure of *Clostridium* spp. was highly stable among cows in the same region and similarity index was as high as 100% in the same farm. Because variation among cows of *Clostridium* spp. was small compared

with *Bacteroides* spp., *Clostridium* spp. could have greater potential to modulate gut function.
Interestingly, *Lactobacillus* spp. appeared to be less diverse in Tay Ninh, a high temperature region. Likewise, a higher population of *Lactobacillus* spp. was found in Lam Dong, where dairy cows showed good milk performance under relatively cool climate in Vietnam. Uyeno *et al.* (2010) reported that relative abundance of *Lactobacillus* spp. decreased as dairy cows aged. Han. *et al.* (2014) indicated that silage lactic acid bacteria composition was not critical in the formation of fecal *Lactobacillus* spp. population. Even though *Lactobacillus* spp. is minor group in the gut microbiota, the population and composition may be influenced by diet formulation and climate.
Methanogenic population was as small as 10^5 copies/g feces and no differences were seen between regions and cow-to-cow. Gill *et al*. (2011) reported that a positive relationship between enteric methane production and fecal methanogen content of steers, but other studies indicated that population structure of methanogens was the main contributor to methane production rather than total methanogen member (Zhou *et al.*, 2011). Further study is necessary to understand methanogenic archaeal structure to reduce methane production through gut fermentation.

Table 1: Regions and feeding regime, average milk performance, and average temperature of collecting samples.

Region	Temp (ºC)	Milk (kg/day)	Elephant grass (kg/day)	Fermented cassava (kg/day)	Corn silage (kg/day)	Brewers grains (kg/day)	Concentrates (kg/day)
Tien Giang	25-30	15-20	30	2-4	none	2-4	8-10
Tay Ninh	25-30	15-20	30	2-4	3-4	2-4	6-8
Lam Dong	14.8-19.9	20-30	30	none	>10	none	< 8

Fig 1. Populations of total bacteria, *Bacteroides* spp., *Clostridium* spp., *Lactobacillus* spp., and methanogens of dairy cow feces collected at Tay Ninh, Tien Giang, and Lam Dong. Difference letters in the same group differ significantly, * $P < 0.001$.



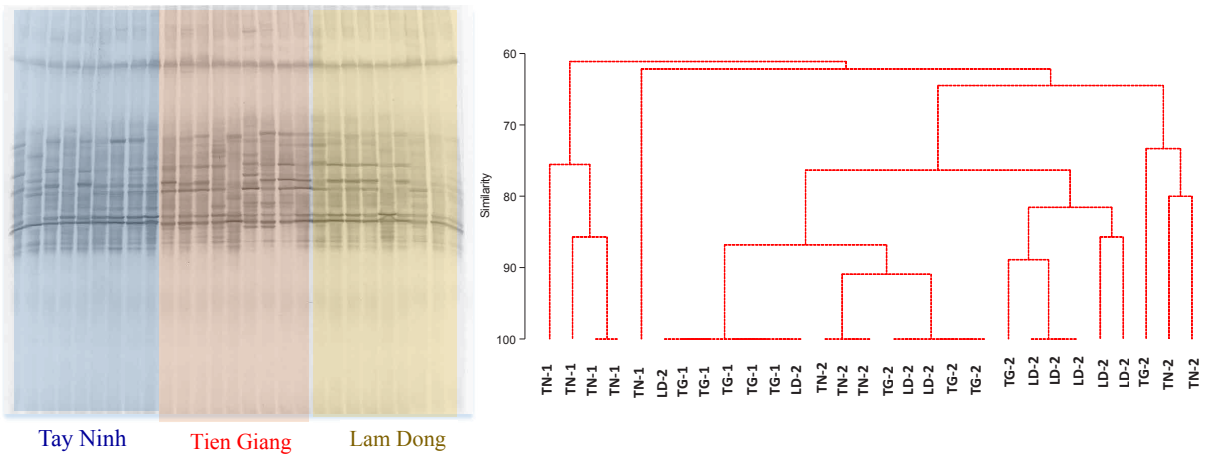


Fig 2. DGGE profile and cluster analysis of *Clostridium* spp. of dairy cow feces collected at Tay Ninh, Tien Giang, and Lam Dong. TN-1 stand for Tay Ninh farm 1; TN-2 stand for Tay Ninh farm 2; TG-1 stand for Tien Giang farm 1; TG-1 stand for Tien Giang farm 2; LD stand for Lam Dong farm 1, LD-2 stand for Lam Dong farm 2.

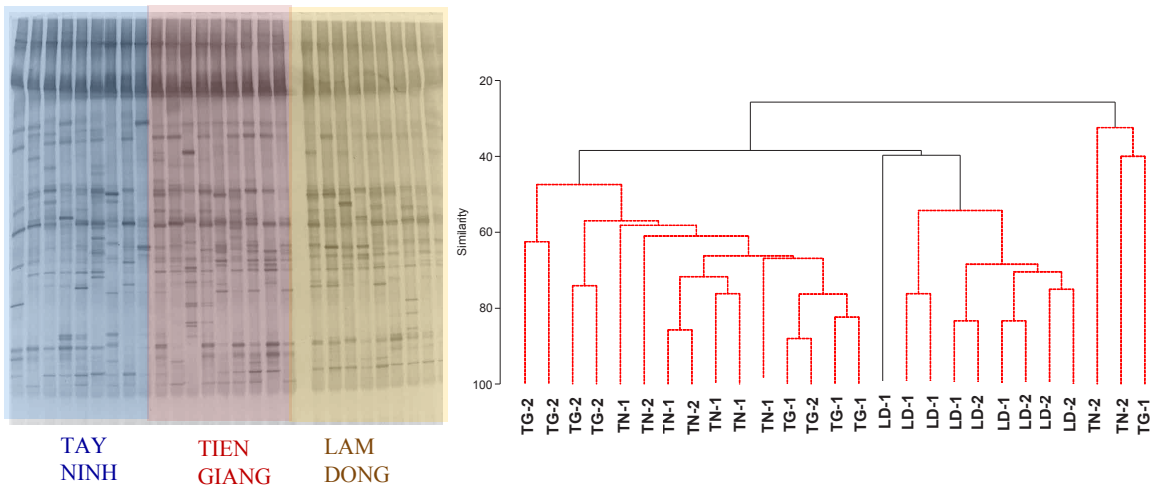


Fig 3. DGGE profile and cluster analysis of *Bacteroides* spp. of dairy cow feces collected at Tay Ninh, Tien Giang, and Lam Dong. TN-1 stand for Tay Ninh farm 1; TN-2 stand for Tay Ninh farm 2; TG-1 stand for Tien Giang farm 1; TG-1 stand for Tien Giang farm 2; LD stand for Lam Dong farm 1, LD-2 stand for Lam Dong farm 2.

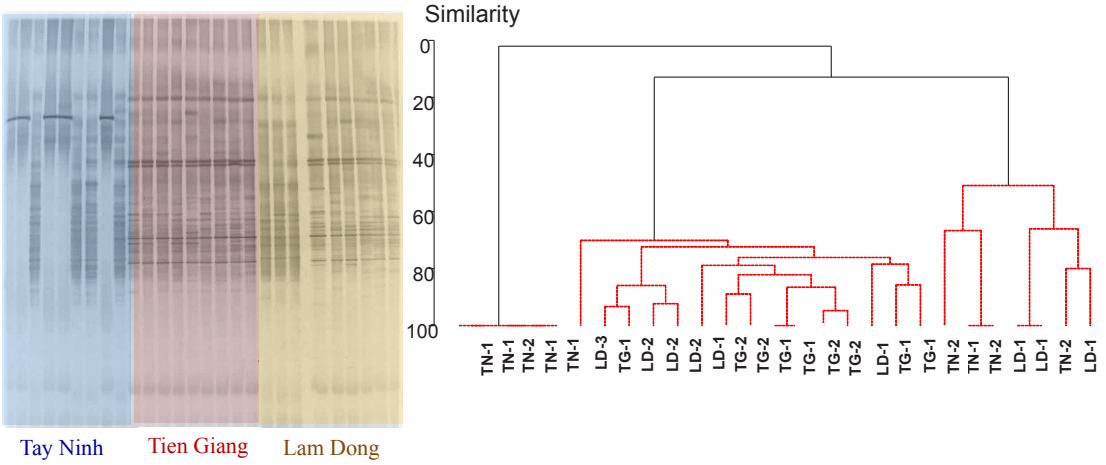


Fig 4. DGGE profile and cluster analysis of *Lactobacillus* spp. of dairy cow feces collected at Tay Ninh, Tien Giang, and Lam Dong. TN-1 stand for Tay Ninh farm 1; TN-2 stand for Tay Ninh farm 2; TG-1 stand for Tien Giang farm 1; TG-1 stand for Tien Giang farm 2; LD stand for Lam Dong farm 1, LD-2 stand for Lam Dong farm 2.

REFERENCES

Bartosch, S. F. (2004). Characterization of Bacterial Communities in Feces from Healthy Elderly Volunteers and Hospitalized Elderly Patients by Using Real-Time PCR and Effects of Antibiotc Treatment on the Fecal Microbiota. *Applied and Environmental Microbiology , 70*, 3575-3581.

Heilig, G. Z. (2002). Molecular Diversity fo Lactobacillus spp. and Other Lactic Acid Bacteria in the Human Intestine as Determined by Specific Amplification of 16S Ribosomal DNA. *Applied and Environmental Microbiology , 68*, 114-123.

Hung, C. C. (2008). Application of Clostridium-specific PCR primers on the analysis of dark fermentation hydrogen-producing bacterial community. *International Jounal of Hydrogen Energy , 33*, 1586-1592.

H. Han, Y. Ogata, Y. Yamamoto, S. Nagao, and N. Nishino (2014). Identification of lactic acid bacteria in the rumen and feces of dairy cows fed total mixed ration silage to assess the survival of silage bacteria in the gut. *J. Dairy Sci*. 97:5754-5762.

Stuart E. Denman, Nigel W. Tomkins and Christopher S. McSweeney (2007) Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. *FEMS Microbiol Ecol* 62, 313-322.

T. Rinttila, A. Kassinen, E. Malinen, L. Krogius and A. Palva (2004) Development of an extensive set of 16S r DNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology* 97, 1166-1177.

Zhou, M., Y. H. Chung, K. A. Beauchemin, L. Holtshausen, M. Oba, T. A. McAllister, and L.L Guan. 2011a. Relationship between rumen methanogens and methane production in dairy cow fed diets supplemented with a feed enzyme additive. *J. Appl. Microbiol*. 111: 1148-1158.

Zhou, M., T.A. McAllister, and L.L. Guan. 2011b. Molecular identification of rumen methanogens: technologies, advances and prospects. *J. Appl. Microbiol*. Anim. Feed. Sci. Technol. 166:76-86

Uyeno. Y, Y. Sekiguchi and Y. Kamagata (2011) r RNA-based analysis to monitor succession of faecal bacterial communities in Holstein claves. *Letter in Applied Microbiology* 51, 570-577.

PO-02-17 Effects of heat-treated soybean meal supplementation on blood urea nitrogen and hematological values in dairy cows

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Introduction

The higher milk yield dairy cows are frequently supplemented with protein rich concentrates. The soybean becomes the main source of protein in animal feed especially, in dairy cows. Besides that they also contain the richest source with ranging from 1.2 to 4.2 mg/kg DM [1] of isoflavones which are secondary plant compounds with estrogenic activity. Furthermore, isoflvones fed to lactating ruminants may be transferred into their milk making dairy products source of isoflavones.

The aim of the present study was to investigate the effects of heat-treated soybean meal supplementation on blood urea nitrogen, hematological value in dairy cows.

Materials and methods

Animal and treatments

The in vivo experiment was carried out on normally cycling crossbred Holstein Friesian cows during 4 weeks at Suranaree University of Technology's farm with the standard feeding system and supplementation of soy bean meal shown in the Table 1. Sixteen healthy dairy cows were used in this study which was randomized complete block design. The animals were divided into 4 groups of 4 animals. The cows were housed in individual pens and had free access to clean water and rice straw.

Data and samples collection

Feed intake of cows and the milk yield were recorded daily during the experimental period. Blood samples were collected from caudal vein of cows at the initiate and the end of the intake. The hematological value and blood urea nitrogen parameters were measured (Table 2, 3)

Statistical analysis

Data were analyzed by ANOVA of SAS. The obtained data from experiments are shown as the means ± S.E.M.

Results and Discussion

The result showed that soybean meal supplementation was no effects on feed intake and milk yield (no shown data). The hematological value in the (Table 2) had no significant difference between treatments but there was a slightly increase in supplemented heat-treated soybean diet. However, RBC was significantly different. Especially, the heat-treated 25% soybean meal supplementation can improve RBC for healthy dairy cows.

The results of blood urea nitrogen are shown in Table 3. During week 1 and 2, there was increase in BUN ($P<0.05$) at treatment non heat-treated soybean (T2) (16.78 ± 0.6mg/dl) and heat-treated soybean (T3; 19.65 ± 0.9mg/dl), T4; 19.18 ± 1.4mg/dl)). More recent studies have demonstrated that the fertility of dairy cow will decrease when the level of BUN is around or above 19 mg/dl [2]. However, the levels of BUN were reduced from week 3 and get the normal level in the week 4 at all treatments.

Conclusions

From the results, the heat-treated soybean meal supplementation caused of a significant increase in blood urea nitrogen during the first 2 weeks. However, the supplementation did not affect feed intake, milk yield and had positive effects on hematological value by improving dairy cow health.

Acknowledgements

Thanks to Suranaree University of Technology (SUT), Thailand, for financial support.

Table 1 Supplementation of soybean meal in the experiments were 4 weeks.

Treatment		Week (kg/day)			
		1	2	3	4
T1	Control	0	0	0	0
T2	20%SBM	1.23±0.07	1.26±0.03	1.29±0.04	1.28±0.04
T3	20% Heat-treated	1.09±0.11	1.17±0.05	1.19±0.05	1.18±0.04
T4	25% Heat-treated	1.50±0.20	1.55±0.16	1.56±0.20	1.55±0.22

Table 2 Effects of supplemented soybean meal in feed dairy cow on hematological value.

Items[1]	Time[2]	Treatments			
		T1	T2	T3	T4
Hct. (%)	Init.	25.25±1.9	23.75±1.9	22.50±0.5	24.50±1.2
	End	25.75±1.5	25.75±2.1	23.00±1.2	25.00±1.2
Hb. (g/dl)	Init.	9.25±0.7	8.78±0.6	7.95±0.2	8.78±0.3
	End	9.45±0.6	9.60±0.6	8.10±0.4	8.78±0.3
WBC ($x10^3$ Cell/cu.mm)	Init.	14.47±5.5	18.97±3.8	11.75±1.2	10.14±1.1
	End	12.62±3.8	20.28±5.2	10.48±1.9	12.42±2.3
RBC ($x10^3$ Cell/cu.mm)	Init.	6.07±0.3	5.85±0.2	5.64±0.2	5.94±0.2
	End	$6.44±0.2^{a}$	$6.28±0.3^{ab}$	$5.68±0.2^{b}$	$6.05±0.1^{ab}$
Platelet ($x10^3$ Cell/cu.mm)	Init.	252.50±90.31	204.25±108.01	135.00±86.84	201.50±89.09
	End	195.25±137.32	231.25±123.68	300.75±158.01	129.00± 117.23

^{a,b} Values within the same row with uncommon superscripts are different (P<0.05).

Table 3 Effects of supplemented soybean meal in feed dairy cow on BUN (mg/dl)**.**

week	Treatments[1]			
	T1	T2	T3	T4
Start	$16.10±1.8^{a}$	$10.70±1.4^{b}$	$13.35±1.2^{ab}$	$13.48±1.5^{ab}$
1	$12.48±1.8^{b,B}$	$17.50±1.4^{a,AB}$	$18.23±0.7^{a,AB}$	$19.15±1.1^{a,A}$
2	$13.60±2.1^{b,B}$	$16.78±0.6^{a,AB}$	$19.65±0.9^{a,A}$	$19.18±1.4^{a,A}$
3	$11.58±2.0^{b,B}$	$14.85±1.3^{ab,AB}$	$18.50±0.8^{a,A}$	$15.80±1.8^{ab,AB}$
4	$10.53±1.6^{b,B}$	$13.48±0.9^{ab,AB}$	$16.88±1.4^{a,A}$	$14.55±0.3^{ab,AB}$

^{a,b} Values within the same row with uncommon superscripts are different (P<0.05).
^{A,B} Values within the same row with uncommon superscripts are different (P<0.01).

References

[1] Kurzer, M.S. and Xu, X. (1997). Dietary phytoestrogens. **Ann Rev Nutr**. 17:353-381.
[2] Canfield, R.W., Sniffen, C.I. and Butler, W.R. (1990). Effects of excess degradable protein on postpartum reproductive and energy balance in dairy cattle. **J**. **Dairy Sci**. 73: 2342-2349.

PO-02-26 Immunohistochemical analysis of the estrogen receptor and Ki-67 antigen in the ovine mammary gland during pregnancy

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1.Objective

Development of the mammary gland occurs in fetal period, and the structure and function changes in puberty and gestational period. Various hormones control the development of mammary gland (Cathrin Brisken et al., 2010). In particular, estrogen and progesterone, secreted by the female reproductive system, regulate the condition of the mammary gland. Several studies have reported that estradiol (E2) from ovary is critical for development of ductal elongation during puberty, and lobuloalveolar development during pregnancy (Bocchinfuso et al., 2000, Shyamala et al., 2002). Estrogen regulates cell proliferation via diffusion through the plasma membrane of target cells and signaling through intra-cellular hormone-specific estrogen receptors (ERα and ERβ)(Chrisie et al., 2005, Marino et al., 2006). However, mammary stem cells have been shown to have no estrogen and progesterone receptors (Asselin-Labat et al., 2006). Therefore, It was proposed that estrogen has an impact on ER-positive cell around mammary stem cells, and the paracrine mechanisms affect the proliferation and differentiation of the stem cells (Tanos and Brisken, 2008). In rodents and primates, ERα was observed in the mammary epithelial cell and stromal cell during pubertal and during gestational period (Shyamala et al., 2002, V Speirs et al., 2002, Guojun Cheng et al., 2009). Colitty (2011) analyzed the expression of ERα mRNA in ovine mammary glands from prepubertal stage to involution. It is confirmed that ERα transcription was significantly down regulated before lambing, during lactation and at involution. However, little is known about the changes in localization of ERα transcript and expression of Ki-67 (a marker of cell proliferation) in the ovine mammary gland during pregnancy. Therefore, in order to understand the mechanism of mammary gland development derived from stem cells during gestation period, we analyzed the expression and distribution of ERα and Ki-67 by immunohistochemistry in the ovine mammary gland during different stages of gestation.

2.Methodology

2.1. Animals, tissue sampling

Tissues were obtained from 10 mixed-breed sheep raised and housed at the University of Utsunomiya Farm (Tochigi, Japan). A piece of the mammary gland was collected by surgically at different developmental stages: days 58, 90, 105, 120, 136 of pregnancy (full term: 147 days). Day of gestation was determined from day of artificial insemination. Pieces of tissue were fixed in 10% (w/v) neutral formalin at room temperature and subsequently processed for embedding in paraffin following tissue preparation procedures.

2.2. Immunohistochemistry

The tissue sections were cut by 5μm using a Leica microtome and mounted on MAS coated glass slide (Matsunami Glass Ind.,Ltd, Japan). Slides were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water , and submitted to antigen retrieved with Histo VT one (Nakarai tesque, Japan) at 90°C for 20 min. After rinsing with PBS (0.1 M), slides were treated with 3% H2O2 in hydrogen peroxide for 1 h to quench the endogenous peroxidase activity, and rinsed in PBS (0.1 M). The ABC technique (Vectastain ABC Elite; Vector Laboratories) was performed with a mouse anti-ERα(C-311, Santa Cruz Biotechnology, CA), mouse anti-Ki-67 (Ki-67P, Dianova GmbH), mouse anti-CK18 (C-04, Abcam, Cambridge, UK) and mouse anti-α-SMA (clone 1A4, Sigma Aldrich, Poole, UK). After endogenous peroxidase and avidin-biotin binding were blocked,the slides were then incubated overnight at 4 ℃ with the following primary antibodies: mouse monoclonal anti-ERa (C-311, Santa Cruz Biotechnology, CA), mouse monoclonal anti-Ki-67 (Ki-67P, Dianova GmbH), diluted 1:500 (ERa), 1:200 (Ki-67). After washing in PBS at three times (each for 5 minutes), the slides were incubated for 30 minutes at room

temperature with a secondary antibody (a 1:200 diluted solution of biotinylated goat anti-rabbit IgG from the Vectastain Elite ABC kit). After washing in PBS (three times, each for 5 minutes), sections were incubated with 1:50 diluted avidin-biotin-HRP reagent (Vectastain Elite ABC, Vector Laboratories, USA) for 30 minutes and processed for color development in diami-nobenzidine (DAB). To determine nonspecific staining, parallel sections were incubated without the primary antibody, as the negative control, with the remainder of the protocol performed as just described. From the sections stained with haematoxylin, the percentage of secretory cells, which line the lumen, was calculated as a percentage of all cells counted per sections.

3.Results and Discussions

Immunolocalization for the ERαrevealed a positive staining in most of the epithelial cells and occasionally in some stromal cells of mammary glands of pregnant ewes (Fig1). ERα-positive epithelial cells were scattered mostly in the inner layer of the luminal structures. Expression of ERα in myoepithelial cells and cells of the vascular system were consistently negative. This finding is in agreement with the results of a previous study about ovine mammary gland indicating that the expression of ERα mRNA in epithelial cell and stromal cell had been observed in late pregnancy (10 days before lambing) (Colitti 2011). The percentages of ERα positive epithelial cells in the luminal epithelium at various stages of gestation, were 50.4%, 13.8%, 30.3%, 23.1%, and 37.4% at day58,90,105,120 and 136 of gestation, respectively (Table 1). Theses results revealed that ERa was expressed in ovine mammary tissue at all stages of pregnancy. E2 level increases steadily throughout pregnancy (Alwan et al., 2010). From mid- to late gestation (from the days 100 to 136 of gestation), The percentages of ERα positive epithelial cells in the luminal epithelium increased. It appears that ERα positive cell proliferation in response to increasing E2. The paper by Cheng et al. (2004) indicated that ERα protein could be detected in very few cells in rhesus monkey at late pregnancy. Such as monkey mammary gland, low levels of ERα protein was also found in pregnant rat (Saji et al.,2000), mouse (Shyamala et al., 2002), and bovine mammary gland (Schams et al., 2003).

The results of the present study differed from previous immunolocalization data in other species. Ki-67, a cell proliferation–associated nuclear marker, has been used for assessment of cell proliferation (Scholzen et al., 2000). Expression of the Ki-67 was localized in the nuclei of epithelial cells and stromal cell in the mammary tissue (Fig 2). The percentages of Ki-67 positive epithelial cells in the luminal epithelium at various stages of the gestation, were 11.3%, 2.8%, 6.3%, 6.4% and 3.1% at day58, 90, 105, 120 and 136 of gestation, respectively (Table1). These data demonstrate that the percentages of Ki-67 positive epithelial cells in the luminal epithelium were changed in a pattern similar to that of the ERα-positive cells until day 120 of gestation. The number of Ki-67-positive cells and ER-positive cells were larger in day 58 of pregnancy than in day 90 day of gestation. Mammary growth during the early gestation is known to be ductal growth (Akers RM et al., 2002). This result indicated that epithelial cells proliferated in response to estrogen for ductal growth. Estrogen drives ductal development during puberty, whereas estrogen/progesterone mediate the proliferative and morphological changes of ductal side-branching and alveologenesis that occur at sexual maturity and during pregnancy (Fendrick JL et al.,1998). Our present data confirmed that increases in epithelial cells, ERα-positive cells, and Ki-67-positive cells occured in alveolus and duct at the day 105 of gestation (Table1). It is assumed that these cell proliferation were response to Estrogen and Progesterone for development of alveolar. At day 120 of gestation, luminal space spreaded and cytoplasmic lipid droplets were observed within luminal mammary epithelial cells. It seems that functional development of mammary gland was completed. Therefore, the number of Ki-67-positive cells was reduced at days 136 of gestation.

4.Conclusion

These results indicate that the expression pattern of ERα and Ki-67 are similar during pregnancy in ovine. Therefore, estrogen may exert effects upon cell proliferation from early to late gestation.

Table 1. Number of mammary epithelial cells and number of ERα, Ki-67 positive cell in ovine mammary gland during gestation.

Days of gestation	epithelial cell/ luminal structure*1	ERα*2	Ki-67*4
58	14.1±1.1	7.1±0.9(50.4%)*4	1.6±0.3(11.3%)
90	14.5±1.1	2±0.4(13.8%)	0.4±0.2(2.8%)
105	20.8±1.4	6.3±1.0(30.3%)	1.3±0.3(6.3%)
120	23.4±1.3	5.4±0.8(23.1%)	1.5±0.2(6.4%)
136	26.2±1.5	9.8±1.1(37.4%)	0.8±0.2(3.1%)

*1 : Number of mammary epithelial cells per luminal structure, expressed as the mean ± SE (n=16).
*2 : Number of ER positive cell in the luminal structure, expressed as the mean ± SE.
*3 : Number of Ki-67 positive cell in the luminal structure, expressed as the mean ± SE.
*4 : The percentages of positive epithelial cells in a luminal.

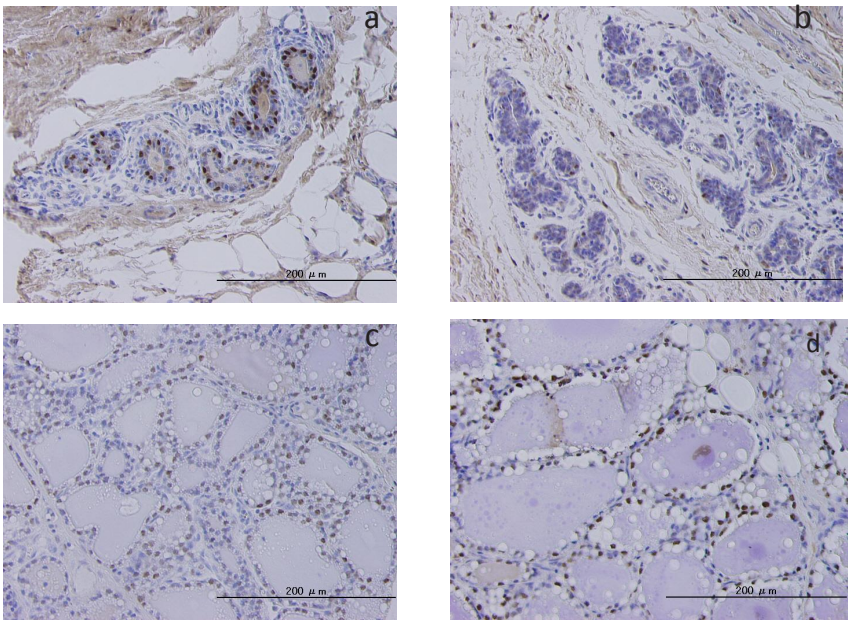

Figure 1. Expression of ERα in mammary epithelial cells.
Receptor positive nuclei were dark brown while receptor negative nuclei were purple.
Panel (a) : day 58 of gestation, panel (b) : day 90 of gestation, panel (c) : day 120 of gestation, panel (d) : day 136 of gestation.

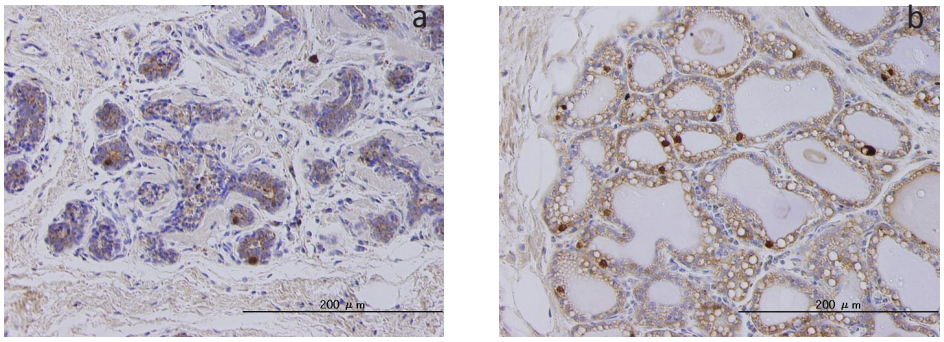

Figure 2. Expression of Ki-67 in mammary epithelial cells.
Ki-67 positive nuclei were dark brown while Ki-68 negative nuclei were purple.
Panel (a) : day 90 of gestation, panel (b) : day 120 of gestation.

References

Akers RM.
Lactation and the Mammary Gland. Ames
Iowa State University Press; 2002.

Alwan AF, Amin FAM, Ibrahim NS. 2010
Blood progesterone and estrogen hormones level during pregnancy and after birth in Iraqi sheep and goat.
Basarah Journal of Veterinary Research. 10:153-157

Asselin-Labat ML, Shackleton M, Stingl J, Vaillant F, Forrest NC, Eaves CJ, Visvader JE, Lindeman GJ. 2006.
Steroid hormone receptor status of mouse mammary stem cells.
Journal of the National Cancer Institute. 98: 1011–1014.

Bocchinfuso, W. P., Lindzey, J. K., Hewitt, S. C., Clark, J. A., Myers, P. H., Cooper, R. & Korach, K. S. 2000
Induction of Mammary Gland Development in Estrogen Receptor- Knockout Mice
Endocrinology 141, 2982–2994.

Cathrin Brisken and Bert O'Malley. 2010
Hormone Action in the Mammary Gland
Cold Spring Harbor Perspectives in Biology. Dec; 2(12): a003178.

Chrisie D. Fowler, Frank Johnson, Zouxin Wang. 2005
Estrogen Regulation of Cell Proliferation and Distribution of Estrogen Receptor-α in the Brains of Adult Female Prairie and Meadow Voles
The Journal of Comparative Neurology. 22; 489(2): 166–179.

Fendrick JL, Raafat AM, Haslam SZ. 1998
Mammary gland growth and development from the postnatal period to

menopause: ovarian steroid receptor ontogeny and regulation in the mouse. J Journal of Mammary Gland Biology and Neoplasia 3: 7-22.

G. Shyamala, Y.-C. Chou, S.G. Louie, R.C. Guzman, G.H. Smith, S. Nandi. 2002.
Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging
Journal of Steroid Biochemistry & Molecular Biology 80 :137–148

Guojun Cheng, Yan Li, Yoko Omoto, Yanling Wang, Tove Berg, Magnus Nord, Pirkko Vihko, Margaret Warner, Yun-Shang Piao, and Jan-Åke Gustafsson. 2009.
Differential Regulation of Estrogen Receptor (ER) and ER in Primate Mammary Gland
The Journal of Clinical Endocrinology & Metabolism 90(1): 435–444

Maria Marino, Paola Galluzzo, Paolo Ascenzi. 2006
Estrogen Signaling Multiple Pathways to Impact Gene Transcription
Current Genomics, 7:497-508

M.Colitti . 2011
Expression of keratin 19, Na-K-Cl cotransporter and estrogen receptor alpha in developing mammary glands of ewes
Journal of Histology & Histopathology. 26: 1563-1573

Saji S, Jensen EV, Nilsson S, Rylander T, Warner M, Gustafsson JA. 2000
Estrogen receptors and in the rodent mammary gland.
Proceedings of the National Academy of Sciences of the United States of America. 97:337–342

Schams D, Kohlenberg S, Amselgruber W, Berisha B, Pfaffl MW, Sinowatz F . 2003
Expression and localisation of oestrogen and progesterone receptors in the

bovine mammary gland during development, function and involution.
Journal of Endocrinology 177:305–317

Scholzen T, Gerdes J. 2000
The Ki-67 protein: from the known and the unknown.
Journal of Cellular Physiology. 182(3):311-22.

Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, Nandi S. 2002;
Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging.
Journal of Steroid Biochemistry and Molecular Biology. 80:137–148.

Tanos T, Brisken C. 2008
What signals operate in the mammary niche?
Breast Disease. 29:69–82.

V Speirs, G P Skliris, S E Burdall, P J Carder. 2002
Distinct expression patterns of ERα and ERβ in normal human mammary gland
Journal of Clinical Pathology, l. 55(5): 371–374.

PO-02-27 Exposure to glyphosate induces oxidative stress and lipid peroxidation in adult male rat

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Objective

The era of weed management with synthetic herbicides began in earnest after World War II with the introduction of 2,4-D. Glyphosate (N-(phosphonomethyl)glycine, GLP) is a global herbicide because of its versatility in controlling economically a very broad spectrum of weeds under varied agricultural, industrial, amenity and domestic situations (Baylis, 2000). Since the introduction of the first GLP herbicide Roundup by Monsanto in the early 1970s, this chemical, in its several salts and many formulations, has transformed global agriculture. GLP, the active ingredient in the herbicide Roundup®, is the main herbicide in use today in the United States, and increasingly throughout the World, in agriculture and in lawn maintenance, especially now that the patent has expired. 80% of genetically modified crops, particularly corn, soy, canola, cotton, sugar beets and most recently alfalfa, are specifically targeted towards the introduction of genes resistant to GLP (Williams et al., 2000). Its residues may thus enter the food chain, and GLP and its metabolite such as aminomethylphosphonic acid (AMPA) and formaldehyde is found as a contaminant in environment such as soil and rivers (Schuette, 1998; Temple and Smith, 1992).

The current study aimed to investigate the effect of GLP exposure on kidney oxidative stress using an in adult male rat. Previous research has suggested GLP induced inflammation (Kumar et al., 2014). Chronic inflammatory processes induce oxidative stress and lipid peroxidation (LPO) (Bartsch and Nair, 2006). Oxidative stress may then trigger inflammatory pathway, which increases hepatic intracellular pro-inflammatory cytokines (Hamsa and Kuttan, 2010). However, the effect of GLP exposure on kidney oxidative stress in rat has rare reported. In the present study, we examined morphology changes, oxidant/antioxidant status in rats in order to explore the mechanism of GLP.

Methodology

Sprague Dawley rats (male, 8 weeks of age, 180–220 g) were purchased from the Nanjing Qinglongshan Experimental Animal Center (Nanjing, China) for use in this study. All rats were housed in separate cages under environmental conditions (23 ± 2°C, 50 ± 10% relative humidity, 12-hr light: dark cycle), and each had ad libitum access to standard rodent chow and filtered water. Prior to experimentation, all rats were allowed to acclimate for at least 1 week. All experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the local ethics committee. Rats were randomly assigned to 4 groups (n = 8/group). Rats were treated orally with 5, 50 or 500 mg/kg body weight of the GLP, on a daily basis for a period of 35 days. Distilled water was used as control treatment. The kidney was collected and weighted after rinsed with ice-cold saline. Samples of kidney tissue was obtained from the animals by surgical excision following euthanasia and fixed in 4% formaldehyde solution for 24hr then dehydrated in an ascending series of alcohol, clarified using xylene, and mounted in paraffin blocks. Paraffin embedded tissues were sectioned into 5 μm slices, stained with hematoxylin-eosin, and evaluated by electron microscopy.

For enzymes determination, the suspension of kidney was centrifuged at 3500 rpm for 15 min. The homogenate collected and used for kidney function assessment employing measurements of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px).

Superoxide (SOD) activity in the homogenates was measured using commercial kits (NJBI) according to manufacturer instructions. The resulting absorbance of each sample was measured at 550 nm in a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and total SOD activity expressed as U/mg protein. Malondialdehyde (MDA) content in each homogenate was measured using a thiobarbituric acid (TBA) method, and results were expressed as nmole MDA/mg protein. The activities of H_2O_2, CAT, GSH, and GSH-Px were also assayed using commercial reagent kits obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China) following the manufacturer's instructions. All operations were done at 4℃ .

Results

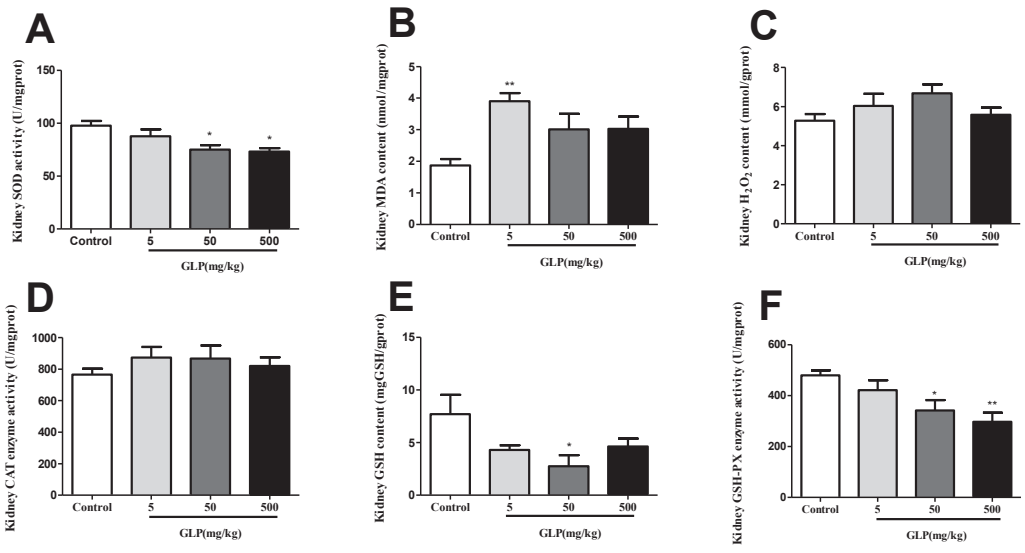
The hematoxylin and eosin staining of renal tissues in control rats demonstrated overall integrity of glomerulus surrounded by Bowman capsule and convoluted tubules. In comparison with control kidney, GLP administration induced marked histological changes, including proximal and distal tubular necrosis and glomerular toxicity.

The activity of antioxidant enzymes in the kidney was examined. As shown in Fig 1, the MDA content in the 5mg/kg GLP-treated group showed significantly increased compared to the control group (Fig. 1B). The SOD and GSH-PX activities were significantly decreased in the 500mg/kg GLP-treated groups compared to the control group ($p < 0.05$) (Fig. 1A and F). And the GSH activity was also showed significantly decreased in the 50mg/kg GLP-treated groups compared to the control group ($p < 0.05$) (Fig.1E). However, there was no difference for the H_2O_2 and CAT activities between the control and treatment group (Fig. 1C and D). In conclusion, these results suggested that exposure to glyphosate induced free radical generation and antioxidant depletion, and caused oxidative stress of rat kidney.

Conclusion

The present study demonstrated that GLP had an effect on the histomorphology, oxidative stress in adult male rat kidney, and then discussed the relationship between them. Chronic inflammation, characterized by the infiltration of adipose tissue by macrophages, endoplasmic reticulum stress, and oxidative stress, plays a relevant part (Trayhurn and Wood, 2004). In a few cases, steatosis leads to lipotoxicity, which causes apoptosis, necrosis, generation of oxidative stress, and inflammation (Marchesini et al., 2008). Animal models of nonalcoholic fatty liver disease (NAFLD) have also suggested a possible role of free fatty acids (FFAs), not triglycerides, in the hepatocytes as factors promoting hepatocellular injury (Yamaguchi et al., 2007). GLP induced inflammation was found to be associated with induction of IL-33, which is known to induce TNF-a, IFN-g and IL-13 upon antigen challenge followed by activation and recruitment of inflammatory cells in the airways (Kumar et al., 2014). Our results showed that the body weight, body weight gain, average daily gain, and liver, spleen and kidney coefficient decreased in 500mg/kg GLP treatment group. These results suggested that treated with 500mg/kg GLP in male rats for 35 days could affect the growth performance of rats. However, in histological sections of the kidney, we have also observed that GLP induced renal tubular damage and glomerular filtration impairment. Taken together, the data demonstrated that GLP could result in kidney damage, the decreased SOD and GSH-PX activities in the kidney tissue, and the MDA content in GLP-treated group increased, the GSH activity was also decreased, indicative of oxidative stress. It showed that exposure to glyphosate caused oxidative stress of rat kidney.

Figure 1



References

Bartsch, H., Nair, J., 2006. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbeck's Archives of Surgery 391, 499-510.

Baylis, A.D., 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Manag Sci 56, 299-308.

Hamsa, T., Kuttan, G., 2010. Ipomoea obscura ameliorates cyclophosphamide-induced toxicity by modulating the immune system and levels of proinflammatory cytokine and GSH. Can J Physiol Pharmacol 88, 1042-1053.

Kumar, S., Khodoun, M., Kettleson, E.M., McKnight, C., Reponen, T., Grinshpun, S.A., Adhikari, A., 2014. Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. Toxicology 325, 42-51.

Marchesini, G., Moscatiello, S., Di Domizio, S., Forlani, G., 2008. Obesity-associated liver disease. The Journal of Clinical Endocrinology & Metabolism 93, s74-s80.

Schuette, J., 1998. Environmental fate of glyphosate. Environmental Monitoring & Pest Management, 1-13.

Temple, W.A., Smith, N.A., 1992. Glyphosate herbicide poisoning experience in New Zealand. New Zealand medical journal 105, 173-174.

Trayhurn, P., Wood, I.S., 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. British Journal of Nutrition 92, 347-355.

Williams, G.M., Kroes, R., Munro, I.C., 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology 31, 117-165.

Yamaguchi, K., Yang, L., McCall, S., Huang, J., Yu, X.X., Pandey, S.K., Bhanot, S., Monia, B.P., Li, Y.X., Diehl, A.M., 2007. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 45, 1366-1374.

PO-02-34 Ceratonia silique(carob) ethanol extract demonstrates anti-inflammatory activity in RAW 264.7 murine macrophage cells

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Objective

The study was conducted to determine the anti-inflammatory activity and corresponding mode of action by *Ceratonia siliqua* ethanol extracts on RAW 264.7 murine macrophage cells.

Methodology

Cell culture

RAW 264.7 murine macrophages cells were cultured and maintained in Dulbeco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin at 37℃ and 5% CO_2.

Plant material and Extract preparation

Two hundred grams (200 g) of carob powder was soaked in 80% ethanol for 48 hours at room temperature. The solution was filtered then subjected to a rotary evaporator before being placed in the deep freezer (-80℃) overnight. The extract was allowed to go through a freeze-drying system to obtain the powdered form. The resulting product (CPE) was then stored in -80℃ prior to use.

Cell viability analysis by CCK assay

Cell counting kit-8 (CCK-8) was used to quantify cell viability. Cells were seeded at a density of 10^4 per well and incubated at 37℃ with 5% CO_2 for 6hrs. The cells were then treated with CPE (0, 50, 100, 200, 400ug/ml) followed by LPS (1ug/ml) stimulation 1 hour after. After 24hrs, fresh media was added to each well and 10uL CCK-8 was added. After 2 hours of incubation at 37℃ absorbance at 450nm was measured using an ELISA plate reader. Viability of the treated cells was expressed as the percentage of control cells.

Nitric oxide determination by NO assay

Nitrite concentration in the medium was measured as an indicator of nitric oxide reaction using the Griess reaction method. 10^5 cells were seeded per well which were incubated for 6hrs. The cells were challenged with LPS (10ug/ml) prior to CPE treatment (0, 50, 100, 200, 400ug/ml) and the plate was incubated for 24hrs. Afterwards, 50ul of media was collected from each well and transferred to a new well with an equal volume of Griess reagent. The plate was covered with aluminum foil and incubated for 10 minutes at room temperature. Absorbance at 540nm was measured using an ELISA plate reader.

RNA isolation and Reverse transcription- polymerase chain reaction

Total RNA was obtained from CPE-treated (0, 50, 100, 200, 400ug/ml) cells using Trizol reagent. RNA concentration was measured with the use of spectrophotometry set with an absorbance of 260/280. 1ug of RNA was used to obtain the cDNA using a commercially available reverse transcriptase. Specific primers were used to amplify the genes cycoxygenase-2 (COX-2), tumor necrosis factor – alpha (TNF-α), inducible nitric oxide synthase (iNOS), nuclear factor – kappa B (NF-κB) and interleukin-1 beta (IL-1β). PCR products were then separated by electrophoresis using 1.5% agarose stained with ethidium bromide and UV transillumination was done afterwards.

Western blotting

CPE-treated (0, 50, 100, 200, 400ug/ml) cells were lysed with a protein extraction solution. The protein concentration was determined through the Bio-Rad protein assay. 30ug of the extracted protein was separated using 10% SDS-PAGE at 100V of constant voltage for 90 minutes. Afterwards, proteins were transferred to a nitrocellulose membrane and blocking was done for 1 hour with 5% skim milk in TBST buffer. The membrane was washed three times with TBST and incubated overnight in 2% skim milk containing primary antibodies (monoclonal anti-TNF-α, anti-iNOS, anti-COX2, anti-NF-κB, anti-IL-1β) at a 1:1000 dilution. Washing with TBST buffer was done

for three times then the membranes were incubated with secondary antibody diluted to 1:2000 for another 2hrs. The membranes were then washed three times with TBST buffer and detection of bands was done with the use of the enhanced chemiluminesence system.

Statistical Analysis

All experiments were done in triplicated and the results were expressed as means ± SD. One-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) was used. Statistical significance was declared at *P*-value less than 0.05.

Results and Discussion

The inflammatory process, being a highly regulated event, involve signals from both pathways that initiate and maintain inflammation as well as signals that shut the process down. During inflammation, macrophages have three major functions: antigen presentation, phagocytosis and immunomodulation through the production of various growth factors and cytokines (Fujiwara and Kobayashi, 2005).

Results of the cell viability analysis (Fig.1) revealed that treatment with *Ceratonia siliqua* increased the viability of the macrophage cell line used in the study. Macrophages are known for their active involvement in the resolution of wounds. The inflammatory response following tissue injury plays pivotal roles in the commencement of normal healing processes (Koh and DiPietro, 2011). An increase in the proliferation rate of macrophages can be positively correlated with an increased rate in the healing of tissues after injurious stimuli. Moreover, a higher cellular proliferation of macrophages is also beneficial for they also play roles in the clearance of pathogens (van Lookeren et al., 2007).

Although inflammation is beneficial to the well-being of the organism, excessive inflammation can result to damage in normal tissues and, when prolonged, even certain disease states. These are mostly due to the toxic effects of certain molecules released by macrophages at the site of inflammation (Iwalewa et al., 2007; Libby, 2007). One of the molecules released in the inflammatory environment is nitric oxide (NO). NO is an important toxic defense molecule against many infectious organisms. It is rapidly oxidized to reactive nitrogen oxide species (RNOS) which mediate most of its immunological effects (Coleman , 2001). However, excessive NO production has been shown to mediate many inflammation-related disease states. The undesired effects of an impaired production of NO include vasoconstriction, inflammation and tissue damage (Sharma et al., 2007). NO assay (Fig. 2) showed that treatment with the ethanol extracts of *Ceratonia siliqua* decreased NO production in a dose-dependent fashion. Moreover, both the mRNA and protein expressions of iNOS also showed a decreasing trend.

Aside from NO, another family of molecules released by macrophages involved in the inflammatory process are cytokines. Cytokines are small proteins that are released by cells that have a specific effect on the interactions and communications between cells (Zhang and An, 2007). In this study, TNF-α and IL-1β were analyzed. RT-PCR (Fig. 3) and Western blot (Fig. 4) analysis show that treatment of cells with *Ceratonia siliqua* ethanol extracts result in the reduction of mRNA expression and protein levels, respectively, for both TNF-α and IL-1β. TNFα promotes inflammation by causing several pro-inflammatory changes which include increased leukocyte adhesion in blood vessels, transendothelial migration and vascular leakage. Also, TNF possesses antitumor and antiviral activities, in addition to its roles in the mediation of systemic responses to sepsis. The central role of TNF in the inflammatory process has been demonstrated in the ability of agents that block the action of TNF to treat a wide array of inflammatory conditions (Bradley, 2008; Winthorp, 2006). IL-1β is one of the prototypic proinflammatory cytokine that exhibit a wide array of effects most notably on acute and chronic inflammatory processes and disorders. Similar to TNF-α, a deranged production of IL-1β is implicated in the pathophysiology of many disease states such as rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer's disease (Ren and Torres, 2009).

Aside from NO and cytokines, another important group of molecules involved in the inflammatory process are prostaglandins. Prostaglandins are generated from arachidonate found in cell membranes through the action of the COX enzymes, including COX-2 (Ricciotti and FitzGerald, 2011). In the present study, dose dependent decrease in the mRNA and protein levels of COX-2 were seen after 24 hours of treatment with the extract.

The NF-κB families of transcription factors possess essential roles in inflammation, stress response, cell differentiation or proliferation, cell death and innate immunity (Hoesel and Schmid, 2013). During inflammation, the canonical NF-κB signaling pathway is activated which leads to an increase in the gene expression of many pro-inflammatory molecules such as several cytokines (interleukins and TNFs), chemokines and adhesion molecules.

In light of these facts, NF-κB has long been considered as the "holy grail" with regard to its potential as a target for novel anti-inflammatory agents (Lawrence, 2009). Our study shows that *Ceratonia siliqua* ethanol extracts reduce the mRNA expression and protein levels of NF-κB dose-dependently. This finding could serve as an explanation for the decrease in the expression of the two aforementioned cytokines analyzed in the study.

Conclusion

Our study show that treatment of RAW 264.7 murine macrophage cells with the concentrations of the extract used in the study resulted in an overall anti-inflammatory effect as shown by the results obtained from NO assay, RT-PCR analysis and Western blotting. *Ceratonia siliqua* can therefore be used further in the development of anti-inflammatory agents or as a functional food in itself.

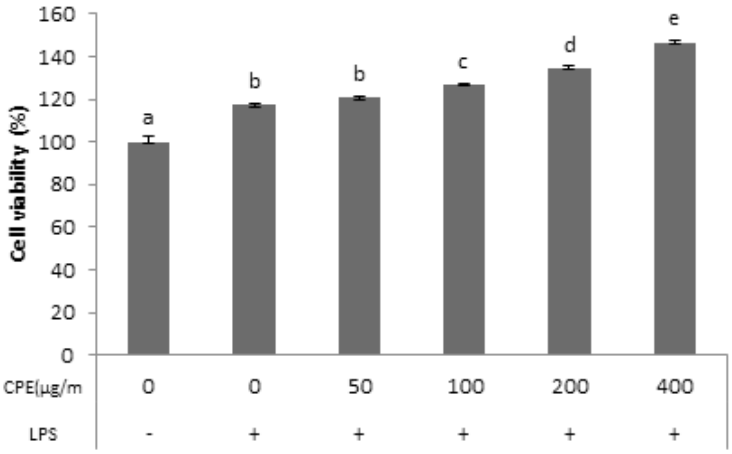

Figure 1. Effects of *Ceratonia siliqua* ethanol extracts on the proliferation rate of RAW 264.7 murine macrophage cells after treatment for 24 hours. Data are means ± SD (n=3). Bars bearing different superscripts are significantly different ($p<0.05$).

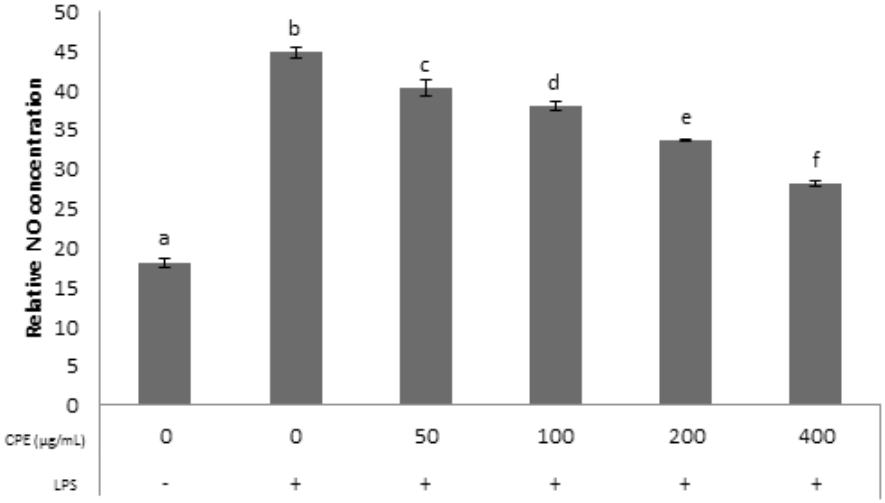

Figure 2. Effects of *Ceratonia siliqua* ethanol extracts on the nitric oxide (NO) released by RAW 264.7 murine macrophage cells after treatment for 24 hours. Data are means ± SD (n=3). Bars bearing different superscripts are significantly different ($p<0.05$).

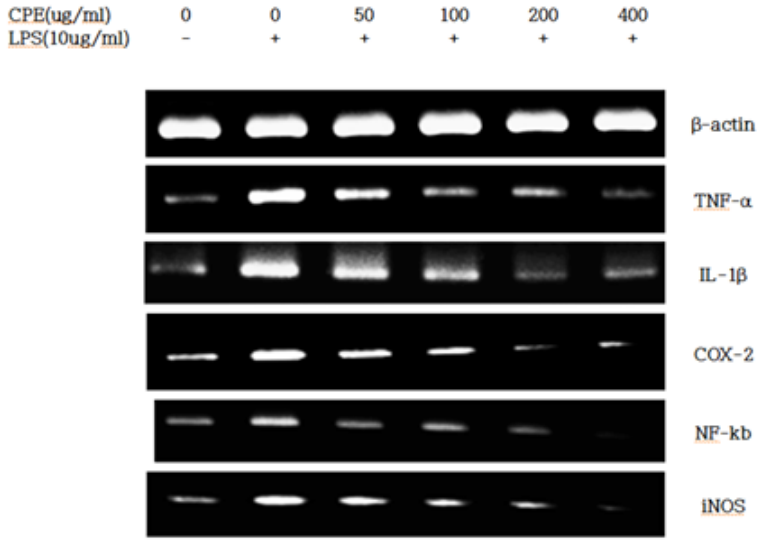

Figure 3. mRNA expression of inflammation-related genes from LPS-treated RAW 264.7 murine macrophage after 24 hours of treatment with *Ceratonia siliqua* ethanol extracts.

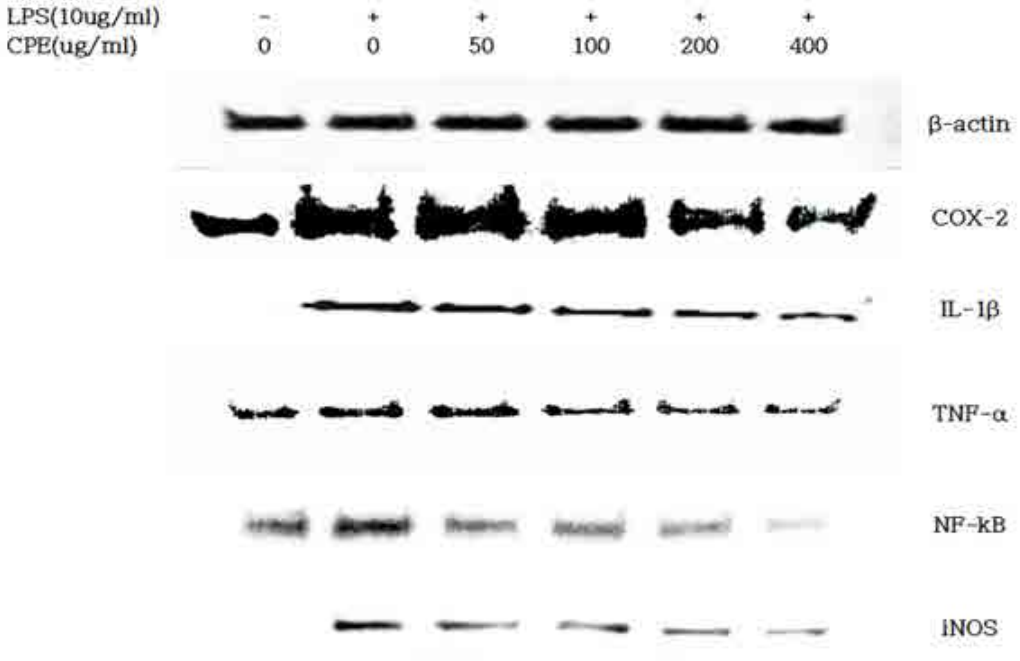

Figure 4. Protein expression of different inflammation-related genes from LPS-treated RAW 264.7 murine macrophage after 24 hours of treatment with *Ceratonia siliqua* ethanol extracts.

References

- Bradley JR. 2008. TNF-mediated inflammatory disease. The journal of pathology. 214(2): 149-160.
- Coleman JW. 2001. Nitric oxide in immunity and inflammation. *International immunopharmacology.* 1(8): 1397-1406.
- Fujiwara N and Kobayashi K. 2005. Macrophages in inflammation. *Current drug targets. Inflammation and allergy.* 4(3): 281-286
- Hoesel B and Schmid JA. 2013. The complexity of NF-κB signaling in inflammation and cancer. *Molecular Cancer.* doi: 10.1186/1476-4598-12-86
- Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN. 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicine of South African origin used to treat pain and inflammatory conditions*. African Journal of Biotechnology.* 6: 2868-2885.
- Koh TJ and DiPietro LA. 2011. Inflammation and wound healing: The role of the macrophage. *Expert Reviews in Molecular Medicine.* 13: e23. doi:10.1017/S1462399411001943.
- Lawrence T. 2009. The Nuclear Factor NF-κB Pathway in Inflammation. *Cold Spring Harbor Perspectives in Biology.* doi: 10.1101/cshperspect.a001651
- Libby P. 2007. Inflammatory Mechnisms: the Molecular Basis of Inflammation and Disease. Nutrition Reviews.
- Ren K and Torres R. 2009. Role of interleukin-1β during pain and inflammation. *Brain Research Reviews.* 60(1): 57-64.
- Ricciotti E and FitzGerald GA. 2011. Prostaglandins and Inflammation. *Arteriosclerosis, Thrombosis and Vascular Biology.* 31(5):986-1000
- Sharma JN, Al-Omran A and Parvathy SS. 2007. Role of nitric oxide in inflammatory disease. *Inflammopharmacology.* 15(6): 252-259. doi: 10.1007/s10787-007-0013-x.
- Van Lookeren CM, Wiesmann C and Brown EJ. 2007. Macrophage complement receptors and pathogen clearance. *Cellular Microbiology.* 9(9): 2095-2102
- Winthorp KL. 2006. Risk and Prevention of Tuberculosis and Other Serious Opportunistic Infections Associated with the Inhibition of Tumor Necrosis Factor. 2(11): 602-610.
- Zhang JM and An J. 2007. Cytokines, Inflammation and Pain. *International Anesthesiology Clinics.* 45(2): 27-37.

PO-02-36

Anti-inflammatory effect of Mongolian Oxytropis altaica (pall) pers ethanol extracts in RAW 264.7 murine macrophage cells

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Objective

The objective of this study was to clarify the effects of Mongolian *Oxytropis altaica* ethanolic extract on the inflammatory response and investigate its anti-inflammatory mehanism in the LPS stimulated RAW 264.7 murine macrophage cells.

Methodology

Plant material and extract preparation

Oxytropis altaica (Pall) pers collected from Mongolia was used in this study. An ethanol extract of *O. altaica* was freeze dried and pulverized to powder form. Briefly, plant was dried and pulverized into powder form. Then the powder was mixed with 80% ethanol and shaked for 24 hours, filtered and evaporated with a rotary vacuum evaporator. The extract was then subjected to freeze drying, and stored in -80°C. The stock solution of *O.altaica* ethanol extract (OAE) was prepared by dissolving extract powder in culture medium (10mg/mL) and the experimental concentrations were diluted in the basal medium.

Cell line and culture media

Murine RAW264.7 macrophage cell was obtained from Korean Cell Line Bank (KCLB). Cells were cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and Penicillin/Streptomycin (100 U/mL) and 3.7mg/mL of $NaHCO_3$ at 37°C in 5% CO_2.

Cell viability analysis

Cell counting kit-8 (CCK-8, Dojindo, Japan) was used to quantify cell viability according to the manufacturer's instruction. Briefly, the cells were seeded at a density of $1x10^4$ cells/ml and allowed to adhere at 37°C for 6 hours. Then the cells were pretreated with OAE extract (0, 25, 50, 100, 200 μg/mL) and after 2 hour with LPS (10 μg/mL). After 24 hour, the media was replaced with fresh media containing CCK-8 reagent and the plate was incubated at 37°C for 2 hour. Absorbance at OD450 was measured using an ELISA plate reader (Tecan, Switzerland). Viability of the treated cells was expressed as the percentage of control cells.

Nitric oxide (NO) determination

Nitrite concentration in the medium was measured as an indicator of NO reaction using the Griess reaction method. Briefly, the cells were seeded at density of $1x10^4$ cells/well and allowed to adhere at 37°C for 6 hours. Then the cells were pretreated with OAE extract (0, 25, 50, 100, 200μg/mL) and stimulated with LPS (10μg/mL) after 2 hour. The cell-free culture medium was collected after 24 hour and used for NO determination. 50μL cell culture medium were mixed with an equal volume of the Griess reagent (equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamideHCl), incubated at room temperature for 10 min, and the absorbance was measured at OD540 using an ELISA microplate reader (Tecan, Switzerland). NO release of the treated cells was expressed as the percentage of control cells.

RNA extraction

Murine RAW264.7 macrophage cells were seeded at a density of 1×10^6 cells/well in 6-well plates and allowed to adhere at 37°C for 6 hours. Then the cells were pretreated with OAE extract (0, 25, 50, 100, 200μg/mL) and stimulated with LPS (10μg/mL) after 2 hour. Total RNA was isolated from cells after 24 hour using Trizol reagent (Invitrogen, MA, USA) according to the manufacturer's instructions.

RT-PCR

1μg of RNA was used to obtain cDNA using by M-MuLV reverse transcriptase (Fermentas, Lithuania) according to the manufacturer's protocol. Then following specific primers were used to quantify mRNA expression: β-actin, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-1β (IL-1β), interleukin-6 (IL-6) and nuclear transcription factor kappa-B (NF-kB). The expression levels of IL-1β, IL-6, INOS, COX-2, and NF- κ B were normalized using β-actin as a control.

Statistical Analysis

All experiments were done in triplicates and data were expressed as means ± standard deviations. The difference between control and AOE-treated cells were evaluated using one-way ANOVA followed by Duncan's Multiple Range Test. *P* value less than 0.05 was considered statistically significant using SAS/STAT® software.

Result

The inflammatory response is an innate defense mechanism against pathogens and chemical or mechanical injury. Main purpose of inflammation is to defend the body against a harmful agent and to promote the renewal of normal tissue (Ashley et al., 2012). Macrophages play important roles in the initiation, maintenance, and resolution of inflammation (Rossol et al., 2011) and can be directly activated by bacterial pathogen such as lipopolysaccharide (LPS) (Fujiwara and Kobayashi, 2005).

In the present study, we evaluated the cytotoxic effect of OAE on LPS-stimulated RAW 264.7 murine macrophage cells using the CCK-8 assay. The concentration of OAE and the duration of OAE treatment used in this study resulted in an increase in the viability of LPS stimulated RAW 264.7 murine macrophage cells (Fig. 1). Several studies showed that an increase in macrophage proliferation promotes wound healing process, and may be helpful in some cases, such as impaired healing, aging and diabetes. In other words, agents that have mitogen effect in macrophages will lead to healthy tissue regeneration (Rodero and Khosrotehrani, 2010, Delavarya et al., 2011 and Shah et al., 2012). Moreover, macrophage participates in innate immune system as main player, due to its ability to recognize, present and remove pathogens from the body, thus increase in macrophage number may result in the improvement of primary defense (Mogensen, 2009).

Chronic inflammation has been reported to initiate the development of various diseases such as asthma, cancer, atherosclerosis, Alzheimer's disease, diabetes and many others (Iwalewa et al., 2007). One of the early chronic inflammation marker is increased level of nitric oxide (NO), produced by many cells involved in immunity and inflammation (Coleman, 2001). NO is involved in the regulation of apoptosis and is released by macrophages through the activity of the iNOS (Tripathi et al., 2007). Thus we investigated effect of OAE on NO production in LPS stimulated RAW 264.7 murine macrophage cells. The result showed a decreasing tendency in NO release in dose dependent manner (Fig. 2). To determine whether the inhibition of NO production in RAW 264.7 cells by OAE is correlated with iNOS mRNA expression, we examined the expression of iNOS mRNA using RT-PCR. As shown in Fig. 3, mRNA expression of iNOS on LPS stimulated RAW 264.7 murine macrophage cells decreased dose dependently after OAE treatment.

Other inflammatory markers aside from NO include pro-inflammatory cytokines (IL-6 and IL-1β), COX-2 and transcriptional factor (NF-kB) (Dinarello, 2010). Here we also evaluated effect of OAE on the above mentioned markers in LPS stimulated RAW 264.7 murine macrophage cells (Fig. 3).

LPS activated macrophages release pro-inflammatory cytokines (IL-1β and IL-6) that up-regulates inflammatory reactions. IL-1β is produced mainly by monocytes and macrophages during cell injury, infection, invasion, and inflammation. It is known to induce the expression of several inflammatory genes and some primary pro-inflammatory mediators such as prostaglandins and NO, which lead to the promotion of inflammation (Rena and Torres, 2009). Another chief cytokine that is produced by macrophages in the acute site of inflammation is IL-6. IL-6 stimulates T cells and B cells, leading to production of acute phase proteins that cause chronic inflammation. Compared to other cytokines released in inflammatory response, IL-6 is a key stimulator for acute inflammatory response (Gabai, 2006). As shown in Fig. 3, LPS substantially increased the production of IL-1β and IL-6. However, OAE significantly inhibited the LPS-induced production of IL-1β and IL-6 in a concentration-dependent manner.

Aside from above mentioned molecules, prostaglandins also play an important role in the inflammation process. COX-2, an enzyme which is induced predominantly in immune cells, such as macrophages and synoviocytes, in response to infection, injury, or other stresses, is a main generator of prostoglanadins (Ricciotti andFitzGerald, 2011). Thus, inhibition of COX-2 expression also suppresses acute inflammation. In our study, result showed that COX-2 mRNA expression in LPS stimulated RAW 264.7 murine macrophage cells decreased in a concentration dependent manner (Fig. 3).

NF-kB is one of the most important transcription factors, and its signaling pathway results in the transcription of pro-inflammatory mediators, such as iNOS, COX-2, IL-1b, and IL-6. LPS stimulates toll-like receptor-4 (TLR-4) on the surface membrane of macrophages, which in return activates NF-κB signaling pathway (Tak and Firestein, 2001). Hence, inhibition of NF-κB in macrophage cells provides us a novel anti-inflammatory therapeutic option. Our study showed that OAE significantly reduced NF-kB mRNA expression in a dose dependent manner (Fig. 3).

Conclusion

In the present study, we demonstrated that *Oxytropic altaica* ethanol extract significantly and dose dependently reduced NO production and mRNA expression of inflammatory genes in LPS stimulated RAW 264.7 murine macrophage cells. Taken together, *Oxytropis altaica* (Pall) pers can be used as a potential herbal component for the development of future anti-inflammatory drugs.

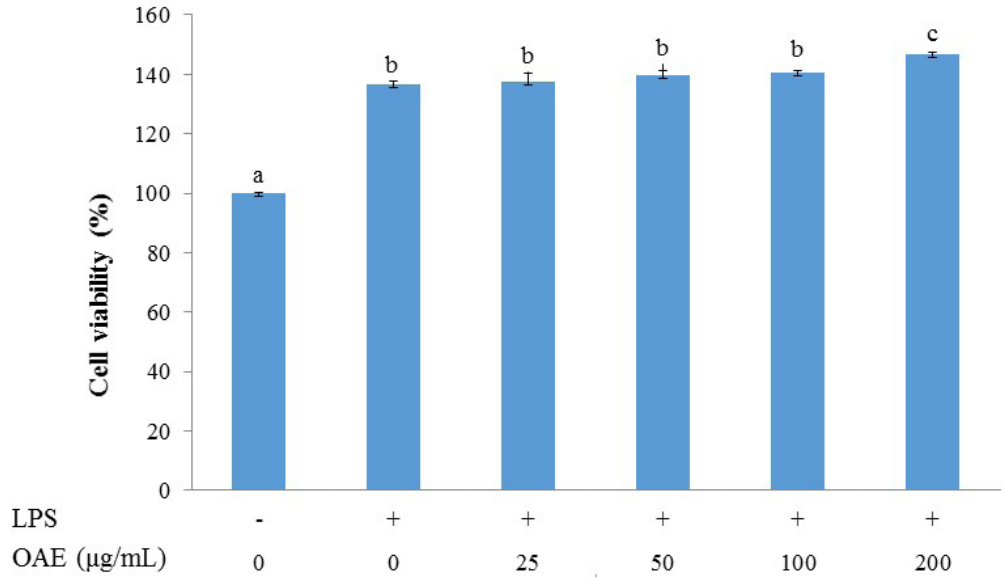

Figure 1. Effect of *Oxytropis altaica* ethanol extract on RAW 264.7 murine macrophage cell proliferation after 24 hour treatment.
Data are means ±SD (n=3). Bars with different superscripts are significantly different ($p<0.05$).

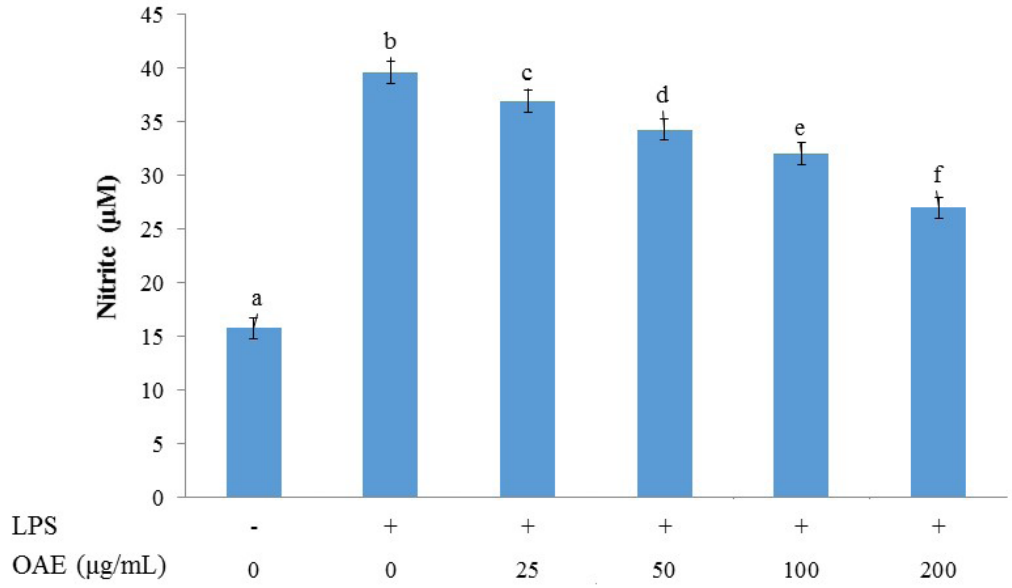

Figure 2. Effect of *Oxytropis altaica* ethanol extract on RAW 264.7 murine macrophage cell NO production after 24 hour treatment.
Data are means ±SD (n=3). Bars with different superscripts are significantly different ($p<0.05$).

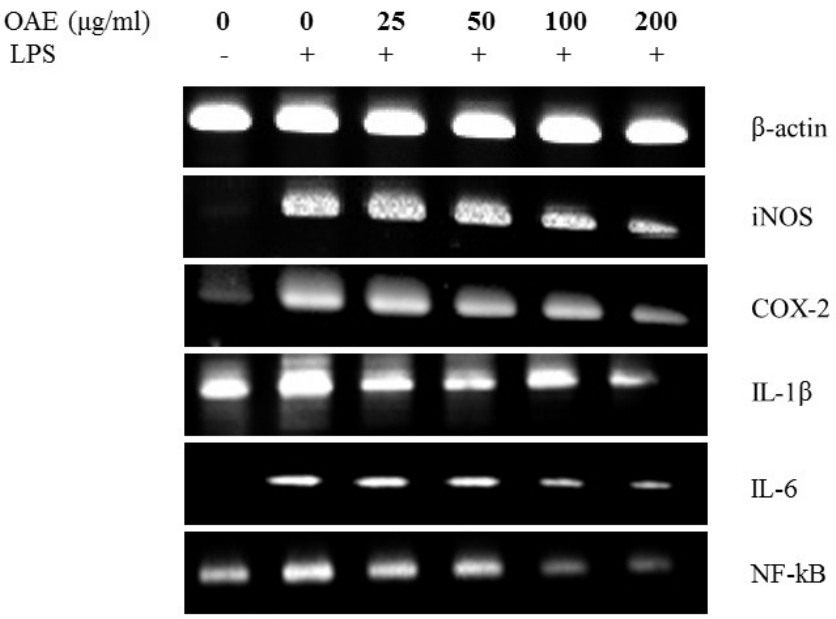

Figure 3. Effect of *Oxytropis altaica* ethanol extract on RAW 264.7 murine macrophage cell inflammation related mRNA expression after 24 hour treatment.

References

1. Ashley NT, Weil ZM, and Nelson RJ. (2012). Inflammation: Mechanisms, Costs, and Natural Variation. *Annu. Rev. Ecol. Evol. Syst*, **43**,385–406.
2. Coleman JW. (2001). Nitric oxide in immunity and inflammation. *Int Immunopharmacol*. **1**, 1397-406.
3. Delavarya BM, van der Veera WM, van Egmonda M, Niessenb MF, and Beelena RHJ (2011). Macrophages in skin injury and repair. *Immunobiology*. **216**, 753–762.
4. Dinarello CA. (2010). Anti-inflammatory Agents: Present and Future. *Cell*, **140**, 935–950.
5. Fujiwara N and Kobayashi K (2005). Macrophages in inflammation. *Curr Drug Targets Inflamm Allergy.* 4,281-6.
6. Gabai C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Res Ther*. 8(Suppl 2): S3.
7. Iwalewa EO, McGaw LJ, Naidoo V and Eloff JN. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*. **6**, 2868-2885.
8. Mogensen TH. (2009). Pathogen Recognition and Inflammatory Signaling in Innate Immune Defenses. *Clinical Microbiology Reviews*. **22**, 240–273.
9. Rena K and Torres R. (2009). Role of interleukin-1β during pain and inflammation. *Brain Research Reviews*, **60**. 57 – 6 4
10. Ricciotti E. and FitzGerald GA. (2011). Prostaglandins and Inflammation. *Arterioscler Thromb Vasc Bio*l.**31,** 986–1000.
11. Rodero MP, Khosrotehrani K. (2010). Skin wound healing modulation by macrophages. *Int J Clin Exp Pathol*. **3**, 643-653.
12. Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet MJ and Hauschildt S. (2011). Lps-induced cytokine production in human monocytes and macrophages. *Crit Rev Immunol*, **31**, 379-446.
13. Shah JMY, Omar E, Pai DR and Sood S. (2012). Cellular events and biomarkers of wound healing. *Indian J Plast Surg*. **45**, 220–228.
14. Tak PP and Firestein GS (2001). NF-κB: a key role in inflammatory diseases. *The Journal of Clinical Investigation*. **107**, 7-11.
15. Tripathi P, Tripathi P, Kashyap L and Singh V. (2007). The role of nitric oxide in in£ammatory reactions. *FEMS Immunol Med Microbiol*, **51**, 443–452

PO-02-37

The growth rate of the male peacock feathers in different body regions

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Introduction

There are many differences of birds' feathers, for example shape, color, length. Especially the lengths of feather are most different. In the vast majority of birds, the tail-coverts are small feathers, just a few centimeters long. However, some birds like the peacock have very large tail-coverts for decorative purposes (Burgess, 2001.). So, we were very interested in the what makes different lengths between feathers. Therefore, this study observed and measured the length growth of feathers to understand the reasons is growing periods or growing rates. And in this study we observed the male peacock, whose feathers' lengths are very different in different body parts to find the significant result.

Materials and Methods

Animal

In this study, we used the blue peafowl (*Pavo cristatus*). Blue peafowl is a specie of peafowl native to South Asia with the special and brightly colored feathers. We observed one male blue peafowl from Department of Animal Science, National Chung Hsing University, Taiwan in this study.

Observation

We chose six different body parts to observe (Figure 1). The six body parts are crown, ventral, dorsal contour, short saddle, long saddle, and tail respectively.

Before the observation, we removed all feathers of measuring parts. Marking the measuring feathers by particular arrangements (Figure 2.A), and continuously removed the other feathers around the measuring ones until they are differences in their length.

There are twelve feathers measured in the crown, dorsal contour, ventral, short saddle, long saddle respectively. And there are eighteen feathers measured in the tail.

Measurement

In this study, we recorded by taking photos, and measured lengths of individual feathers by straight ruler directly (Jenni and Winkler, 2009.). There are two ways to measure the feathers. One way is measuring the length from the base to the tip of the feather (Figure 2.B). The other way is measuring 'T' feather (Burgess, 2001) from the base of feather to the top of rachis.

Statistical analysis

In this study, we used SAS GLM system to operation analysis. Respectively, calculating the mean, standard deviation, maximum, and minimum of each measurement.

The feathers in peacock, especially in the male, show diversity of feather length and morphology. Therefore, in order to analyze if growth rate or growth period results in the difference in feather length, feathers from six regions, including crown, ventral, dorsal, anterior saddle (short), posterior saddle (long) and tail feathers, were plucked and measured their growth. According to the average length, the feathers are grouped into three. Group 1 includes crown, ventral and dorsal feathers. Tail feathers are in Group 2 and short saddle and long saddle feathers are in Group 3.

Growth curve

The records made into growth curves. The X-axis is measuring date, and Y-axis is length of feather. Six body parts made into six graphs respectively, and each feather sample made into individual growth curve.

Results and Discussions

To reach the average length of mature feathers. The crown feathers spent ten weeks; the dorsal contour feathers spent seven weeks; the ventral feathers spent five weeks, and the short saddle feathers took twenty-six weeks. The tail feathers stopped growing in fourteenth week, but didn't reach average length. The long saddle feathers stopped recording in twenty-sixth week because they still far from the average length (Figure 3).

The results showed that the growth period of Group 3 is 2.36 times longer than Group 1, and 1.53 times longer than Group 2. Also, Group 2 is 1.55 times longer than Group 1. In terms of growth rate, Group 3 is 4.74 times

faster than Group 1, and 1.51 times faster than Group 2. And, Group 2 is 3.15 times than Group 1.

Conclusions

In conclusion, Group 3 feathers show the longest growth period and fastest growth rate. In addition, it was found that the multiple of growth rate is much bigger than of growth period. Hence, the results in this study demonstrated that the growth rate has a greater effect on growth of male peacocks' feathers.

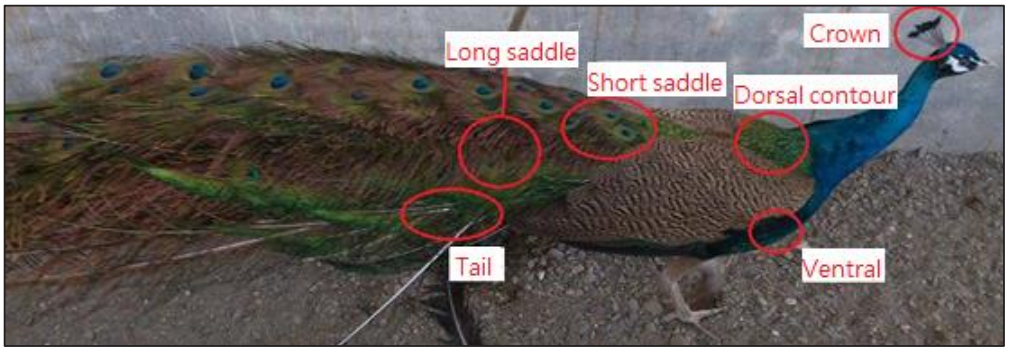

Figure 1. The six body parts of this observation.

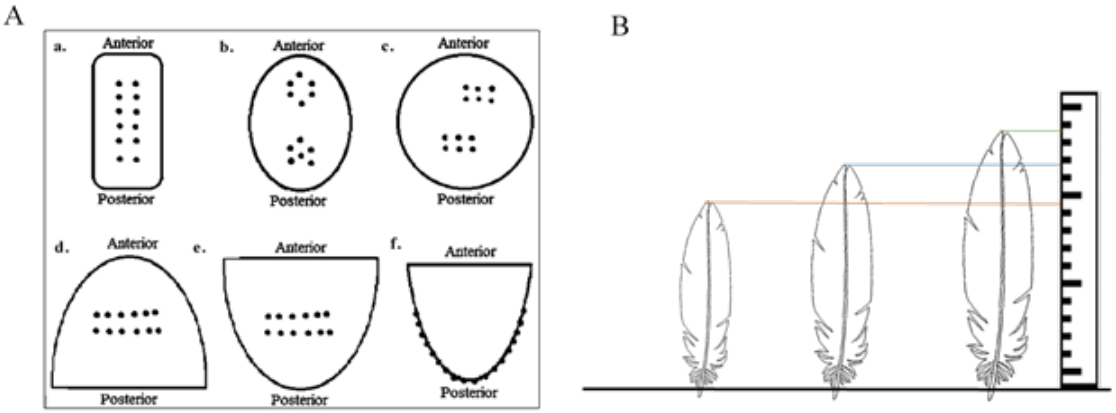

Figure 2. The measurement of feathers. (A) The particular arrangements of measuring feathers in the different body parts. Each dot means one measuring feather. (a) Crown. (b) Dorsal contour. (c) Ventral. (d) Short saddle. (e) Long saddle. (f) Tail. (B) The measuring method of feathers.

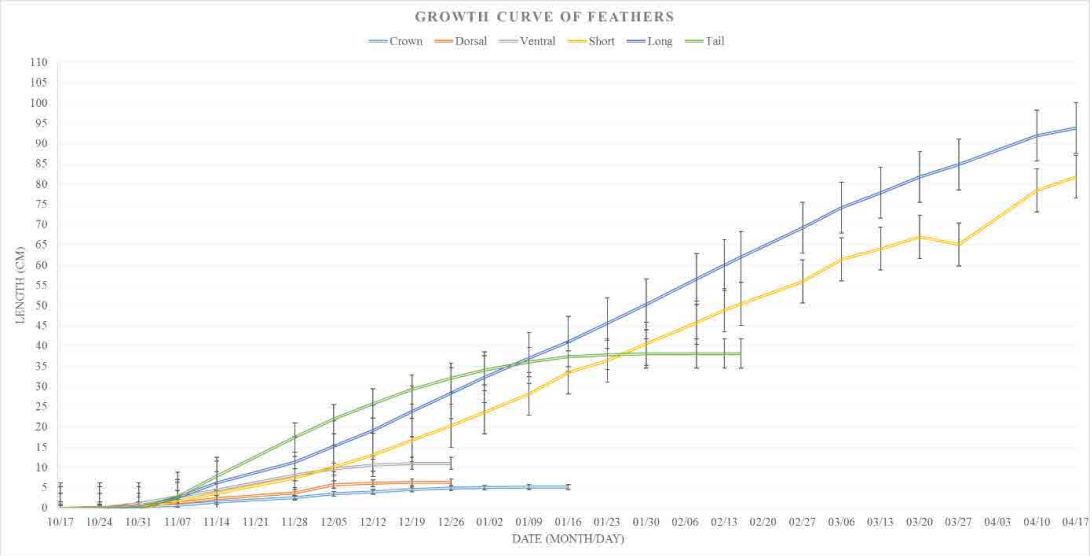

Figure 3. The growth curve of feathers.

References

Burgess, S. 2001. The beauty of the peacock tail and the problems with the theory of sexual selection. Journal of Creation. 15(2): 94-102.

Dakin, R., and R. Montgomerie. 2011. Peahens prefer peacocks displaying more eyespots, but rarely. Animal Behaviour 82: 21-28.

Jenni, L., and R. Winkler. 2009. The feather-length of small passerines: a measurement for winglength in live birds and museum skins. Bird Study.

Loyau, A., M. S. Jalme, and G. Sorci. 2005. Intra- and Intersexual Selection for Multiple Traits in the Peacock. Ethology 111: 810-820.

Loyau, A., M. S. Jalme., C. Cagniant, and G. Sorci. 2005. Multiple sexual dvertisements honestly reflect health statusin peacocks (*Pavo cristatus*). Behav Ecol Sociobiol 58: 552–557.

Petrie, M., and P. Cotgreave.1996. Variation in the train morphology of peacocks (*Pavo cvistatus*). J. Zool., Lond. 238, 365-371.

PO-02-38

Effects of agar-added pickling solution on pidan quality

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INTRODUCTION

Taiwan is located on subtropical area and high temperature and relative humidity during summer season causes the depression of pidan yield rate. The main reasons of depressed pidan yield rate are the poorer egg qualities and the pickling periods were shortened. Some references indicated that high ambient temperature caused adverse effects on laying ducks (Singh et al., 1991) and meat type ducks (Bouverot et al., 1974; Hester et al., 1981). It has been proven that the decreased eggshell thickness in laying hens during the summer (North and Bell, 1990) and the characteristic poor eggshell quality of fowls subjected to thermal stress can be partially accounted for by low feed intake (Oguntunji and Alabi, 2010). And our previous study results indicated that agar-addedpickling solution might improve the pidan yield rate and its quality during the summer season. According to this, this experiment was conducted to confirm the effects of adding agar in pidan pickling solution, hoping to alleviate the depressed pidan yield and its quality during summer season.

MATERIALS AND METHODS

Animals

The ducks used in this experiment were bred and hatched at the Ilan Branch, Livestock Research Institute. Eighty female ducks were given starter diets (ME, 2900 kcal/kg; CP, 19.5%) before three weeks of age in the brooding house. After three weeks of age, ducks were transferred to a semi-opened duck house and given grower diets (ME, 2660 kcal/kg; CP, 13.5%). Ducks were further transferred to an artificial climate-controlled duck house and kept in individual cage at 12 weeks of age. Layer diets (ME, 2700 kcal/kg; CP, 20%) were adopted after 17 weeks of age. The temperature and relative humidity setting value in the duck house was the average record of four southern counties (Chiayi, Tainan, Kaohsiung and Pintung) in Taiwan, July, 2011.

Traits determined

This experiment was conducted from 33 weeks of age and lasted for three weeks. Each week, all eggs were collected for further processing. Data regarding as pidan coagulation score, pH value of egg white, pH value of egg yolk and egg white absorbance at 600 nm were collected at 9, 12, 15 and 18 days of pickling.

The coagulation score was determined by an experienced technician, the range from 1 to 5 and higher score means better coagulation condition of the pidan.

The pH was determined followed method developed by Ockerman (1974). Ten grams of sample added with 90 ml of distilled water, pH value was determined after homogeneous for 30 seconds. After pH determination, the same sample was used for egg white absorbance determination at 600 nm.

Pickling solution

The formulation of pickling solution are based on eggs weight and described as below: water 100%, Sodium chloride (NaCl) 7 %, Sodium hydroxide (NaOH) 4.2 %, Zinc sulfate ($ZnSO_4$) 0.3 % and additional 1.5% agar added in the agar-added group.

Statistic analysis

The data were analyzed by SAS software (SAS Institute, 2008) and significant effects were further explored using Tukey's honest significant difference.

RESULTS AND DISCUSSION

The results of this experiment were shown in the Table 1. For the pidan coagulation score, we found that adding 1.5% agar in the pickling liquid solution would improve the coagulation of pidan. This may resulted from agar-added in pickling liquid increased the viscosity of pickling solution, therefore, the speed that alkaline transferred from liquid into egg may become more steady. It's already know that over-alkaline in the egg is one of the main reason decreasing the pidan yield rate. This may partially explain the higher coagulation score found in the agar-added group in the late pickling stage. Besides, we observed that adding some agar in the pickling solution can

prevent the failure pidan processing caused by the tiny eggshell crack. This may become another application of agar-added pickling solution. The results of pH of the egg weight and egg yolk didn't differ significantly. In fact, we can find the pH value of egg white were all higher than 11, represented the coagulated speed were very fast and these results were similar with Wang et al. (1998) and Chen (2000). The results of egg white absorbance indicated that the color would become deeper with the increased pickling time. Besides, the egg white absorbance of agar-added group showed a trend to become deeper color steadily. In contrast, the value of control group showed a huge variance.

In conclusion, adding 1.5% agar in the pidan pickling solution could stable the pidan color in processing and slightly improve the coagulation score in the late pickling stage.

Table 1. The pidan qualities at different pickling time of agar-added and control groups[1]

Traits	Treatments	
	Agar-added	Control
	9 days of pickling	
Pidan coagulation score	4.0±0.4	4.1±0.4
pH value of egg white	11.25±0.15	11.19±0.13
pH value of egg yolk	10.53±0.16	10.20±0.08
Egg white absorbance at 600 nm	0.070±0.020	0.141±0.116
	12 days of pickling	
Pidan coagulation score	3.8±0.5	4.2±0.4
pH value of egg white	11.26±0.09	11.21±0.08
pH value of egg yolk	10.50±0.20	10.41±0.30
Egg white absorbance at 600 nm	0.088±0.029	0.091±0.012
	15 days of pickling	
Pidan coagulation score	4.1±0.6	3.7±0.2
pH value of egg white	11.03±0.37	11.02±0.56
pH value of egg yolk	10.36±0.23	10.22±0.60
Egg white absorbance at 600 nm	0.106±0.008	0.201±0.202
	18 days of pickling	
Pidan coagulation score	4.7±0.1	4.3±0.2
pH value of egg white	11.29±0.04	11.32±0.01
pH value of egg yolk	10.59±0.09	10.51±0.06
Egg white absorbance at 600 nm	0.172±0.023[a]	0.120±0.030[b]

Means ± SD.

[1] Average results of 3 determined experiment weeks

[a,b] Means in the same row without the same superscript differ significantly（$P < 0.05$）.

References

Bouverot, P., B. Hildwein and D. LeGoff. 1974. Evaporative water loss, respiratory pattern, gas exchange and acid-base balance during thermal panting in Pekin ducks exposed to moderate heat. Respir. Physiol. 21:255-269.

Chen, J. W. 2000. A new process for preparing pidan without black spots on the shell and its quality. Master's thesis. National Taiwan Univ., Taiwan.

Hester, P. Y., S. G. Smith, E. K. Wilson and F. W. Pierson. 1981. The effect of prolonged heat stress on adrenal weight, cholesterol and corticosterone in white Pekin ducks. Poult. Sci. 60:1583-1586.

North, M. D. and Bell, D. 1990. Commercial Chicken Production Manual. 4th edn. Van Nostrand Reinhold, New York, New York.

Ockerman, H. W. 1974. Quality control of post-mortem muscle tissue. p.51. The Ohio Agriculture Research Development Center, U.S.A.

Oguntunji, A. O. and O. M. Alabi. 2010. Influence of high environment temperature on egg production and shell quality: a review. World's Poult. Sci. J. 66:739-749.

SAS Institute. 2008. SAS/STAT® Enterprise Guide. Release 4.1 Edition. SAS Institute Inc., Cary, NC.

Singh, N. J., G. Sahoo, H. K. Kanungo and J. B. Nayak. 1991. Performance of Khaki Campbell ducks in hot and humid climate. Indian J. Anim. Prod. Manage. 7:230-232.

Wang, C. T., J. H. Lin, H. P. Su and C. W. Lin. 1998. To stabilize the processing procedure of alkalized egg without added heavy metal by temperature control. Food Sci. 25:601-609.

PO-02-39 Red mite population: Increase has a direct correlation to a decrease in egg production

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Objective

Global population is forecast to exceed 97 million in 2050, which is predicted to induce a food-supply crisis (UN, World Population Prospects: The 2015 Revision, 2015). Furthermore, due to changing dietary habits and increasing world population, there is an increase in demand for lipid and animal protein foods (FAO STAT Fat supply quantity and protein supply quantity in selected country 1992 – 2013, 2014).
The poultry chicken egg is high quality protein and global production has increased by about 280% since 1970. Even at the current level of increasing egg production, egg production will not be sufficient to meet demand by 2050. So it is necessary to further increase the production volume before 2050 (International Egg Commission. FAO STAT Egg production 1979-2013). To meet these demands, the poultry industry needs more stable production which will require an improved environment for layers and better health controls. One major health and environmental concern is caused by ectoparasites. *Dermanyssus gallinae* (De Geer, 1778), known as the "red mite," is a hematophagia ectoparasite, commonly found in laying hens and is one of the most important epidemiological and economic problems. Red mite infection induces a decrease in egg production, poor egg quality and can ultimately lead to premature death of hens from substantial blood loss (Chauve, 1998; Cosoroaba, 2001). Additionally, the red mite is a transporter various diseases (Moro et al., 2007; Tomasz, 2003). So an increase in amount of red mites, may lead to a higher outbreak risk. The red mite causes large economic losses worldwide (Bruneau et al., 2001). In Japan, the total annual economic losses were estimated at 6 million yen (Chiba Prefectural Livestock Experiment Station, 2011). The red mite has been recognized as a new threat in the poultry industry that must be understood and controlled (Shimmura et al., 2011). However, red mite contamination in industrial farms is difficult to evaluate and excessive use of insecticides may contribute to the problem because of the occurrence of chemical resistance red mites (Marianna et al., 2012). Furthermore, the most effective timing for control applications of red mites, and the most effective extermination method is unknown.

We have developed an electrostatic charged device (*i*-Trap®, Kondo-Electric Co., Ltd.) which can attract and capture red mites without the use chemicals or insecticides. This device has an electrical charge from static electricity that is created by the polyurethane composition of the material. The *i*-Trap® attracts and traps the red mites. This allows for quantification of the red mite infestation population from which the contamination level for red mites can be determined.

In this study, we conducted two experiments to establish what times red mites are active in an industrial farm by a full day sampling (24h) of red mite behavior and the effects of humidity and temperature (Experiment 1) on red mite activity in an industrial farm. Then we analyzed the relationship between the number of red mites trapped in the *i*-Trap® and egg quality and quantity (Experiment 2).

Material and method

For Experiment 1, one *i*-Trap®, a polyurethane trap that uses electrostatic charge to attract red mites, was installed on one chicken cage on June-29, 2014 at a semi-windless poultry farm with 120 Boris Brown layers. Four cameras (XG-200C and lens CA-LA8, Keyence Corporation) were set at the four sides of the *i*-Trap® and fixed point photography was used (Fig.1). Photos were taken from the four points every 30 minutes. A full day sampling (24h) of red mite behavior was conducted and the humidity and temperature were measured. The red mites were counted by image analysis application (Win ROOF ver7.4.5). The number of red mites was determined by the software targeting a circular area. Then, a circular outline in the area was set and the red mites are identified and

counted. A separate circular figure was set up (Filter size 3 × 3, Edge threshold 100, Binarize edge Off, Minimum radius 2, Maximum radius 30, Candidacy center points80, Delete near circle80, Delete circle which an edge is rare10, Delete circle which a binary is rare off, Approximate polygon off). The number was then extrapolated based upon the density of red mites in the circular area to give an overall count of the number of red mites. For Experiment 2, one *i*-Trap® was set up 2 times per month for field tests at the same site between June and October of 2014. After each test, the *i*-Trap® was collected put in a sealed box with chloroform to kill red mites inside the *i*-Trap®. The *i*-Trap® was then opened and photos were taken of both sides of the polyurethane with a digital camera (WG-30W, Ricoh Co., Ltd) from a height of 20 cm. Then, red mites were counted by image analysis application (Win ROOF ver7.4.5 Mitani Corporation) as described above. Furthermore, egg production was recorded 2 times per month. Then, egg weight and Haugh unit and egg York collar were measured with an Egg Multi-Tester (EMT-5000**,** Robotmation Co., Ltd.) and egg shell-breaking strength was measured using an egg shell strength meter (Fujihira Industry Co., Ltd.). Shell thickness was measured with an egg shell thickness meter (Fujihira Industry Co., Ltd.).

Results

Time-lapse tracking of red mite movement and red mite behavior showed an increase in activity 4-5 hours after the lights were turned off. However, humidity and temperature did not affect red mite behavior (Fig.2). We could establish a significant link between the red mite population and egg production (Fig.3). Egg production decreased the most when red mite population was highest, in August. Furthermore, a simple regression analysis using the Least-Squares Method demonstrated that there was a correlation between the red mite population and egg production. We found that if the red mite population exceed 100, there was an 8.71% decrease in egg production (confidence coefficient approx. 48%) (Fig.4). Additionally, egg weight (g), egg shell strength (kg/cm^2), shell thickness meter ($\times 10\mu$) dropped in August. However, there was no significant changed for Haugh unit and egg yolk color in August (Fig.5).

Conclusion

In this study, we showed that there is a correlation between red mite behavior and egg quality, egg weight, egg shell strength, and shell thickness. Shell thickness is of particular interest for producers because egg breakage can occur during transport. Time-lapse tracking of red mites indicated when red mites were active. We succeeded in producing a red mite behavior visualization. So, the *i*-Trap® can be used as a monitoring system at industrial poultry farms. Use of this monitoring system allows for a better understanding of when countermeasures are needed. This means that insecticide treatment can be conducted only when necessary rather that at regularly scheduled intervals which can lead to cost savings, and reducing the use of insecticides. More effective use of insecticides may also reduce the numbers of insecticide resistant red mites. The *i*-Trap® may be an integral part of a HACCAP program for industrial farming.

Fig.1 camera were set up for time-lapse tracking

Fig.2 Time-lapse tracking and humidity and temperature measurement

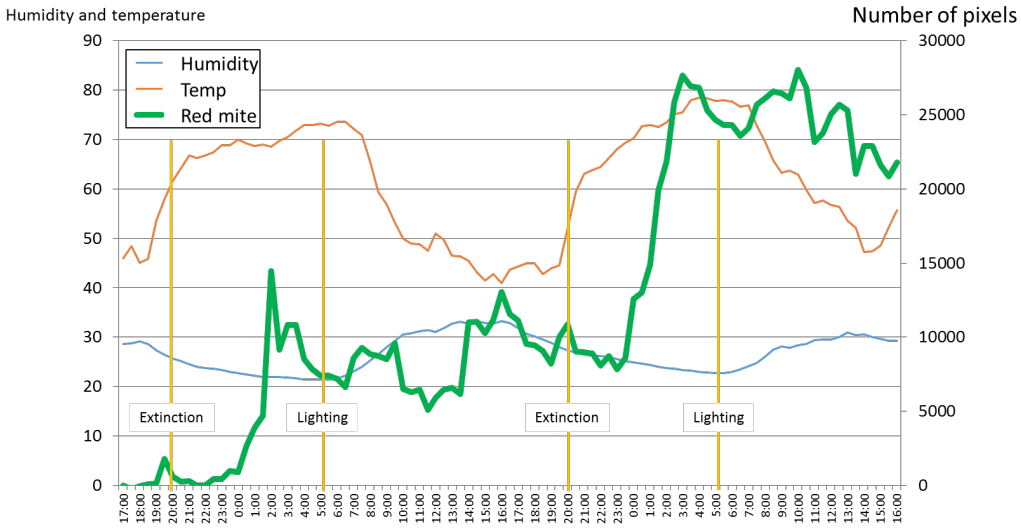

Fig.3 Number of red mites and the correlation with egg production

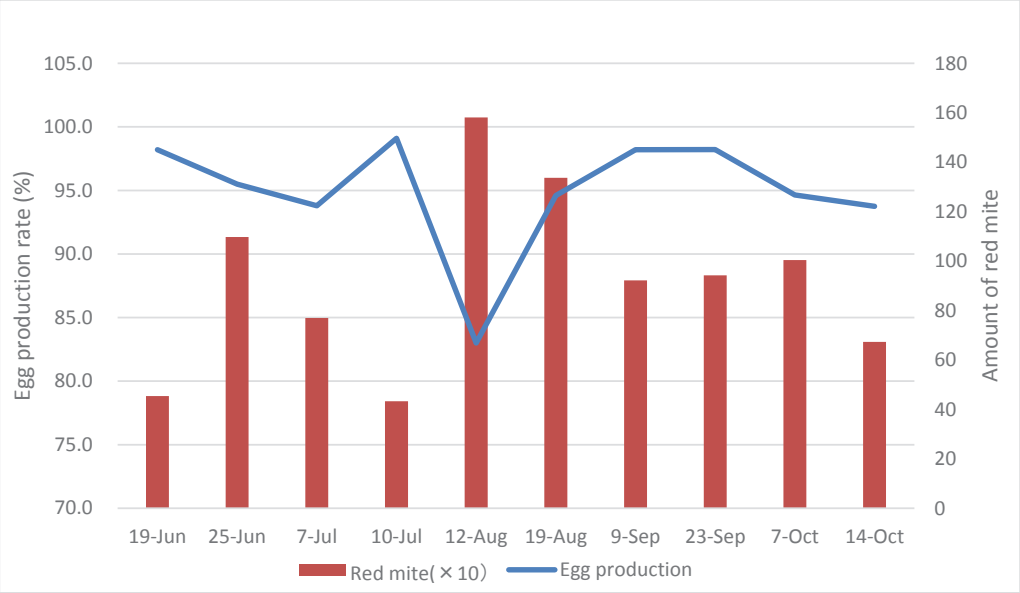

Fig.4 Simple regression analysis using the Least-Squares Method

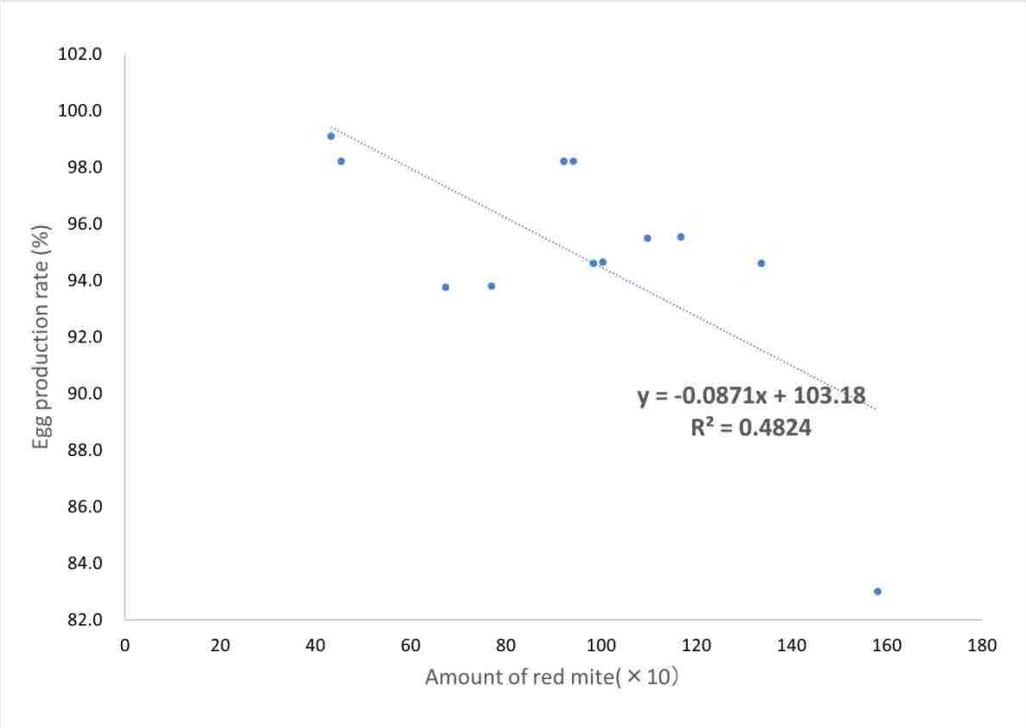

Fig.5 Egg quality test

Month	Jun	July	August	September	October
Egg weight(g)	54.7±3.0	54.5±3.0	52.5±3.1	53.3±3.0	56.4±3.0
Haugh unit	95.9±10.2	94.7±6.0	96.3±5.3	94.5±12.5	87.7±18.9
Egg yolk color	13.0±0.5	13.0±0.5	12.9±0.4	13.0±0.0	13.6±0.5
Egg shell strength (kg/㎠)	4.2±0.6	3.8±0.5	3.1±0.5	3.9±0.5	4.0±0.8
Shell thickness meter(×10μ)	43.3±2.6	40.0±3.5	35.9±2.3	37.8±3.6	38.3±2.8

References

Bruneau, A., Denburg, A., C, Chauve., and Zenner, L. (2001). First in vitro cycle of the chicken mite, Dermanyssus gallinae (De Geer, 1778), utilizing an artificial feeding device. *Vet Parasitology*, 123, pp. 583–589

Chauve, C. (1998). The poultry red mite Dermanyssus gallinae (De Geer, 1778): Current situation and future prospects for control. *Vet Parasitol*, 16;79(3), Nov:239-45

Cosoroaba, I. (2001). Massive Dermanyssus gallinae invasion in battery-husbandry raised fowls. *Revue De Medecine Veterinaire*. 152(1): 89-96

Cencek, T. (2003). Prevalence of Dermanyssus Gallinae in Poultry Farms in Silesiaregionin Poland. *Bull. Vet Inst. Pulawy* 47, June, 465-469

Chiba Prefectural Livestock Experiment Station. (2011). Countermeasures were invented for red mites in Japan. Retrieved from http://www.naro.affrc.go.jp/narc/kenkyu_koryu/files/kantoseika_h24CB03.pdf

Food and Agriculture Organization of the United Nations Statistics Division. (2015). Food Balance / Food Supply - Livestock and Fish Primary Equivalent / Average 1992-2013. Retrieved from http://faostat3.fao.org/browse/FB/CL/E

Food and Agriculture Organization of the United Nations Statistics Division. (2015). Production / Livestock Primary / Average 1979-2013. Retrieved from http://faostat3.fao.org/browse/Q/QL/E

Moro, C.V., Fravalo, P., Amelot, M., Chauve, C., Zenner, L., and Salvat, G. (2007). Colonization and organ invasion in chicks experimentally infected with Dermanyssus gallinae contaminated by Salmonella Enteritidis. *Avian Pathol*. 36(4), Aug: 307-11

Marangi, M., Morelli, V., Pati, S., Camarda A., Cafiero, M. A., and Giangaspero, A. (2012). Acaricide Residues in Laying Hens Naturally Infested by Red Mite Dermanyssus gallinae. *PLoS ONE* 7(2): e31795

Shimmura, T., Bracke, M. B., DeMol, R. M., Hirahara, S., Uetake, K., and Tanaka., T. (2011). Overall welfare assessment of laying hens: comparing science-based, environment-based and animal-based assessments. *Anim Sci J*. Feb;82(1):150-60

United Nations. (2015). World Population Prospects The 2015 Revision. Working Paper No. ESA/P/WP.241

International Egg Commission. (2012). Nutrition. Retrieved from http://thinkegg.com/index.php/nutrition/

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Survival of lactic acid bacteria isolated from fermented meat products in gastrointestinal tract model

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ABSTRACT

The aim of this study was to investigate the survival of lactic acid bacteria (LAB) isolated from traditional fermented meat products in gastrointestinal tract model. The 8 LABs were isolated from traditional fermented meat products, Thai traditional fermented sausages and Nhams, and screened preliminary probiotic property. Their survival in artificial gastric juice at pH 2, 3, 4 and 7 for 0, 30, 60, 90 and 180 min and intestinal fluid at pH 8 for 180 min were investigated. The result showed that the viability of all strains tended to remain stable, in the range of 8.23 – 9.53 log cfu/ml, in gastric model at pH 3, 4 and 7 for 180 min. After simulated intestinal fluid at pH 8 for 180 min, their viabilities tended to remain stable excepted isolate no. 2021B1 decreased in the range of 0.93 - 1.01 log cfu/ml. Isolate no. 10111C2, 601-21B1 and 8031C1 exhibited gastric juice at pH 2 and intestinal fluid tolerance as well as more viability and survival in gastrointestinal tract model. They can be probiotic and applied to fermented meat products.

INTRODUCTION

The probiotic concept has been defined as a living microorganism which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Lactic acid bacteria are regard as a major group of probiotic bacteria (Collin et al., 1998). Lactic acid bacteria (LAB) are the most commonly used microorganisms in food preservation techniques such as fermentation. Most scientists agree that probiotic strains shall be able to survive transit through the gastric acid environment as well as exposure to bile and pancreatic juice in the upper small intestine to be able to exert beneficial effects in the lower small intestine and the colon, although there are convincing data on beneficial immunological effects also from dead cells (Mottet and Michetti, 2005). The viability and survival of probiotic bacteria are the most important parameters for providing therapeutic functions. Several factors have been claimed to affect the viability of probiotic bacteria in fermented meat products including low pH and bile salts. In order to be used as potential probiotics (Chou and Weimer, 1999). The aim of this study was to investigate the survival of lactic acid bacteria isolated from Thai traditional fermented meat products in gastrointestinal tract conditions.

MATERIALS AND METHODS

Lactic acid bacteria isolates and growth condition

The lactic acid bacteria were previously isolated from traditional fermented meat products including 6 traditional fermented sausages and 16 Nhams (traditional fermented pork). The cultures have already screened and assessed preliminary probiotic property, well-growth selection, antagonistic activity of foodborne pathogen and survival of LAB in pH conditions and bile salt concentrations in previous study (Nithisantawakhupt et al., 2015). The LAB isolates were stored in MRS broth (de Man Rogosa and Sharp, Merck, Germany) at -20°C.

Tolerance test of LAB isolates in gastrointestinal tract model

Simulated gastric and intestinal digestion was tested essentially as modified method of Zárate et al. (2000). A 1 ml of LAB was transferred in 35 ml of MRS broth with 1% NaCl (w/v) and incubated at 37°C in anaerobic condition for 16 hr that the initial concentration of approximately 10^9 cfu/ml. After washing in 3.5 ml of sterile saline solution (0.9% NaCl) and centrifugation at 5,000 x g for 10 min, the cell suspension was added to 25 ml of gastric juice with the following composition: 125 mM of NaCl, 7 mM of KCl, 45 mM of $NaHCO_3$, and 0.3 % of pepsin (Sigma, USA). The final pH was adjusted with HCl solution to pH 2, 3, 4 and 7. The bacterial suspension was agitated to simulate peristalsis by shaking water bath (Vision scientific, Korea). Aliquots were taken for enumeration of viable at 0, 30, 60, 90 and 180 min by pour plate technique on MRS agar in anaerobic condition for 48-96 hr. Simulated intestinal fluid was prepared by suspending the cells (after 180 min of gastric digestion) in 0.1% (w/v) of pancreatin (Sigma, USA) and 0.15 % (w/v) of bile salts (Sigma, USA) in water and adjusted it to pH 8.0 with 1 N

NaOH solution. The suspension was incubated as described above and samples for total viable counts were taken for 0, 30, 60, 90 and 180 min in gastric model and 30, 60, 90 and 180 min in intestinal model exposure using pour plate technique with MRS agar in anaerobic condition. The total incubation in gastrointestinal tract model was 360 min. The experiment was performed in triplicate and mean were calculated. Then their percentages of survival of LAB were calculated as below equation.

% Survival of LAB = (Total viable count of LAB in gastric or intestinal fluid model x 100)/Total viable count of LAB at 0 min in gastric model

RESULTS AND DISCUSSION

Tolerance test of LAB isolates in gastrointestinal tract model

The study of LAB survival in gastrointestinal tract model at pH 2, 3, 4, and 7 was demonstrated. A viability of LAB isolate no. 1011C2, 1012C2, 10111C2, 2021B1, 5031A2, 601-21B1, 73-21A2 and 8031C1 tended to remain stable, in the range of 8.23 – 9.53 log cfu/ml, in gastric model at pH 3, 4 and 7 for 180 min. After simulated intestinal fluid at pH 8 for 180 min, viabilities of all isolates tended to remain stable except viability of isolate no. 2021B1 decreased in the range of 0.93 - 1.01 log cfu/ml (Table 1). On the other hand, in gastrointestinal tract model at pH 2 for 180 min, the survival percentages of LAB isolate no. 8031C1, 10111C2 and 601-21B1 were more than 95 %. Then their percentage of survival have a moderate decrease in intestinal fluid for 180 min that they were 53.11, 52.14 and 47.68%, respectively. On the contrary, the survival of other LAB isolates sharply decreased in gastric model for 180 min and slightly decreased in intestinal model for 180 min expect their LAB isolate no. 1012C2 and 73-21A2 moderately sharply decreased in gastric and intestinal model, respectively (Fig. 1). The criteria key in the selection of a probiotic are therefore considered acid and bile tolerance, also gastrointestinal conditions. (Zárate et al., 2000). Besides, Pancreatic juice inhibits growth of multiresistant bacterial strains and for some probiotic bacteria (Kruszewska et al., 2004). Most LABs were susceptible to bovine and porcine bile in vitro and were resistant to human bile which correlated with the survival in the human GIT (Dunne et al., 2001). Thus, bile secreted in the small intestine reduces the survival of bacteria by changing the composition of lipids and fatty acids in their cell membranes. In this study, both acid and bile stresses were assayed in a sequential way, also the simulating gastrointestinal movement. These results was similar to Charteris et al. (1998) found *Lactobacillus* sp. and *Bifidobacterium* sp. have a moderate tolerance to acid pH during 90 min incubation which decreased after 2 h but individual strains vary considerably. Kawther et al. (2010) reported that the simulated gastric transit tolerance of *L. johnsonii*, *L. gasseri* and *L. salivarius* strains was pH dependent and showed lower viability at pH 2.0 after 180 min compared with pH 3.0 and pH 4.0.

CONCLUSION

LAB isolate no. 10111C2, 601-21B1 and 8031C1 exhibited acid and bile tolerant as well as more viability and survival in gastrointestinal tract model specially in artificial gastric juice at pH 2. Thus, they tended to be probiotic and will be applied in fermented meat products in the future.

Keyword: LAB, Gastrointestinal tract model, Fermented meat product, Nham, Probiotic

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Table 1 Survival of LABs in gastrointestinal tract model[a]

LAB isolate no.	Gastrointestinal tract condition	Incubation time (min)	Survival of LABs (log cfu/ml) at different pH condition			
			pH2	pH3	pH4	pH7
1011C2	Pepsin	0	9.15 ± 0.10	9.40 ± 0.01	9.42 ± 0.06	9.37 ± 0.02
		30	8.80 ± 0.02	9.47 ± 0.01	9.36 ± 0.06	9.29 ± 0.02
		60	7.00 ± 0.00	9.38 ± 0.01	9.33 ± 0.01	9.24 ± 0.05
		90	5.68 ± 0.07	9.37 ± 0.02	8.90 ± 0.04	9.19 ± 0.01
		180	4.88 ± 0.08	9.35 ± 0.03	9.34 ± 0.02	9.44 ± 0.01
	Bile salt + Pancreatin	180	4.88 ± 0.07	9.31 ± 0.01	9.38 ± 0.01	9.48 ± 0.00
1012C2	Pepsin	0	9.64 ± 0.01	9.55 ± 0.00	9.58 ± 0.00	9.57 ± 0.00
		30	9.15 ± 0.00	9.39 ± 0.03	9.42 ± 0.02	9.40 ± 0.01
		60	8.59 ± 0.07	9.39 ± 0.03	9.37 ± 0.01	9.38 ± 0.00
		90	8.44 ± 0.06	9.32 ± 0.01	9.32 ± 0.00	9.34 ± 0.00
		180	8.11 ± 0.03	9.30 ± 0.01	9.29 ± 0.01	9.34 ± 0.02
	Bile salt + Pancreatin	180	3.39 ± 0.09	8.91 ± 0.01	8.88 ± 0.04	9.16 ± 0.00
10111C2	Pepsin	0	9.57 ± 0.01	9.58 ± 0.00	9.59 ± 0.00	9.59 ± 0.00
		30	9.49 ± 0.05	9.56 ± 0.02	9.59 ± 0.00	9.59 ± 0.00
		60	9.53 ± 0.01	9.57 ± 0.01	9.55 ± 0.00	9.58 ± 0.00
		90	9.55 ± 0.02	9.56 ± 0.02	9.54 ± 0.01	9.58 ± 0.00
		180	9.51 ± 0.05	9.53 ± 0.03	9.53 ± 0.01	9.57 ± 0.01
	Bile salt + Pancreatin	180	4.95 ± 0.01	9.45 ± 0.02	9.49 ± 0.00	9.53 ± 0.04
2021B1	Pepsin	0	8.86 ± 0.04	9.41 ± 0.01	9.39 ± 0.00	9.43 ± 0.00
		30	7.92 ± 0.13	9.27 ± 0.01	9.22 ± 0.04	9.26 ± 0.01
		60	7.07 ± 0.02	9.07 ± 0.02	9.11 ± 0.02	9.12 ± 0.04
		90	7.01 ± 0.02	8.99 ± 0.01	9.03 ± 0.02	9.05 ± 0.06
		180	5.99 ± 0.02	8.23 ± 0.03	8.77 ± 0.06	9.02 ± 0.05
	Bile salt + Pancreatin	180	3.39 ± 0.09	7.30 ± 0.00	7.76 ± 0.15	9.11 ± 0.01
5031A2	Pepsin	0	9.51 ± 0.01	9.55 ± 0.01	9.51 ± 0.01	9.51 ± 0.00
		30	9.35 ± 0.02	9.40 ± 0.01	9.44 ± 0.05	9.46 ± 0.01
		60	8.61 ± 0.10	9.20 ± 0.01	9.31 ± 0.05	9.13 ± 0.03
		90	5.76 ± 0.03	9.45 ± 0.06	9.22 ± 0.06	9.41 ± 0.03
		180	5.57 ± 0.13	9.45 ± 0.01	9.42 ± 0.09	9.44 ± 0.01
	Bile salt + Pancreatin	180	4.90 ± 0.05	8.77 ± 0.06	8.96 ± 0.18	9.21 ± 0.07
601-21B1	Pepsin	0	9.61 ± 0.00	9.61 ± 0.00	9.64 ± 0.04	9.71 ± 0.00
		30	9.59 ± 0.01	9.50 ± 0.05	9.60 ± 0.01	9.70 ± 0.00
		60	9.56 ± 0.02	9.52 ± 0.01	9.56 ± 0.00	9.66 ± 0.01
		90	9.55 ± 0.02	9.60 ± 0.00	9.54 ± 0.00	9.64 ± 0.00
		180	9.56 ± 0.00	9.46 ± 0.00	9.53 ± 0.00	9.63 ± 0.00
	Bile salt + Pancreatin	180	4.58 ± 0.05	8.70 ± 0.06	8.92 ± 0.01	9.45 ± 0.00
73-21A2	Pepsin	0	9.45 ± 0.01	9.48 ± 0.02	9.52 ± 0.01	9.54 ± 0.00
		30	8.40 ± 0.06	9.46 ± 0.01	9.48 ± 0.02	9.53 ± 0.01
		60	8.18 ± 0.17	9.44 ± 0.01	9.43 ± 0.09	9.43 ± 0.03
		90	7.64 ± 0.15	9.44 ± 0.02	9.48 ± 0.00	9.41 ± 0.01
		180	7.64 ± 0.15	9.38 ± 0.03	9.46 ± 0.00	9.44 ± 0.02
	Bile salt + Pancreatin	180	4.09 ± 0.05	9.24 ± 0.01	9.38 ± 0.02	9.45 ± 0.02
8031C1	Pepsin	0	9.53 ± 0.01	9.54 ± 0.01	9.55 ± 0.01	9.57 ± 0.00
		30	9.49 ± 0.01	9.43 ± 0.01	9.42 ± 0.00	9.57 ± 0.01
		60	9.48 ± 0.01	9.38 ± 0.01	9.49 ± 0.01	9.41 ± 0.01
		90	9.12 ± 0.03	9.42 ± 0.01	9.46 ± 0.02	9.58 ± 0.01
		180	9.14 ± 0.02	9.41 ± 0.05	9.48 ± 0.01	9.47 ± 0.01
	Bile salt + Pancreatin	180	5.06 ± 0.04	9.26 ± 0.04	9.32 ± 0.03	9.40 ± 0.01

[a] LAB isolates were incubated in gastric model at pH2, pH3, pH4 and pH7 for 180 min and in intestinal model at pH 8 for 180 min.

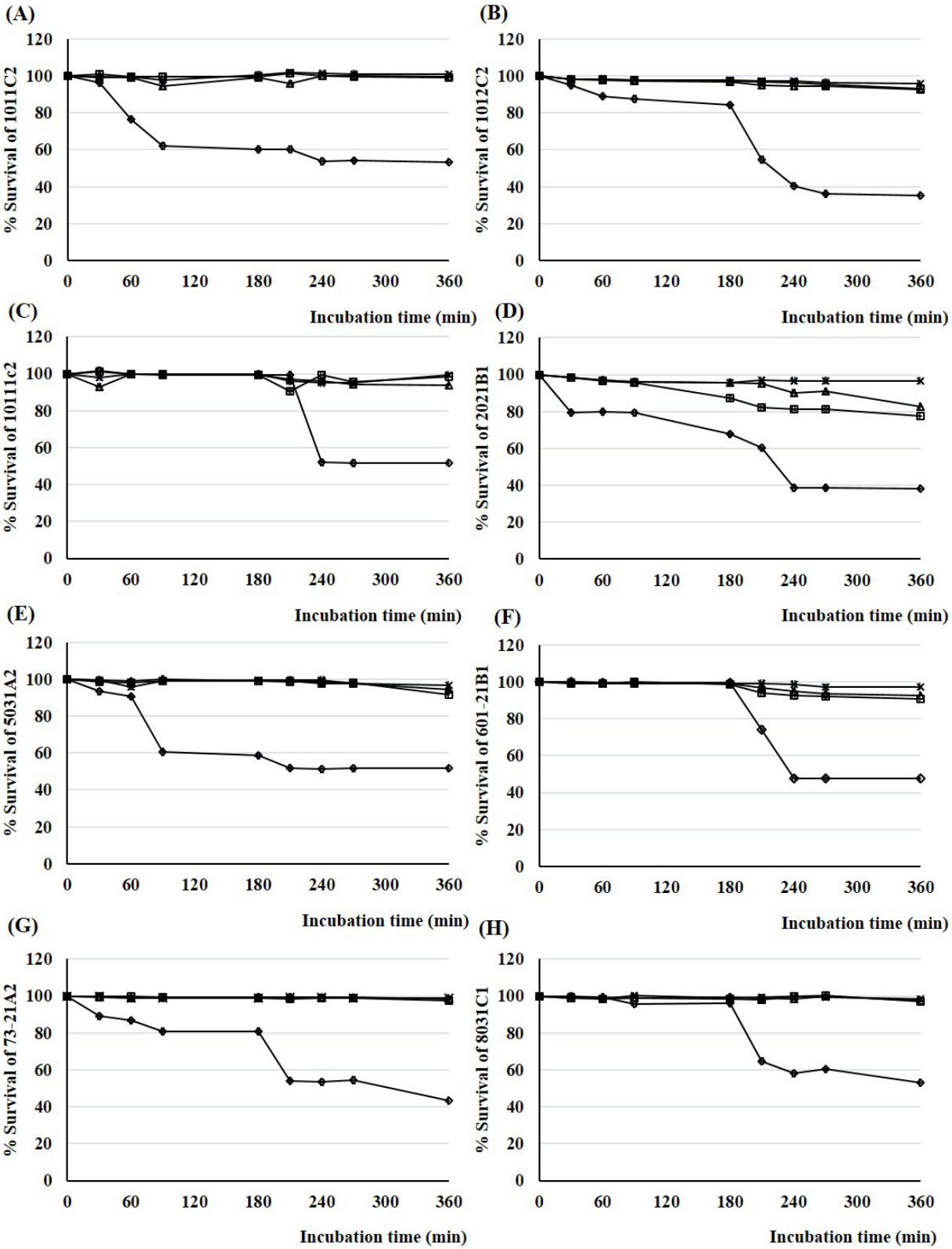

Fig. 1 Survival percentage of LABs (A) isolate no. 1011C2, (B) isolate no. 1012C2, (C) isolate no. 10111C2, (D) isolate no. 2021B1, (E) isolate no. 5031A2, (F) isolate no. 601-21B1, (G) isolate no. 73-21A2 and (H) isolate no. 8031C1 in gastrointestinal tract model for 360 min; in gastric model at (⊸) pH2, (⊟) pH3, (⊿) pH4 and (⊸) pH7 for 180 min and in intestinal model at pH 8 for 180 min.

REFERENCES

Charteris, W. P., P. M. Kelly, L. Morelli and J. K. Collins. 1998. Development and application of an *in vitro* methodology to determine transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper gastrointestinal tract. J. Appl. Microbiol. 84: 759–768.

Chou, L. and B. Weimer. 1999. Isolation and characterization of acid and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. J. Dairy Sci. 82: 23-31.

Collin, J. K., G. Thornton and G. O. Sullivan. 1998. Selection of probiotic strains for human applications. Int. Dairy J. 8: 487-490.

Dunne, C., L. O'Mahony, L. Murphy, G. Thornton, D. Morrissey, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. C. O'Sullivan, F. Shanahan and J. K. Collins. 2001. *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings. Am. J. Clin. Nutr. 73: 386-392.

Guarner, F. and G. J. Schaafsma. 1998. Probiotics. Int.J. Food Microbiol. 39: 237–238.

Kawther, EL-Shafei, N. F. Tawfik, Nadia, M. A. Dabiza, O. M. Sharaf and B. A. Effat. 2010. In vitro assessment of gastrointestinal viability of potentially probiotic Lactobacilli. J. Ame. Sci. 6: 357-367.

Kruszewska, D., J. Lan, G. Lorca, N. Yanagisawa, I. Marklinder and Å. Ljungh. 2002. Selection of Lactic Acid Bacteria as probiotic strains by *in vitro* tests. Microb. Ecol. Health Dis. 29: 37–49.

Kruszewska, D., Å. Ljungh, S. O. Hynes and S. G. Pierzynowski. 2004. Effect of the antibacterial activity of pig pancreatic juice on human multi-resistant bacteria. Pancreas 128: 191–199.

Mottet, C. and P. Michetti. 2005. Probiotics: wanted dead or alive. Dig. Liver Dis.37: 3–6.

Narakaew, T., K. Pilasombut, N. Ngamyeesoon and A. Swetwiwathana, 2010. Preliminary characterization of *Lactobacillus salivarius* K7 for probiotic properties. KKU Res. J. 15: 878-888.

Nithisantawakhupt, J., P. Tangwatcharin and K. Suksupath. 2015. Screening of lactic acid bacteria from traditional fermented products. In: Proceedings of the 2nd International Symposium on Agricultural Technology (ISAT2015). 1-3 July. A-One Royal Cruise hotel Pattaya, Thailand.

Zarate, G., A. Perez-Chaia, S. Gonzales and G. Oliver. 2000. Viability and B-galactosidase activity of dairy propionibacteria subjected to digestion by artificial gastric and intestinal fluids. J. Food Prot. 63: 1214-1221.

PO-02-41 Resistant starch and dietary fiber extracted from by-products of banana processing enhance probiotic viability in fermented sausage model

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ABSTRACT

Synbiotic fermented sausage model (FSM) containing 1% of resistant starch (RS) and 1% of dietary fiber (DF), extracted from banana pulp and peel, with *Lactococcus lactis* subsp. *lactis* were studied. The growth profile and acidification activity of *L. lactis* subsp. *lactis* were monitored in MRS broth, FSM supplemented without and with RS and DF for 24 h at 30°C. The results showed that *L. lactis* subsp. *lactis* grew during the first 2 h in MRS broth and SFM supplemented with RS and DF, whereas this probiotic bacterium grew after 2 h in SFM without supplementation. However, higher growth rates were observed for SFM supplemented with RS and DF than MRS broth. Similarly, RS and DF supplementation raised the maximum specific growth rate and lowered the generation time significantly ($p<0.05$). In addition, FSM supplemented with RS and DF yielded bacterial growth with higher values of *L. lactis* subsp. *lactis* than FSM and MRS broth. Furthermore, the decrease in the extracellular pH and increase total acid again con?rmed the results of growth profile, being lower and higher, respectively, than in the case of the FSM containing RS and DF. The low amount of acid produced in FSM grown culture could be due to the slower and prolonged fermentation by this probiotic bacterium. This present study revealed that RS and DF extracted from by-products of banana processing are two of the greatest prebiotics and can be applied to the fermented sausage.

INTRODUCTION

Lactococcus lactis subsp. *lactis* is widely used mesophillic meat starters in fermented meat. It has been included in the Generally Regard as Safe (GRAS) and also serves as probiotics (Aslam and Qazi, 2010). The intake of RS, DF and probiotics exert a positive impact on the development of the intestinal microbiota and are reported to relieve constipation and reduce the incidence of colon cancer (Farnworth, 2008). Finally, the beneficial effects on probiotics viability exerted by some ingredients such as fruit by-product to pork sausage and fermented sausage have been reported (Ribas-Agustí et al., 2014).

The waste of food is an unlucky reality worldwide. In particular, during the processing of fruit for pulp production, around 65–70% by weight of the raw material is lost, leading to serious environmental problems. However, it was demonstrated that some fibers of fruit by-products show functional properties such as water-holding, swelling, gel forming, bile acid binding, and cation-exchange capacities (Lamsal and Faubion, 2009). Among the promising fruit by-products are low quality pulp of banana and the peels of banana, mainly because of their content of resistant starch (RS), insoluble and soluble dietary fibers (DF) and fructooligosaccharides. These prebiotics are in fact able to selectively stimulate the growth and activity of the gut microbiota, particularly lactobacilli and bi?dobacteria (Davis and Milner, 2009). It was reported that RS production from green banana pulp contains high quantity of RS (approximately 45.71%) and DF production from green banana peel contains high quantity of DF (approximately 78.62%) (Suksathit and Tangwatcharin, 2015). Furthermore, peel of banana (Musa sp., Musaceae) contains around 43–49 g of total DF per 100 g of dry matter, in addition to significant amounts of α -linolenic acid (ALA), essential amino acids and micronutrients (Emaga et al., 2007, Mohapatra et al., 2010).

Considering the continuous search for efficient, safe, and cost-effective RS and DF for application in the meat industry and the opportunities that RS and DF might open with regard to these concerns, the present study investigates the potential effects of RS and DF from banana by-products that are abundantly available in nature with regard to the quality characteristics of fermented sausage model. The effects of the addition of these RS and DF to fermented sausage model in term of probiotic viability enhancement are reported.

MATERIALS AND METHODS

Bacterial strain. *L. lactis* subsp. *lactis* from traditional fermented meat product of culture collection of Meat Microbiology Laboratory, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabangwas used in this study and its preliminary potential probiotic was determined by antagonistic

activity of foodborne pathogen, bacteriocin production and survival of LAB in pH conditions and gut model. Furthermore, its identification was confirmed by 16S rRNA gene sequence analysis of *Lactococcus*. This probiotic was maintained on de Man, Rogosa & Sharpe agar (MRS) (Merck, Germany) in anaerobic condition. The overnight culture in anaerobic condition was prepared by inoculating approximately 10 ml of MRS broth adding NaCl (10 g/l) with 2-3 colonies taken from MRS agar that the count of bacteria was approximately 10^9cfu/ml was adopted for growth profile and acidification activity of *L. lactis* subsp. *lactis* in fermented sausage model (SFM) supplemented with RS and DF.

Extraction of resistant starch and dietary fiber. Edible green (unripe) banana (Kluai Namwa, ABB) pulps and peels were collected from a local banana processing factory in Chachoengsao province, Thailand. In processing into powder, the banana pulps and peels were sliced into 1-mm-thick pieces, spread evenly on a stainless steel tray, dried in a hot-air oven at 50°C for 8 h, and then milled and passed through a 1 mm sieve (Wachirasiri et al., 2009).

Resistant starch (RS) from banana pulp prepared through two autoclaving–cooling cycles was dispersed in distilled water in a ratio of 1:4 (w/v). Some hydrochloric acid or citric acid or acetic acid was added until acid concentration was 0.1 mol/l. The mixture was stored at room temperature for 12 h, and then 1 mol/l sodium hydroxide solution was added to neutralize acid until pH 7.0. The neutralized mixture was stored at 4 °C for 24 h, dried in an oven (105 °C) and then ground and passed through a 1mm sieve (Zhao and Lin, 2009).

Dietary fiber (DF) from banana peel was extracted using the method of Yoshimoto et *al.* (2005). The banana peel powder samples were defatted for 12 hrs using hexane as a solvent (5 ml/g sample). The residue was dried at 50 °C in a hot air oven to assure complete removal of the solvent. The defatted peel powder was mixed with water (1: 20 w/v ratio). The pH was adjusted to 5.8 by adding 1 N HCl solution. An alpha-amylase was added (0.1 ml/g sample). The sample was incubated at 95°C for 30 min. after cooling down to 60°C, the pH was adjusted to 7.5 by adding 1 N NaOH. Neutrase was then added (10 mg/g sample) and incubated for 30 min at 60°C. After that, the pH was adjusted to 4-4.5 by using 1 N HCl solution. An amyloglucosidase solution was added (0.1 mg/g sample) at 60°C for 30 min. Finally, the mixture was filtered through Whatman No.4 filter paper and dried in the hot air oven at 50°C for 12 hrs. The dried samples were then powdered in an Udy cyclone mill (Udy cooperation, Colorado USA) using a 1 mm sieve.

Growth condition. The growth profile and acidification activity were determined in FSM including meat extract (10.0 g/l); tryptone (10.0 g/l); glucose (10.0 g/l); NaCl (25.0 g/l); sodium tri-polyphosphate (3.0 g/l); sodium ascorbate (0.5 g/l); sodium nitrite (0.1 g/ml); and cooked rice (2.0 g/l). After medium sterilization, shopped garlic (50.0 g/l) was added in the medium. After that 300 ml of FSM were divided into 3 groups as follows: (1) non supplemented (FSM), (2) supplemented with RS (10 g/l) and (3) supplemented with DF (10 g/l). *L. lactis* subsp. *lactis* was inoculated in FSM and MRS broth (control). The initial count of bacteria in each group was approximately 10^5 cfu/g. The growth of the test inoculates was investigated by checking the evolution of viable cell counts of *L. lactis* subsp. *lactis* in FSM. The sampling for determination of cfu, pH and total acid was carried out in triplicate after 0, 2, 4, 6, 8, 12 and 24h of incubation at 30°C in anaerobic condition.

Growth profile. The sample suspensions were submitted to count for *L. lactis* subsp. *lactis*. Enumeration of bacteria was done on MRS agar (de Man Rogosa and Sharp, Merck, Germany) adding NaCl (10 g/l). The plates were incubated at 30 ± 2°C for 24-48 h in anaerobic condition before colonies were counted. The results were transformed log cfu/ml. The maximum specific growth rate (μ_{max}) was calculated during the exponential growth phase as$\mu_{max}=\ln(X_2/X_1)/(t_2-t_1)$, being$X_2andX_1$the counts (cfu/ml) at timet_2 andt_1, respectively. The generation time ($l=\ln 2/\mu_{max}$) was calculated for each culture from the corresponding value of μ_{max} (Oliveira et al., 2011).

Acidification activity. The pH of each sample suspension was determined at room temperature using electrodes of a pH meter (Mettler mini scan EZ, Germany) placed directly into each suspension. The determination was performed in triplicate to find the mean pH of the sample. The total acid was determined as described by Thomas et al. (2015) using colorimetric acidity titration.

Statistical analysis. Data of bacterial count, μ_{max}, l, pH value and total acid were presented as means and standard deviations. All statistical computations were performed to determine significant differences ($p < 0.05$) by ANOVA

followed by Duncan's new multiple range test.

RESULTS AND DISCUSSION

Growth profile of *L. lactis* subsp. *lactis*. The growth of *L. lactis* subsp. *lactis* in fermented sausage model supplemented without and with 1% (w/v) RS or 1% (w/v) DF are shown in Fig. 1. MRS broth was also included in this study for comparative purpose. This probiotic grew during the first 2 h in MRS broth and SFM supplemented with RS and DF, whereas it grew after 2 h in SFM. Higher growth rates were observed for SFM supplemented with RS and DF than MRS broth. According to Fig. 1B and 1C, RS and DF supplementation raised the maximum specific growth rate (μ_{max}) and lowered the generation time (l) significantly ($p<0.05$) which means they exerted a prebiotic effect (Oliveira et al., 2011). Moreover, green banana flour is high RS content which is important on growth of probictic bacteria. Generally, food with high RS content (green banana flour, retrograded rice flour, and RS standard) would be better to promote the survival of probiotic bacteria (Dangsungnoen et al., 2012). DF extracted from green banana peel contains around 78.62 g of total DF, 1 g of inulin, 6 g of fructooligosaccharide and 10–20 g of pectin per 100 g of dry matter, in addition to significant amounts of α -linolenic acid (ALA), essential amino acids and micronutrients such as Mg, K, P and Ca (Emaga et al., 2007; Mohapatra et al., 2010; Suksathit and Tangwatcharin, 2015). In addition, FSM supplemented with RS and DF yielded bacterial growth with higher values of *L. lactis* subsp. *lactis* than FSM and MRS broth. This probiotic in all groups reached a stationary state within 10–12 h, and constancy in cell concentration was observed during the ?nal stages of fermentation (Fig. 1A).

Acidification activity of *L. lactis* subsp. *lactis*. The decrease in the extracellular pH and increase total acid again con?rmed the results of growth profile (Fig. 2A and 2C), being lower and higher, respectively, than in the case of the FSM containing RS and DF. The low amount of acid produced in FSM grown culture could be due to the slower and prolonged fermentation by this bacterial strain. *L. lactis* has a homofermentative metabolism and produce lactic acid which the acid decrease the pH (Roissart and Luquet, 1994).

IMPLICATIONS

The capabilities to enhance growth and produce acid by *L. lactis* subsp. *lactis* are two of the reasons why RS and DF extracted from banana pulp and peel, by-products of banana processing, are two of the greatest prebiotics and can be applied to the fermented sausage.

Keywords: Probiotic bacteria, Resistant starch, Dietary fiber, Fermented sausage model, By-product of banana processing

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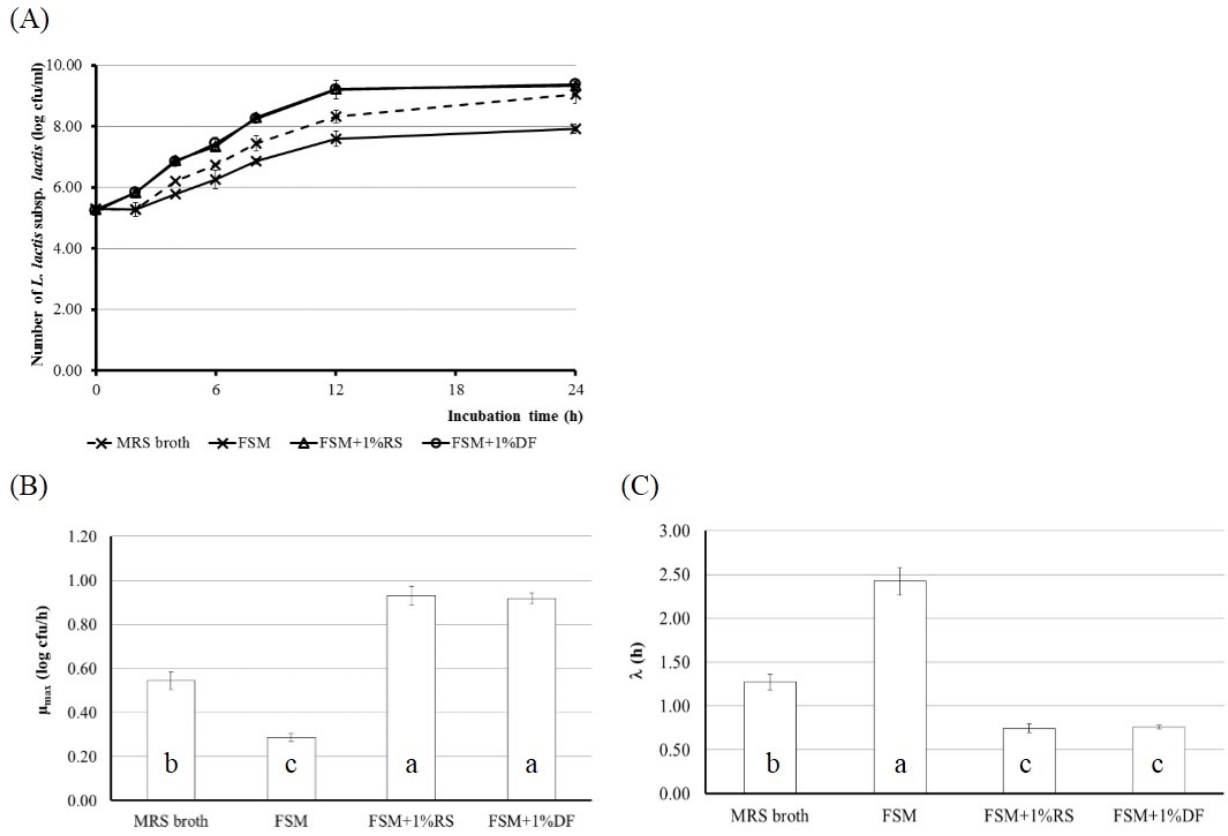

Fig. 1 Growth (A), maximum specific growth rate (μ_{max}) (B) and generation time (λ) (C) of *L. lactis* subsp. *lactis* in MRS broth and fermented sausage model supplemented without and with 1% (w/v) RS or 1% (w/v) DF. The results are presented as means of three independent experiments and standard deviations (bar). [a-c] Different letters indicate that values are significantly different ($p < 0.05$).

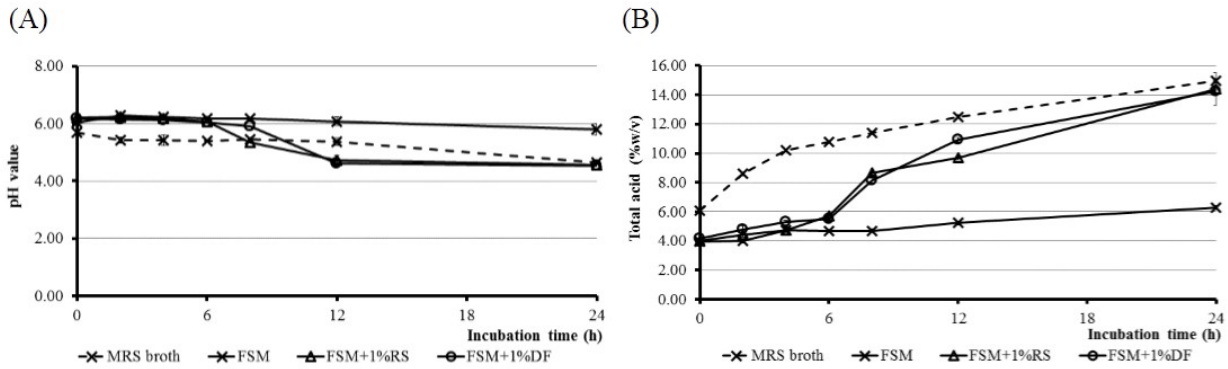

Fig. 2 The pH value (A) and total acid (B) of changing by *L. lactis* subsp. *lactis* in MRS broth and fermented sausage model supplemented without and with 1% (w/v) RS or 1% (w/v) DF.

REFERENCES

Aslam, S. and J. I., Qazi, 2010. Isolation of acidophilic lactic acid bacteria antagonistic to microbial contaminants. Pakistan J. Zool. 42: 567-573.

Dangsungnoen, P., A. Moongngarm and S. Deeseenthum. 2012. Comparison of resistant starch content and survival of *Lactobacillus spp.* on four different sources of resistant starch. 2012 International Conference on Life Science and Engineering. 23-24 July 2012. Singapore.

Davis, C. D. and J. A. Milner. 2009. Gastrointestinal microflora, food components and colon cancer prevention. J. Nutr. Biochem. 20: 743-752.

Emaga, T. H., R. H. Andrianaivo, B. Wathelet, J. T. Tchango and M. Paquot. 2007. Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. Food Chem. 103: 590–600.

Farnworth, E. R. 2008. The evidence to support health claims for probiotics. J. Nutr. 138: 1250–1254.

Lamsala, B. P. and J. M. Faubion. 2009. The beneficial use of cereal and cereal components in probiotic foods. Food Rev. Int. 25: 103-114.

Mohapatra, D., S. Mishra and N. Sutar. 2010. Banana and its by-product utilization: an overview. J. Sci. Ind. Res. 69: 323–329.

Oliveira, R. P. D. S., P. Perego, M. N. D. Oliveira and A. Converti. 2011. Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. LWT - Food Sci. Technol. 44: 520–523.

Wachirasiri, P., S. Julakarangka and S. Wanlapa. 2009. The effects of banana peel preparations on the properties of banana peel dietary fibre concentrate. Songklanakarin J. Sci. Technol. 31: 605-611.

Ribas-Agustí, A., M. Gratacós-Cubarsí, C. Sárraga, M. D. Guàrdia, J. García-Regueiro and M. Castellari. 2014. Stability of phenolic compounds in dry fermented sausages added with cocoa and grape seed extracts. LWT - Food Sci. Technol. 57: 329-336.

Roissart, H. and F. M. Luquet 1994. Bactéries lactiques: aspects fondamentaux et technologiques. Uriage, Lorica, France. p. 605.

Suksathit, S. and P. Tangwatcharin. 2015. Chemical composition, functional properties and preliminary prebiotic properties of dietary fiber and resistant starch from green banana 'Kluay Khai' peel and pulp. King Mongkut's Agric. J. 33: 49-60.

Thomas, B. T., M. O. Oni, G. C. Agu, O. D. Popoola and A. Davies. 2015. Multivariate analysis of intrinsic parameters of Garri for the quantitative prediction of its microbial loads. Int. J. Microbiol. Res. 6: 195-199.

Yoshimoto, M., O. Yamakawa and H. Tanoue. 2005. Potential chemopreventive properties and varietal difference of dietary fiber from sweet potato (*Ipomoea batatas* L.) root. Japan Agric. Res. Quarterly. 39: 37-43.

Xin-Huai Zhao and Y. Lin. 2009. Resistant starch prepared from high-amylose maize starch with citric acid hydrolysis and its simulated fermentation *in vitro*. Eur. Food Res. Technol. 228: 1015–1021.

PO-02-42 Quality of Reduced-Fat Thai-fermented Sausage (Isan sausage) Substituted with Konjac Gel

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INTRODUCTION

Thai-fermented sausage or Isan-sausage is traditional food in the north and northeastern of Thailand. All ingredients are stuffed in natural casing or synthetic casing and made dry in the air about 3-4 days and typically eaten as a light meal served with bird's eye chilis, raw cabbage, and sliced ginger. Thai-fermented sausage usually contain very high fat as it is made from 50% pork, 35% back fat, cooked rice and seasoning. However, this product presents some negative health effects because of their high fat. The kind of saturated fat from meat is often assumed to contribute to the increase in these health problems, for example, the cardiovascular disease, coronary heart disease and obesity (WHO, 2003). Nowadays, consumers are interested in good health that the food industry to develop a new formulation to contain less fat. Therefore, they use fat replacers such as modified starch, fiber, gum and cellulose. Fat replacer can be divided into two kinds: 1) Fat substitute and 2) Fat mimetic. Fat substitute consists of a physical structure and a chemical structure which resemble the fat but it can't digestive by an enzyme and low calorie. Fat mimetic is protein or carbohydrate which shows similar properties of the fat such as flavor, texture and mouthfeel (Akoh, 1999).

Konjac glucomannan (KGM) is a neutral polysaccharide produced by the Amorphophallus konjac, a native plant of East Asia, where it has been used since ancient times.Although KGM can be used for different purposes on account of its technological properties, it forms gels which combined with other ingredients (starch, carrageenan, gellan gum) can be used as 'fat analogs' in the formulation of reduced/low-fat meat products. Additionally, konjac gel, when ground down to the desired particle size can give the appearance of visible granulated fat required for use as a raw material to substitute animal fat. There have been researchers using fat analog in frankfurter frankfurters (Jiménez-Colmenero et al., 2010), bologna sausage (Chin et al., 2000), fresh sausages (Osburn and Keeton, 1994), pork nuggets (Berry and Bigner, 1996) or pâté (Delgado-Pando et al., 2011).

The aim of this study was to investigate the effect of the fat reduction achieved by substitute pork backfat as 0%, 50% and 75% with the same proportion of konjac gel, on the process and quality characteristic of reduced-fat Thai-fermented sausage, with no change in the proportion of lean meat used.

MATERIALS AND METHODS

Preparation of konjac gel

Konjac gel was made with konjac flour, corn starch, carrageenan and food grade calcium hydroxide. Its preparation was based on that described by Osburn and Keeton (2004) with slight modifications by Jiménez-Colmenero et al. (2010). The resulting konjac gel was stored at 4 ± 1 °C and used for Thai-fermented sausage within 24 h of preparation.

Preparation of Thai-fermented sausage

Three different formulations of Thai-fermented sausage were produced. The first group was a control sample, prepared with normal fat content, mainly using 60% meat and 25% pork backfat. The second group was a reduced-fat sample designed as 50% konjac gel substituted fat, the formulation using 60% meat, 12.5% pork backfat and 12.5% konjac gel. The final group was 75% konjac gel substituted fat, which it formulated with 60% meat, 6.25% pork backfat and 18.75% konjac gel. All samples also contained 15% cooked rice and seasoning.

Monitoring quality during fermentation and analyzing product quality

During 4 days of fermentation, pH, total acidity, weight loss, water activity (a_w) and moisture content of all groups were monitored. At the end of fermentation, uncooked products were used to determinations of color (CIE L*a*b*) and texture profile analysis, and cooked samples were used to sensorial evaluation by a 9-point hedonic score test. Seven panelists were recruited and trained before evaluating samples.

Statistical analysis

All experiments in this study were carried out by completely randomized design**.** Mean values were compared by the Duncan's multiple range test. The analysis was performed using the SPSS package (SPSS 11.5 for windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSTION

The changes in characteristics during fermentation

All Thai-fermented sausage showed a gradually pH decline during 4 days of fermentation from around 5.78-6.03 to around 4.66-5.11 depending on their treatments (Fig. 1a). This evident was supported by the increasing in total acidity of samples from 0.33-0.50% at the beginning and rising to 0.93-0.96% at days 4 of fermentation as shown in Figure 2b. At the end of the process, although there was no significant differences in total acidity among samples ($P>0.05$), the effect of substituted fat with konjac gel was found. Products substituted with 75% konjac gel had the highest pH value, followed by 50% konjac gel and control, respectively. This result might be obtained because the konjac gel had a higher pH than the pork backfat that it was replaced.

As a result of pH decrease along fermentation, the increasing of weight loss in all sausage was found (Fig. 1c). However, sample substituted fat with 75% konjac gel had the highest weight loss, followed by 50% konjac gel and control, respectively ($P<0.05$). Regarding a_w and moisture content of samples, there were gradually decreased during fermentation. Additionally, at the end of fermentation, it was found that fat reduction with 75% konjac gel presented the lowest a_w value as compared to 50% konjac gel and control ($P<0.05$). The result implied that the addition of the high-konjac gel content in the product was inferior ability to hold water itself than pork backfat.

Quality characteristics of Thai-fermented sausage

Products substituted fat with konjac gel (both 50% and 75%) provided a higher redness and yellowness than control ($P<0.05$) (Table 1). Additionally, the substituted fat with 75% konjac gel had a lower lightness value than control ($P<0.05$), but the substituted as 50% konjac gel showed an intermediate value. The differences in color among samples as represented by product picture were shown in Figure 1f. Other studies had shown that when fat content was reduced, the product was darker and redder than a high-fat product. This effect was observed in cooked sausage (Carballo et al., 1996) and dry fermented sausage (Muguerza et al., 2004).

Concerning texture profile analysis, springiness and chewiness were not affected by fat reduction ($P>0.05$). It can be observed that the decrease in the fat content or increase level of konjac gel resulted in the decrease in the cohesiveness of product ($P<0.05$) (Table 1). In addition, the product with 75% konjac gel had an inferior quality with respect to higher hardness and gumminess as compared to control ($P<0.05$). The formation of harder structures has been reported as fat content decreases in dry fermented sausages (Liaros et al., 2009; Muguerza et al., 2004), and in cooked meat products (Carballo et al., 1996).

Sensory scores of Thai-fermented sausage was shown in Table 1. No significant differences in color, appearance, odor and flavor were found among products ($P>0.05$). Nevertheless, the substituted fat with 75% konjac gel provided lower scores of tenderness, juiciness and overall acceptability than control ($P<0.05$), but the 50% konjac gel showed a similar quality as compared to the control one ($P<0.05$).

CONCLUSION

Reduced-fat Thai-fermented sausage (Isan sausage) substituted with konjac gel opens up new possibilities for fat reduction in this product. The substituted pork backfat with 50% konjac gel into product had acceptable sensory characteristics similar to a control. However, the usage of the konjac gel for fat reduction was limited. The high proportion of konjac gel, more than 50% to reduce fat, it might present an undesirable result in pH, weight loss, color hardness and juiciness as well as overall acceptability.

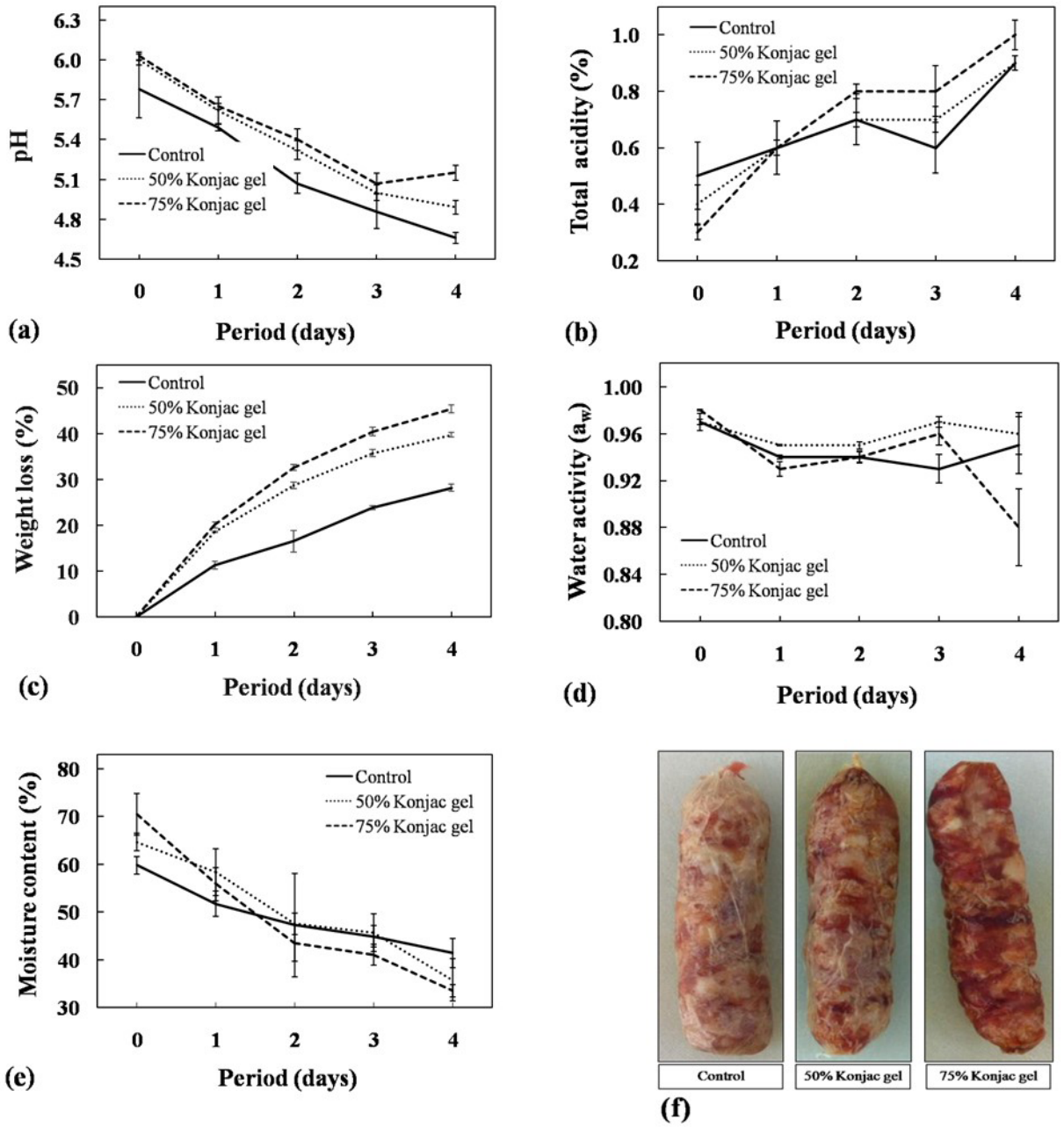

Figure 1. Changes in pH (a), total acidity (b), weight loss (c), a_w (d) and moisture content (e) of Thai-fermented sausages during 4 days of fermentation. Representative picture of Thai-fermented sausages (uncooked) from different treatments at 4 days for fermentation (f).

Table 1. Physical and sensorial properties of Thai-fermented sausage from different treatments

Characteristics	Control	50% konjac gel	75% konjac gel
Uncooked product color			
- Lightness (L*)	55.19 ± 3.46[a, †, ‡]	50.89 ± 1.77[ab]	49.02 ± 2.89[b]
- Redness (a*)	3.78 ± 1.38[b]	7.22 ± 0.63[a]	6.16 ± 1.05[a]
- Yellowness (b*)	6.55 ± 0.37[b]	12.01 ± 0.10[a]	11.08 ± 1.10[a]
Texture characteristic of cooked product			
- Hardness (N)	44.85 ± 8.52[b]	57.23 ± 4.53[ab]	71.40 ± 18.35[a]
- Cohesiveness (ratio)	0.51 ± 0.02[a]	0.43 ± 0.02[b]	0.38 ± 0.01[c]
- Gumminess (N)	23.02 ± 4.46[b]	24.55 ± 2.08[ab]	28.58 ± 3.45[a]
- Springiness (ratio)	0.55 ± 0.03[a]	0.51 ± 0.04[a]	0.53 ± 0.05[a]
- Chewiness (N)	12.64 ± 2.64[a]	12.65 ± 1.66[a]	15.08 ± 2.63[a]
Sensorial attributes			
- Color	8.00 ± 0.82[a]	7.29 ± 1.80[a]	7.43 ± 1.99[a]
- Appearance	7.43 ± 2.15[a]	8.00 ± 0.58[a]	7.29 ± 0.95[a]
- Odor	8.14 ± 1.07[a]	8.14 ± 0.90[a]	8.29 ± 0.76[a]
- Flavor	8.14 ± 0.69[a]	7.71 ± 1.25[a]	7.71 ± 1.11[a]
- Tenderness	6.57 ± 1.81[a]	6.50 ± 1.76[a]	3.86 ± 2.19[b]
- Juicy	8.14 ± 0.69[a]	6.64 ± 1.44[ab]	4.86 ± 2.41[b]
- Overall acceptability	7.71 ± 0.49[a]	7.00 ± 1.15[ab]	5.43 ± 2.15[b]

† Values are given as means ± SD of each meat batch (n=3).
‡ Different superscripts in the same row indicate significant differences among treatments (P<0.05).

REFFERENCES

Akoh, C.C. 1999. Fat substitute. Food Ingredients Anal. Int. 21: 13-22.

Berry, B. W. and M. E. Bigner. 1996. Use of carrageenan and konjac flour gel in low-fat restructured pork nuggets. Food Res. Int. 29: 355–362.

Carballo, J., P. Fernández, G. Barreto, M. T. Solas and F. Jiménez-Colmenero. 1996. Morphology and texture of bologna sausage containing different levels of fat, starch and egg white. J. Food Sci, 61: 652–655.

Chin, K. B., J. T. Keeton, R. K. Miller, M. T. Longnecker and J. W. Lamkey. 2000. Evaluation of konjac blends and soy protein isolate as fat replacements in low-fat bologna. J. Food Sci. 65: 756–763.

Delgado-Pando, G., S. Cofrades, C. Ruiz-Capillas, M. T. Solas, M. Triki and F. Jiménez-Colmenero. 2011. Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation. Meat Sci. 89: 65–71.

Jiménez-Colmenero, F., S. Cofrades, I. López-López, C. Ruiz-Capillas, T. Pintado and M. T. Solas. 2010. Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. Meat Sci. 84: 356–363.

Liaros, N. G., E. Katsanidis, and J. G. Bloukas. 2009. Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. Meat Sci. 83: 589–598.

Muguerza, E., O. Gimeno, D. Ansorena and I. Astiasarán. 2004. New formulations for healthier dry fermented sausages: A review. Trends Food Sci. Technol. 15: 452–457.

Osburn, W. N. and J. T. Keeton. 1994. Konjac flour gel as fat substitute in low-fat prerigor fresh pork sausage. J. Food Sci. 59: 484–489.

Osburn, W. N. and J. T. Keeton. 2004. Evaluation of low-fat sausage containing desinewed lamb and konjac Gel. Meat Sci. 68: 221–233.

WHO.2003. Diet, nutrition and the prevention of chronic diseases. WHO Technical report Series. 916. Geneva.

PO-02-44

Water-holding Capacity and Tenderness of Pork during Storage Times

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Introduction

Meat palatability is affected by several factors and tenderness is a one of the most important. Consumers are willing to pay a premium for a guaranteed tender product with the potential to increase the value of the meats (Miller et al., 2001). The primary mechanism of postmortem improvement in tenderness is through the degradation of the structure of the muscle.

Biochemical processes proceed during handling, processing and storage of meat. This directly affects the quality of the products (Toldra et al., 1997; Toldra and Flores, 2000). Proteolytic activity during the conversion of muscle to meat and the subsequent aging of meat is of importance for several meat quality parameters. These include the development of flavor, tenderness, and water-holding capacity of meat (Bauchart et al., 2006; Gill et al., 1996; Koohmaraie, 1994).

During storage times, meat characteristics were changed by the action of enzymes on the proteins. As postmortem metabolism continues, the activity of proteolytic enzymes continues as long as the important factors such as suitable pH, temperature, substrate availability and presence of specific ions or inhibitors are present (Ertbjerg et al., 1999; Rosell et al., 1996). The degradation of proteins in meat is mainly influenced by the calpain and the cathepsins. The calpain is mainly active during the early postmortem (pH > 6), while the cathepsins are mainly active at low pH. (Spanier et al., 1990). Pork tenderness increases rapidly in the first 48 hours postmortem. According to the finding of Dransfield (1994), 80 percent of tenderization occurs in about five days for pork. Normally, consumers in Thailand buy pork from the market and cook it daily or keep in refrigerator for a week. Under this condition, therefore, this present study was aim to evaluate the meat quality especially water-holding capacity and tenderness of pork during storage times.

Materials and Methods

Forty-eight fattening pigs (22.25 ± 3.27 kg BW), 24 barrows and 24 gilts, were assigned to the experiment of this study. All animals were fed with basal diet and drinking water *ad libitum*. At 99.08 ± 11.82 kg BW, pigs were slaughtered at the standard slaughterhouse. Muscle pH at 45 min (pH_{45min}) and 24 h (pH_{24h}) postmortem were measured (Metler-Toledo: SevenGoTM SG2 pH meter) directly on the carcasses at the 10-11th rib. *Longissimus dorsi* (LD) and *Semimembranosus* (SM) muscles were collected from each pig. The whole muscle was cut into 1¼ inches thickness and storage in vacuum bag at 0-4°C for 2 and 5 days postmortem. After storage times, the samples were kept at -20°C for further analysis. Meat samples were used to determine thawing loss, cooking loss and Warner-Bratzler shear force (WBSF) according to the methods of Wheeler et al. (2005). All data were analyzed using independent t-test. Values of $P \leq 0.05$ were considered significant.

Results and Discussion

The results showed that pH_{45min} and pH_{24h} of LD and SM muscle were generally accepted (Figure 1). The incidence of PSE and DFD pork did not find in this study. Several studies indicated that pH_{45min} and pH_{24h} (ultimate pH) of normal pork muscle were 6.4 and 5.7-5.8, respectively (Adzitey and Nurul, 2011; Faucitano et al., 2010; Warriss et al., 1987).

The data of present study showed that there were no significantly different ($P>0.05$) on thawing loss, cooking loss and WBSF of pork LD and SM muscle during storage times (Table 1). Whereas, the findings of Ellis et al. (1998); Frenzel et al. (2014) reported that increasing of ageing time decreased ($P<0.01$) shear force values of pork loin. And the report of Juárez et al. (2011) indicated that the increase in duration of wet/dry ageing also decreased ($P<0.05$) drip loss, cook loss and shear value of pork LD. For the result of this study, storage times did not affect the water holding capacity and WBSF of pork loin. It might be the inappropriate factors (pH, temperature,

substrate, etc.) for proteolytic enzymes activity (Ertbjerg et al., 1999; Rosell et al., 1996). However, the several studies concluded that pH at postmortem was highly correlated (P<0.01) with water holding capacity and water loss (Huff-Lonergan and Lonergan, 2005; Bulotien? and Jukna, 2008; Adzitey and Nurul, 2011).

Conclusion

According to the results were obtained in this study, storage time effect was no observed on water-holding capacity and WBSF of pork LD and SM muscle. It was concluded that pork quality could not be improved during 2 and 5 days of storage times.

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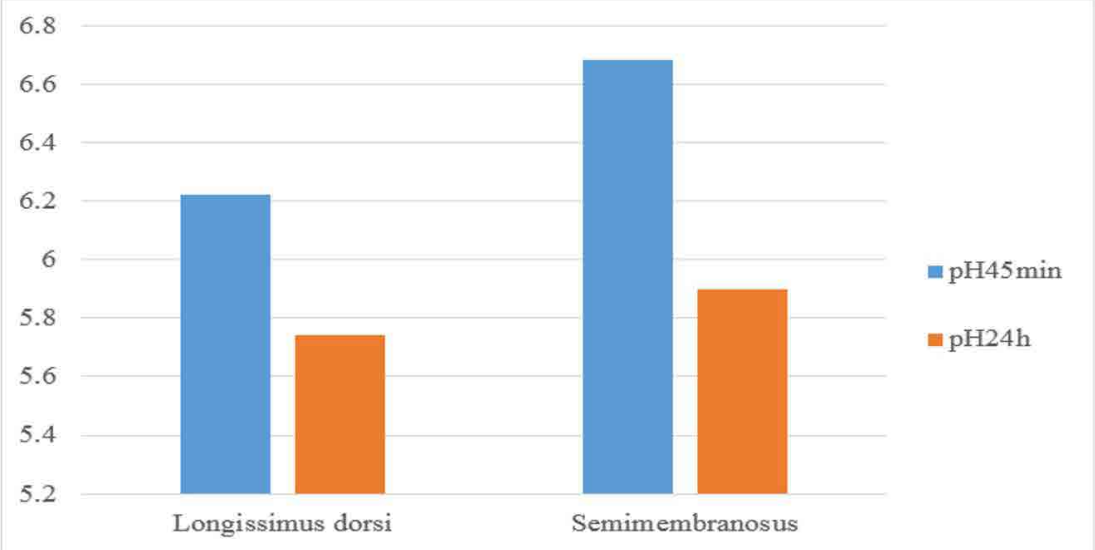

Figure 1 Meat pH of LD and SM muscle at 45 min and 24 h postmortem

Table 1 Water-holding capacity and WBSF of pork LD and SM muscle during 2 and 5 days of storage times

Parameters	Storage times		P-value
	2 days	5 days	
Longissimus dorsi			
Thawing loss (%)	8.84	9.70	0.12
Cooking loss (%)	22.11	22.44	0.27
WBSF* (kg)	6.37	6.03	0.70
Semimembranosus			
Thawing loss (%)	11.64	11.42	0.21
Cooking loss (%)	25.53	25.53	0.99
WBSF* (kg)	5.79	5.80	0.93

*WBSF = Warner-Bratzler shear force

References

Adzitey, F. and H. Nurul. 2011. Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to redu ce these incidences - a mini review. J. Inter. Food Res. 18: 11-20.

Bauchart, C., D. Remond, C. Chambon, P. Patureau Mirand, I. Savary-Auzeloux, C. Reynes, and M. Morzel. 2006. Small peptides (<5 kDa) found in ready-to-eat beef meat. Meat Sci. 74(4): 658-666.

Bowker, B. C., A. L. Grant, J. C. Forrest, and D. E. Gerrard. 2000. Muscle metabolism and PSE pork. In Proceedings of the American Society of Animal Science.

Bulotienė, G., and V. Jukna. 2008. The influence of muscle fibre area on pork quality. Veterinarija Ir Zootechnika. T. 42: 34-37.

Dransfield E. 1994. Optimization of tenderization, ageing and tenderness. Meat Sci. 36: 105-121.

Ellis, M., M. S. Brewer, D. S. Sutton, H. Y. Lan, R. C. Johnson, and F. K. McKeith. 1998). Aging and cooking effects on sensory traits of pork from pigs of different breed lines. J. Muscle Foods. 9(3): 281−291.

Ertbjerg, P., P. Henckel, A. Karlsson, L. M. Larsen, and A. J. Møller. 1999. Combined effect of epinephrine and exercise on calpain/calpastatin and cathepsin B and L activity in porcine longissimus muscle. J. Anim. Sci. 77(9): 2428-2436.

Faucitano, L., M. C. Ielo, C. Ster, D. P. Lo Fiego, S. Methot and L. Saucier. 2010. Shelf life of pork from five different quality classes. Meat Sci. 84(3): 466-469.

Frenzel, L. L., R. M. Harp, B. D. Lambert, J. T. Sawyer., and M. A. Frenzel. 2014. Effects of wet aging and temperature on Warner-Bratzler shear force, sensory characteristics, and microbial shelf-life of pork loin chops. The Texas J. Agri. Natural Resources. 27: 24-35.

Gill, I., R. Lopez-Fandino, X. Jorba, and E. N. Vulfson. 1996. Biologically active peptides and enzymatic approaches to their production. Enzyme. Microb. Technol. 18(3): 162-183.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71: 194-204.

Juárez, M. W. R. Caine, M. E. R. Dugan, N. Hidiroglou, I. L. Larsen, B. Uttaro, and J. L. Aalhus. 2011. Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Sci. 88(2011): 117–121.

Koohmaraie, M. 1994. Muscle proteinases and meat aging. Meat Sci. 36: 93-104.

Miller, M.F., M.A. Carr, C.B. Ramsey, K.L. Crockett, and L.C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79: 3062-3068.

Rosell, C.M., M. Flores, and F. Toldra. 1996. Myoglobin as an endogenous inhibitor of proteolytic muscle enzymes. J. Agri. Food Chem. 44(11): 3453-3456.

Spanier, A. M., K. W. McMillin, and J. A. Miller. 1990 Enzyme activity levels in beef: effect of post-mortem aging and end-point temperature. J. Food Sci. 55: 318-322.

Toldra, F., and M. Flores. 2000. The use of muscle enzymes as predictors of pork meat quality. Food Chem. 69(4): 387-395.

Toldra, F., M. Flores, and Y. Sanz. 1997. Dry-cured ham flavour: enzymatic generation and process influence. Food Chem. 59(4): 523-530.

Warriss, P. D., and S. N. Brown. 1987. The relationships between initial pH, reflectance and exudation in pig muscle. Meat Sci. 20(1): 65-74.

Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 2005. Shear Force Procedures for Meat Tenderness Measurement. Online available: http://www.ars.usda.gov/SP2UserFiles/Place/30400510/protocols/ShearForceProcedures.pdf

PO-02-47

GLOVE DETECTOR CAPACITANCE-BASED TIREN CHICKEN MEAT AS CHICKEN MEAT SAFETY SOLUTION IN INDONESIA

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INTRODUCTION

Almost of all Indonesian communities like chicken food. Chicken meat is liked because it can be cooked into various types of foods, the numbers that much, and the price is reasonable. But the currently problem in Indonesia is low food safety applied by traders in traditional markets. Many cases of the sale of chicken meat which is not feasible as sale of tiren chicken meat. Tiren Chicken (mati kemarin) or carcass chicken is chicken that does not through the process of good slaughter. Tiren chicken meat does not through good slaughter so that the three channels on the neck of the chicken (gastrointestinal tract, respiratory tract, and blood vessels) are not cut off completly. This causes the blood do not out from the body and the blood settles on the meat. The blood settles in the meat will be a source of nutrients for the growth of microorganisms (bacteria, viruses, and fungus) faster. This causes problem to consumer health who consume it.

Food safety that must be applied in Indonesia must fulfill the criteria of safe, healthy, whole, and halal (SHWH). The Government's policy of providing meat that is SHWH with the objective of protecting public health and inner peace of mind guarantee society. As for the meat in question was: safe i.e. meat does not contain the danger of chemical and physical biology, which can lead to disease and disrupt human health; healthy i.e. meat have substances that are needed and useful to the health and growth of the human body; whole i.e. not meat mixed with parts of other animals; and halal i.e. animals or their meat is slaughtered and handled in accordance of Islamic jurisprudence (The Minsitry of Agriculture, 2012). Tiren chicken meat which is sold in traditional markets surely don't fulfill the criteria of SHWH because: tiren chicken contain dangers that can lead to disease and disrupt human health; substances that your body needs on tiren chicken meat damaged; chicken meat mixed with tiren chicken meat, tiren chicken meat is not halal because the meat not slaughtered and not handled according of Islamic jurisprudence.

Tiren chicken meat sales in traditional markets are banned because that would be detrimental to consumers. Government through legislation of criminal law (KUHP) Article 501 paragraph 1 States the party authorities would catch the perpetrators (trader) sells goods damaged or carrion (tiren). Islam forbids such things because of the elements of fraud and the sale of meat are not halal. In accordance with the Hadith, the mother of Syihad "that Ubeidullah Ibn 'Abd convey to him that Abdullah ibn Abbas told him that the Prophet passed on a goat that has become the carcass, and then he said: "why do not you capitalize on his skin?" They said, "it was a wreck." Then his words were: "the unlawful it is just eat it!" (Narrated By Al-Bukhari Muslim). Tiren chicken meat should be utilized, but cannot be sold or consumed because it contains elements of fraud and not in accordance with the criteria of SHWH. Meat is one of the farm products that is composed of a network of animal and can be processed so that it can be consumed, without interfering with the body's health. Chicken has a complete nutrient content and high protein. Chicken meat has a lot of good nutrition for health due to the presence of essential amino acids are complete and balanced, water, carbohydrates, and inorganic components (Soeparno, 2009). Tiren chicken meat is a chicken not through a process of deduction in accordance with provisions that exist or can be said to has died before the wreck to be slain. The case of buying and selling tiren chicken meat by traders in traditional markets is done by reason of traders can avoid losses because chicken is not sold so that any gains can be obtained. Traders that cheating will be mixing the chicken meat is fresh with tiren chicken meat that does not meet the criteria of SHWH. So many consumers will be harmed because obtaining an unworthy chicken meat consumption. A dead chicken that we often know with tiren chicken (mati kemarin) that is a dead chicken before slain this is due to an assortment of dead getting hit by car, pain, starvation, poisoning, too old or died en route, and died while awaiting execution killed. The chain of trade and marketing chicken pieces still largely through the hands of distributors. This can cause the death of chicken occurs before the slain. Moreover, coupled with a means of shelter, handling and transportation less adequate (Nurkholis, 2009).

Chicken tiren should be avoided, according to the word of Allah SWT in the Quran Surat Al-Baqarah 168, 172, and 173. In addition the Prophet said in a Hadith narrated by muslim halal Which reads "it is already clear, and no matter whom it was clear; and in between there are things which are musytabihat (syubhat, vague, unclear halal covenants), most people do not know the law. He who carefully from syubhat, really he has saved religion and her self esteem". Therefore, consumers need to be smart in choosing chicken meat in traditional markets opportunities. But the consumers are difficult generally to distinguish chicken tiren with chicken meat is fresh in traditional market because there are already cut up and mixed together.

Chicken meat is usually sold as a whole or in pieces. Most communities prefer to buy Chicken already cut pieces. That is because not all people fond of all parts of the chicken, the chicken so the purchase already cut up into a solution, there is asection from on the thrown. Part of the usual pieces provided by the traders chicken pieces including legs, thighs (drumstick), thighs, chestwith ribs, back, and kamip (Dwiatmaja, 2012).

Research on the characteristics of chicken physics tiren and the fresh chicken meat that has been done, the measurement process is done by touching the measuring instrument with the object being measured. This has a disadvantage when made tool detection because the detection tool should come into contact with the object to be detected. Chicken traders usually do not want chicken determining if touched with a tool by prospective buyers.

Based on explanation above, needed a solution to tackle the problem of rampant buying and selling tiren chicken meat by traders who are not responsible. One method that is often applied to the Foundation of the system of detection tiren chicken meat is the way biological and chemical nature by observing the color of chicken meat and observed the tenderness of chicken meat, but this way is relatively expensive. In addition, the difference in the way the value of the capacitance can be relied upon distinction tiren chicken and chicken meat as fresh as research conducted by Frida Agung Rakhmadi, Widayanti Anggar, and Astika Rusma. Therefore making gloves tiren chicken based capacitance detection is one of the solutions for detecting chicken meat with tiren utilization technologies that are increasingly developing. The gloves are used as media practical use because more and more superior compared to other tools because traders don't want to if he touched the chicken using the tool by consumers. These gloves are also useful to cover the hands of consumers of chicken so it does not need to wash your hands after touching the chicken meat. The purpose of this writing is to know the capacitance value of chicken meat and fresh tiren chicken meat, as well as making gauntlets tiren chicken detector.

METHODOLOGY

Writing method such as: the study of the literature, observation, and field experiments. The cornerstone of the theory of reading the form obtained from the results of research, books, and scholarly articles by searching, recording, an inventory and study to obtain data secondary. Field observation i.e. observation and interviews conducted in person to know the actual state of the meat chicken traders in traditional markets, a chicken slaughterhouse, and a chicken farm. Method study of the literature and observation field used for complementary sources of literature and data required in this writing.

Experiments carried out for 4 months at the Faculty of Animal Husbandry and the Faculty of Mathematics and Natural Sciences of Universitas Padjadjaran, Sumedang, Indonesia. Experiments are conducted to measure the capacitance value of chicken meat and the manufacture of gloves. Measurement of the capacitance value is performed using either a capacitance meter, thin aluminum plates, knives, cutting boards, trays, and plastic. As well as materials in the form of chicken meat and chicken meat fresh tiren. The knife is used to cut the chicken, cutting board for chicken meat trays while cutting, tray for chicken meat, containers and plastic container for dirt. Capacitance meters used to measure capacitance value in parts of chicken meat and chicken meat fresh tiren. Chicken meat is flanked by thin aluminum plates are connected with a capacitance meter. The glove-making is done by using the tool in the form of a sewing tool, scissors, laptops, data cable. As well as materials in the form of gloves made from leather, indicator light color green/red, probe (a thin aluminum plates), cable, and a microcontroller. The gloves are made with store and manage data acquired on a microcontroller using a laptop connected via data cable. Microcontroller that is already saved on the hard-wired parts of gloves. The cable will connect the microcontroller with the probe. At the top of the microcontroller mounted indicator light. Sewing tools used to sew glove.

RESULT

1. Capacitance Value

Table 1 and Table 2 show the value of capacitance of tiren chicken is higher than the value of the capacitance of

the fresh chicken meat on all parts of the chicken meat. These differences appear to be more clear on the graph of Figure 1.

A capacitor is an electrical component that consists of two conducting plates is partitioned parallel to each other with an electrical materials. This component is very important in electronics or electricity because it has properties: can store an electric charge, can withstand a direct current, and skipped the flow back and forth (Bisman, 2003).

A parallel plate capacitor with dielectric air and given the voltage of Vs. shown in Figure 3. The number entered to charge the capacitor is proportional to the voltage provided by the source. Capacitance of a capacitor load represented by the following equation:

C = Q/V

Description:

C = capacitance value in F (Farad)

Q = electron charge in C (Coulomb)

V = voltage in V (Volts)

It appears that the unit of capacitance is the Coulomb/Volt or (C/V) or the Farad (F). A farad is the amount of electric charge of one coulomb stored in electric (an intermediate substance) with a potential difference of one volt. So the capacitance of a capacitor is the ability of the capacitors to store charge on its plates. Capacitance of a capacitor depends on: dielectric material used, the area of plates, and the distance between the plates (Bisman, 2003).

The value of capacitance of chicken tiren is bigger than the fresh chicken shows that the size of the storage capacity of charge tiren chicken is bigger than the size of the storage capacity of a fresh chicken charge, so the chicken has tiren permittivity bigger than on a fresh chicken meat. The magnitude of the permittivity chicken tiren when compared to a fresh chicken meat due to the process of decay and decomposition of chemical substances in chicken meat tiren faster than fresh chicken meat. That is because the total microorganisms on meat chicken tiren is more than the fresh chicken (Rakhmadi, 2013).

2. Glove Sensor Capacitance-Based Tiren Chicken Meat (Figure 2)

Glove Sensor Capacitance-Based Tiren Chicken Meat

The difference capacitance value of fresh chicken meat and tiren chicken meat is used as a concept in gloves chicken tiren detector. The bigger the capacitance tiren chicken compared fresh chicken shows that the size of the storage capacity of charge chicken tiren is bigger than the size of the storage capacity of a fresh chicken charge. With the reference, mikrokontroller can count and decide whether the chicken tested positive or not with tiren informed through the color of the green light for the fresh chicken and red meat for chicken tiren.

On the gloves that are made there is a sensor (probe) consists of two main parts, namely part of the sensor (probe) and indicators. Part of the probe sensor embedded in a glove that will come into contact with chicken meat. The value of the capacitance of the flesh will be unreadable by a microcontroller and obtained a value of 90% capacity. Microcontroller is a chip that serves as an electronic circuit controller and keep the program inside. Microcontroller generally consists of a CPU (Central Processing Unit), memory, I/O and supporting units like Analogto-Digital Converter (ADC) that are already integrated in it. The indicator lamp in the form of small partsthat produce red/green color.

System working gloves chicken tiren detection when consumers want to buy chicken meat to the traditional market, then consumers can detect fresh by touching the chicken meat chicken meat sold by using gloves. Part of the sensor probe is embedded in the form of these gloves will come into contact with meat and transfer the information to a microcontroller to provide information the value of capacitance in the form of a different color lights on the indicator. The indicator is red if positive tiren (more value from 1.0008 μF) and green color if not.

CONCLUSIONS

Conclusion

1. The value of capacitance tiren chicken meat all different parts with a capacitance value of fresh chicken meat, chicken meat capacitance value tiren capacitance value is bigger than the fresh chicken meat.

2. Gloves detection detection of chicken meat tiren uses the concept of the bigger value of the capacitance on tiren chicken meat then fresh chicken meat, Indicator is red if positive tiren and green color if not.

Suggestion

Adjustment of the glove sensor capacitance-based tiren chicken meat making needs to be done. So it can be

utilized by the Government of Indonesia in repressing the circulation of tiren chicken meat given to consumers either for itself consumption or for the of processed chicken meat seller.

Table 1. The capacitance value of tiren chicken meat

				C (µF)	0.6779		ΔC	0.3503
Parts	**[C] Capacitance (µF)**							
	1	**2**	**3**	**4**	**5**	**6**	**C**	**ΔC**
Thigh	0.365	0.848	0.811	0.589	0.543	0.664	0.6367	0.1792
Breast	0.637	1.227	0.692	0.684	0.667	1.588	0.9158	0.3980
Wings	0.429	0.659	0.71	0.839	0.655	0.678	0.6617	0.1328
Head	1.083	0.467	0.735	1.019	1.063	1.514	0.9802	0.3542
Back	0.677	1.520	0.559	0.665	0.727	0.970	0.8530	0.3542
Foot	0.013	0.015	0.016	0.019	0.031	0.025	0.0198	0.0069

Table 2. The capacitance value of chicken meat

				C (µF)	1.0008		ΔC	0.3772
Parts	**[C] Capacitance (µF)**							
	1	**2**	**3**	**4**	**5**	**6**	**C**	**ΔC**
Thigh	0.846	0.771	1.003	1.087	0.712	0.732	0.8585	0.1539
Breast	1.124	1.396	1.120	1.355	0.990	1.814	1.2998	0.2953
Wings	0.821	0.834	0.785	1.002	0.962	0.578	0.8303	0.1501
Head	1.459	1.379	1.660	1.688	0.771	1.830	1.4645	0.3768
Back	0.494	1.978	1.091	1.382	1.136	0.721	1.1337	0.5207
Foot	0.445	0.537	0.126	0.177	0.728	0.494	0.4178	0.2281

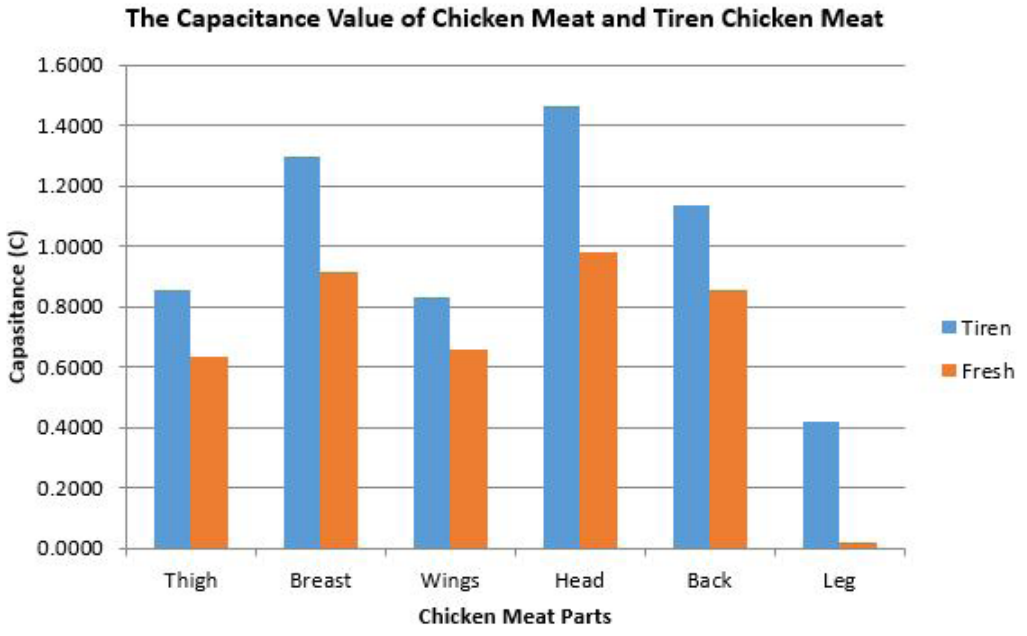

Figure 1. Graph the capacitance value of chicken meat and tiren chicken meat

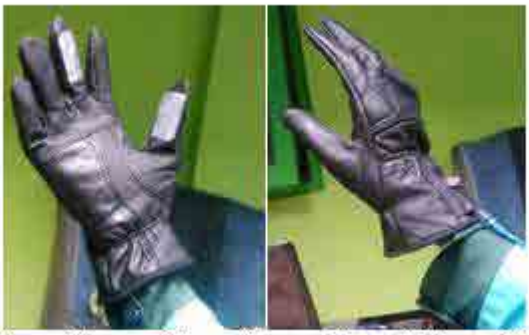

Figure 2. Glove Sensor Capacitance-Based Tiren Chicken Meat

REFERENCES LISTS

Bisman. 2003. *Design of Digital Capacitance Meter.* Sumatera Utara: Faculty of Mathematics and Natural Sciences, Department of physics Universitas Sumatera Utara.

Dwiatmaja, Anggara Wahyu dan Rakhmadi, Frida Agung. 2013. *The Resistance Characteristic of Chicken Meat and Tiren Chicken Meat*. Yogyakarta: Faculty of Science and Technology, UIN Sunan Kalijaga.

The Ministry of Agriculture. 2012. *Chicken Meat as Source of Nutritious Food*. Directorate General of Livestock And Animal Health.

Nurkholis. 2009. *Review of Islamic Law Against Selling Tiren Chicken (Studies Sase of Chicken Sellers at the Market of Rejomulyo, Semarang).* Semarang: Faculty of Shariah, Institut Agama Islam Negeri Walisongo.

Rakhmadi, Frida Agung,dkk. 2013. *The Characteristic Capacitance Identification of Chicken Meat and Tiren Chicken Meat*. Yogyakarta: Faculty of Science and Technology, UIN Sunan Kalijaga.

Soeparno. 2009. *Science and Meat Technology*. Yogyakarta: Gadjah Mada University Press.

PO-02-48 Application of ELISA and LC-MS/MS Methods for Ensuring the Food Hygiene and Safety of Pig Production

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Introduction

Based on the actual demands of the society and productions, the human health and life are directly affected by the hygiene and food safety (HFS). The HFS affects not only to the maintenance and development of the human lineage but also to the development process and integration of the international economy. The HFS problem of a fresh pork production chain is sensitive and important in Vietnam. Presently, Viet Nam became the WTO's 150th member and enjoined the Trans-Pacific Partnership Agreement, the commitment of ensuring the HFS is therefore increasing. Due to the less consciousness of breeders about the HFS, the abuse and the illegal utilization of chemicals and veterinary medicines in the stock breeding which supply in Vietnam domestic market are very popular (Pham K. D. et al., 2013). There are some factors that contaminated to the fresh pork as bacteria, heavy metals and residual antibiotic, hormone by veterinary medicines, the feed, drinking water, equipment and facilities from the farm to the food (Thuy T. Q., 2007; Hoa N. V., 2008; Dang P. K. and Kiem N. V., 2009; Dang P. K. et al, 2013; Fahrion A. S., et al, 2013). However, those risk factors are not clearly affected the HFS of fresh pork in Lamdong province. We hypothesized that the HFS of fresh pork is not ensured Vietnam standards by the microbial contamination in the feed, equipment, used water as well as the chemical residues in the feed and the heavy contamination in drinking water for the chain pig products. The present study was designed to test the hypothesis by assessing the contribution of risk factors in the feed, equipment, used water and drinking water to the HFS of fresh pork.

Methods

In this study, a cross-sectional was designed as an investigation method in this study, in which the setting was Lamdong province from December 2014 to June 2015. Lamdong is a province located at 11°57'N, 108°26'E in the southern part of the Central Highlands(*Tay Nguyen*) region of Vietnam. It is in a typical tropical climate. The weather is cool all year with an average temperature of 19°C, with two main seasons: the rainy and the dry season. The province can be divided into three main areas with five strong points: development of long-term industrial trees and bushes, forestry, minerals, tourism-services, and livestock.

Total of 90 farms, 40 feed leaders, 24 slaughterhouses and 31 food markets were selected by stratified random sampling. Based on the level of pig production, economic development, and geographic characteristics, three villages were chosen from three representative districts, which cover both rural and urban areas. The surveyed percent of the feed leaders, the farms, the slaughterhouses and the food markets was 50%, 10%, 50% and 100% following of the lists in nine villages, respectively. If the slaughterhouses in each village were low three, we had surveyed 100%. In the 10% the surveyed farm, we selected two farms for each location at five incidences as the north, the east, the south, the west and the center of each village.

The sampling was collected randomly and represented in the following Table 1. The sampling was analyzed and was assessed by different methods as Table 2.

The log data were analyzed by means of the Tukey-Kramer with $P < 0.05$, and the data were analyzed by percentage not achieved sampling of Chi-square testes with $P < 0.05$ using the SAS program (version 9.0)

Results and Discussion

The Figure 1 presents that the percentage of total aerobic bacteria (TAB) pollution in fresh pork was 89.29% and the average density of TAB reflecting the difference between the small slaughterhouse (5.6 logs CFU/g) and the suitable slaughterhouse (6.27 log CFU/g). Also, the proportion of Salmonella pollution in fresh pork was 5.56% and 26.67% in a ratio of 1:4 ($P = 0.006$) in the small slaughterhouse and the suitable slaughterhouse, respectively. This proportion was higher than the ones that were researched of two studies in Hai Phong and Bac Giang cities (Ngo V.B and Thuong Q, 2008; Duong T. T. et al., 2010). Consequently, this result might be affected due to the slaughter methods.

It is clear that there was no residual bacteria and hormone in fresh pork that was researched in those slaughterhouses. However, there were still two exceptional cases. One was 2.38 % salbutamol residue, and the other was a sample with a positive test in the small slaughterhouse. This result matched the fresh pork that could be found in the rural area which can be the cause of bacteria residue (Duong V.N., 2006)

Salmonella was not found in the used water. However, the percentage of polluted coliform found in the water was 75%. In addition, the average density in the small slaughterhouse and the suitable slaughterhouse was 1.55 log CFU/100 ml and 1.61 log CFU/100 ml, respectively. There is not much difference ($P > 0.05$) of the TAB polluted in the water density between the small slaughterhouse (5.66 logs CFU/100 ml) and the suitable slaughterhouse (2.97 log CFU/100 ml). In fact, the slaughterhouse used buckets and water that did not go through the filter system throughout the slaughter process.

The density of TAB and Enterobacteriaceae pollution in the knife and cutting board were 5.28-5.66 log CFU/cm^2 and 2.55-3.00 log CFU/cm^2, and 5.13-5.79 log CFU/cm^2 and 1.98- 2.84 log/cm^2, respectively. Exceptionally, there was only one case in which the density of Enterobacteriaceae pollution in the cutting board was different between the small slaughterhouse and the suitable slaughterhouse both ($P = 0,045$). The slaughterhouse in Lamdong that has a slaughtering method of a pig lying on a floor can be cross-contaminated on the pork by the microbe from the equipment, floor, and water distribution. The contamination is higher than the slaughtering method in Hue city that the pig lying on the table (Le H. N., 2005).

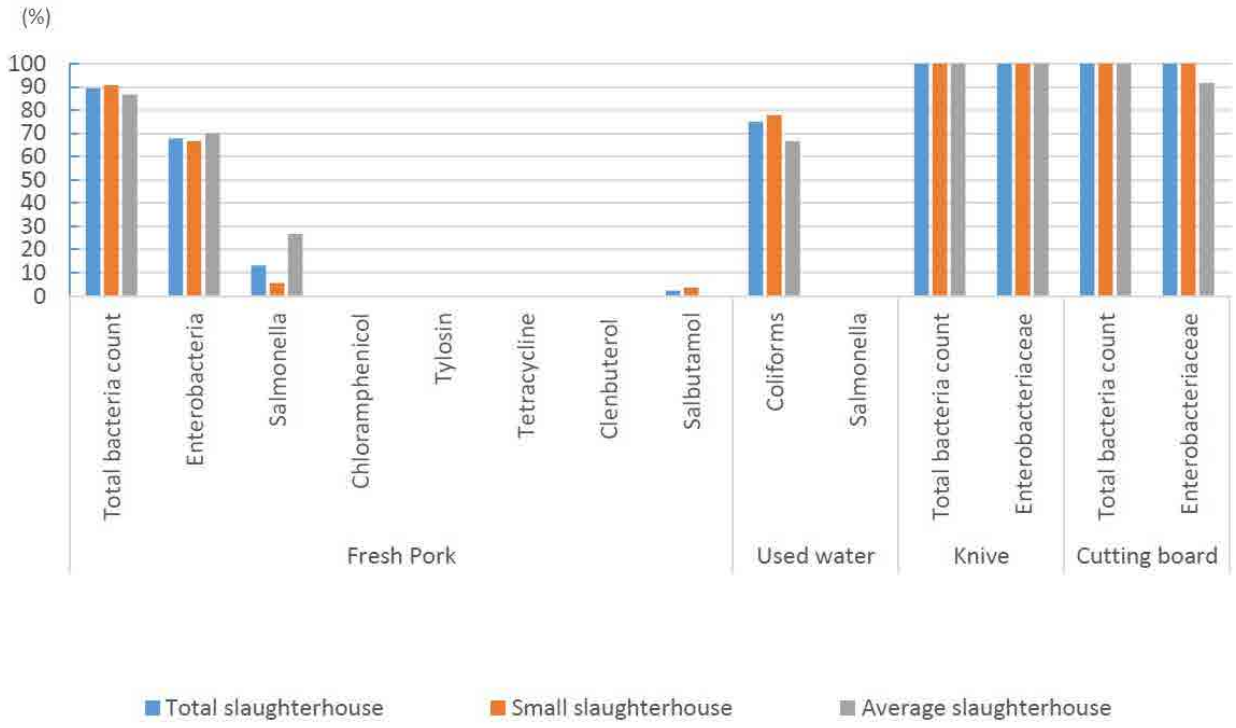

Fig. 1 Current status of the hygiene and food safety in the slaughterhouse.

Fig. 2 presents that the microbial contamination of fresh pork was different between the small market and centralized markets. It is same with the results from Hanoi city, where have the difference among the urban markets, rural markets and supermarkets (Fahrion A. S., 2013). In detail, the pollution of TAB as 92.59% and 100% at the centralized and small markets, respectively. The pollution of E. coli in the pork has a ratio of 6:1 ($P < 0.0001$) with the average density of 0.77 log CFU/g and 2.21 log CFU/g at the centralized and small markets ($P < 0.0001$), respectively. Salmonella was not found in the centralized market, but it was found as 27.27% in the pork samples from the residential markets ($P = 0.0025$). The results are higher than ones of Hanoi city (Fahrion A. S. et al., 2013) and in the same with other studies in Vietnam (Le Bas C. et al., 2006).

There is no chemical contamination with the pork from the market, and the results are better than other studies in Vietnam that found the antibiotic residues in the pork by using the qualitative test (Le Bas C. et al., 2006). However, due to effect of sample size factor ($n = 40$), the ration fluctuates within 0.00 ~ 8.76% (confidence at

95%).

Moreover, the Salmonella was not found in the used water. However, the total counts of bacteria and Coliforms were 2.54 -2.82 log CFU/100 ml (P = 0.3277) and 1.37-1.73 log CFU/100 ml (P = 0.4565). The microbial contamination of the used water was not different between two market models.

Besides, there was a difference in the microbial density in the equipment between two models of the markets. Exceptionally, Enterobacteriaceae was 2.85-3.15 log CFU/cm^2 in knives (P = 0.1534). In which, total bacteria count was 6.01 – 6.68 log CFU/cm^2 in knives (P < 0.0001) and 6.13 – 6.69 log CFU/cm^2 in cutting board (P = 0.0003), respectively. Enterobacteriaceae was 2.94 – 3.50 log CFU/cm^2 (P = 0.0094) in cutting board for the centralized market and the residential market, respectively.

In the drinking water for a pig in a total of three districts, Fig. 3 indicated the percentages of total bacteria count, coliform, Fe, Cd, As and Pd were 7.78%, 51.11%, 1.11%, 0.00%, 1.11%, 0.00%, respectively. The percentage of bacteria and heavy metals, in which were contaminated in the drinking water, was not different among various districts (P > 0.05). The coliform contamination in drinking water was the cause of the microbial contamination in pig that affects the HPS of fresh pork.

Besides, E.coli, aflatoxin, aflatoxin B1, tetracycline, chloramphenicol, clenbuterol, salbutamol, and ractopamine were not found in the feed of the food agents and farms. Tylosine has 2.5% in the feed of the food agents. The results were higher than a studied in Hanoi (Nguyen V. K. and Pham K. D., 2009) due to overusing antibiotics in livestock by the food producer. In the while, Vietnam permit to mix some antibiotics to the feed (Vietnam Ministry of Agriculture and Rural Development, 2006).

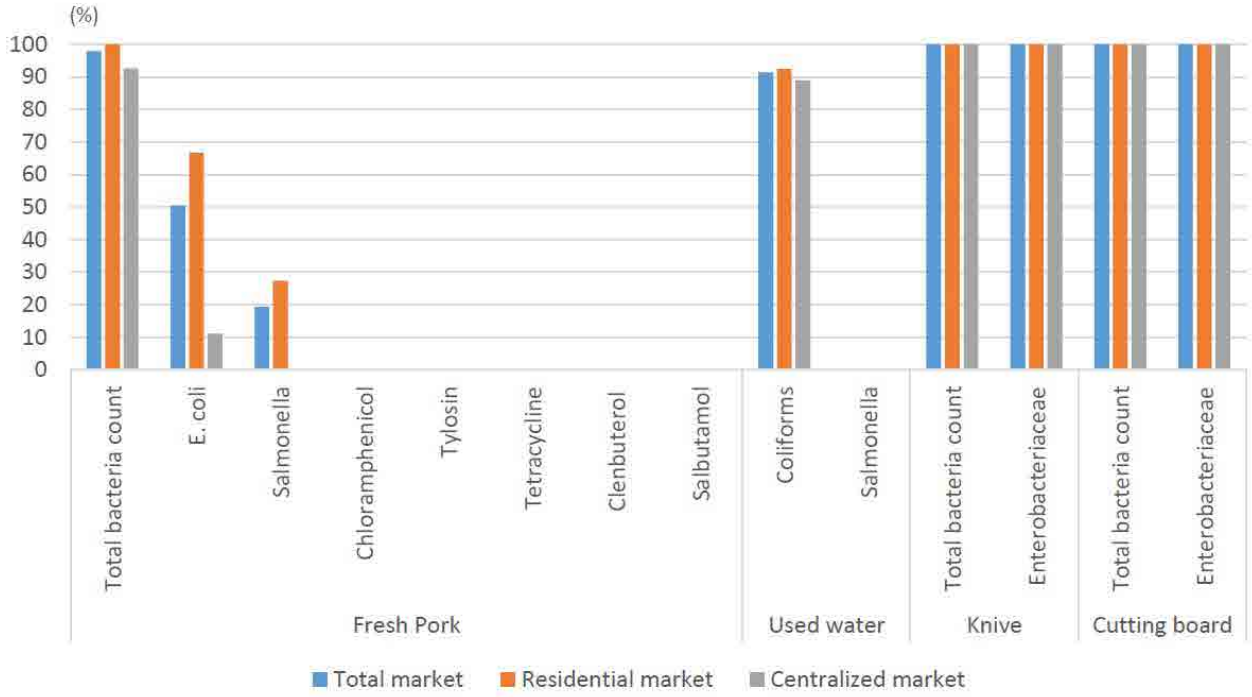

Fig. 2 Current status of food safety in pork distribution system

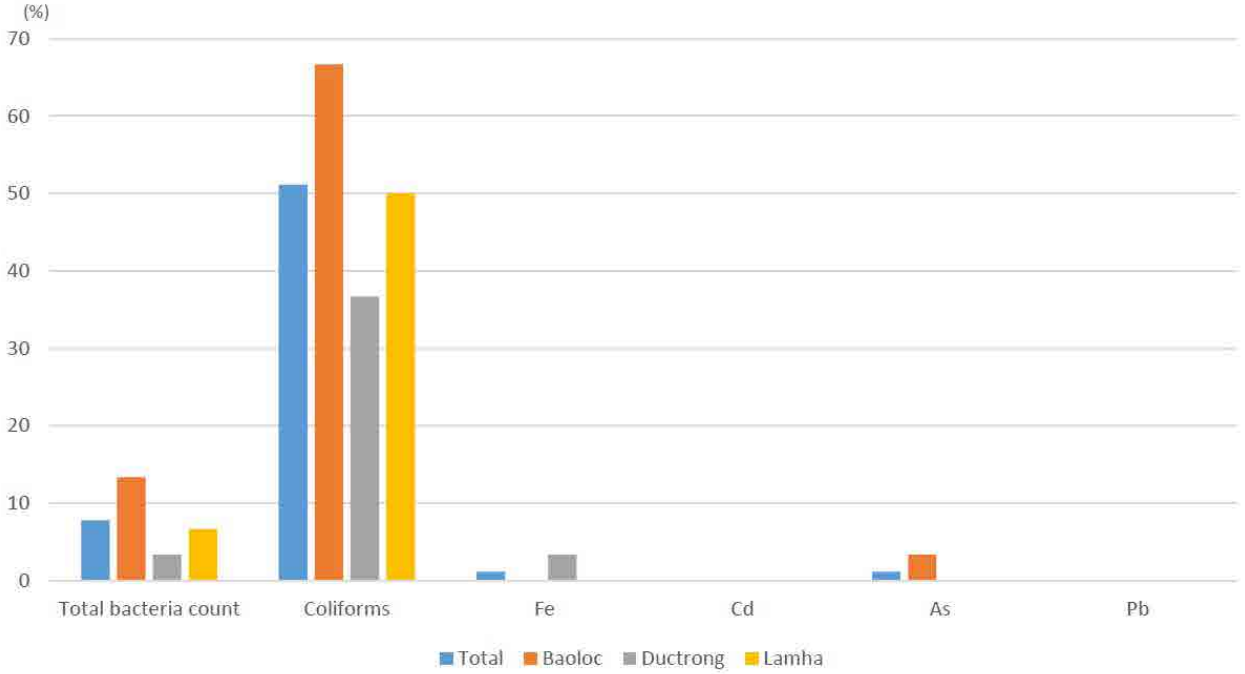

Fig. 3 Current status of the hygiene and food safety in drinking water for the pig.

Conclusions

This study presents that the fresh pork was contaminated by the bacteria and residual chemical in the slaughterhouse and the market (excepting salbutamol with 2.38%). The feed as well as the equipment and used water caused some effects to the residual antibiotic (tylosine with 2.5%) and the microbial contamination in the fresh pork, respectively. Coliforms of the drinking water was a factor that directly affects to the fresh pork. The models of the market and slaughterhouse were also affected by the microbial contamination in fresh pork. Besides that, the sanitary conditions and people's awareness will force the HFS in various models of the market and slaughterhouse that should be studied continually.

Table 1. Distribution of the pig production chain chosen for investigation.

Parameters	Districts in Lamdong province			Total
	Lamha	Baoloc	Ductrong	
Feed	22	10	8	40
In:				
The first feed leader	4	1	0	5
The second feed leader	9	4	4	17
Small sales feed leader	9	5	4	18
Drinking pig water in farm	30	30	30	90
Feed in farm	30	30	30	90
Surface fresh pork	35	32	17	84
In:				
Average slaughterhouse	5	20	5	30
Small slaughterhouse	30	12	12	54
Used water	22	16	10	48
In:				
Average slaughterhouse	2	8	2	12
Small slaughterhouse	20	8	8	36
Equipment (knife, cutting board)	12	12	6	30
In:				
Average slaughterhouse	2	8	2	12
Small slaughterhouse	10	4	4	18
Fresh pork	35	32	17	84
In:				
Average slaughterhouse	5	20	5	30
Small slaughterhouse	30	12	12	54
Surface fresh pork	27	30	36	93
In:				
Residential markets	18	21	27	66
Centralized markets	9	9	9	27
Fresh pork	12	13	15	40
In:				
Residential markets	6	7	9	22
Centralized markets	6	6	6	18
Used water	12	13	15	40
In:				
Residential markets	6	7	9	22
Centralized markets	6	6	6	18
Equipment (knives, cutting boards)	27	30	36	93
In:				
Residential markets	18	21	27	66
Centralized markets	9	9	9	27

Table 2. The method of analyzed and assessed sampling in the studied parameters.

Target	Analyzed method	Request to reach	Assessed method
In Feed			
Tetracycline	ELISA LC/MS/MS	≤ 50 ppm	QCVN 01 – 12:2009/BNN
Tylosin		≤ 40 ppm	
Total Aflatoxin		≤ 100 ppb	
Aflatoxin B1		≤ 50 ppb	
Chloramphenicol		Negative	QD 54/2002/BNN
Clenbuterol		≤ 50 ppb	
Salbutamol		≤ 50 ppb	
Ractopamine		≤ 50 ppb	
In Drinking water			
Fe	TCVN 6177:1996	≤ 0.5 mg/l	ISO 6332:1988
E. coli	ISO 07251:2005	Negative	QCVN 01 – 12:2009/BNN
As	AOAC 957.22	≤ 2 mg/kg	
Pb	TCVN 7602:2007	≤ 5 g/kg	
Cd	TCVN 7603:2007	≤ 1 g/kg	
TVKHK	TCVN 6187:1996	10000 CFU/ml	
Coliforms		30 MPN/100 ml	
In Surface fresh pork			
Total bacteria count	ISO 4833:2003	≤ 10^5 CFU/g	TCVN 7046:2009
Enterobacteriaceae *Escherichia coli*	ISO 16649:2001	≤ 10^2 CFU/g	
Salmonella	ISO 6579:2007	Negative/25g	
In Fresh pork			
Chloramphenicol	ELISA LC/MS/MS	Negative	QD54/2002/BNN
Clenbuterol			
Salbutamol			
Tetracycline	AOAC 995.09 – 2005		
Tylosin	AOAC 995.09 – 2005	≤ 100 ppb	TT 15/2009/BNN
In Equipment			
TVKHK	SMEWW 9215B:2005	≤ 10 CFU/cm^2	TT 60/2010/BNN
Enterobacteriaceae	TCVN 5518:2007	≤ 1 CFU/cm^2	
In Used water			
Coliforms	ISO 9308:1990	0 MPN/100 ml	QCVN 01:2009/BYT
Salmonella	SMEWW 9260B:1995	Negative	

Reference

Vietnam Ministry of Agriculture and Rural Development (2006). Animal feeding stuff – maximum levels of antibiotics and drugs in complete feed. 10TCN 861:2006.

Cam N. H., Nguyen T. T. Thuy., Nguyen B. T. (2014). Assessment of current slaughtering status and bacterial contamination in pork meat in slaughter places in Nam Dinh province. J. Sci. & Devel. 12(4):549-557

Duong T. T., Nguyen V. L., Truong Q. (2010) A survey on the Sanitation Indicator Bacterial contamination in pork, buffalo meat and beef in some slaughter houses in Bac Giang province. J. Sci. & Devel. 8(3):466-471.

Duong V. N., Paulsen P., Suriyathporn W., Smulders F. J., Kyule M. N., Baumann M. P., Zessin K. H., Pham H. N. (2006) Preliminary analysis of tetracycline residues in marketed pork in Hanoi, Vietnam. Ann N Y Acad Sci. 1081:534-542.

Fahrion A. S., Lapar M. L., Nguyen N. T., Do N. T., Grace D. (2013). Food-borne hazards in a transforming pork value chain in Hanoi: Basis for future risk assessments. Journal of preventive medicine. Volume XXII. 4(140):18-25.

Hoa N. V. (2006) A survey conducting on the situation of antibiotics in livestock in Ho Chi Minh City. J. Labour protection. 178:17-20.

Le Bas C., Tran T. H., Nguyen T. T., Dang D. T., Ngo C. T. (2006). Prevalence and epidemiology of Salmonella spp. in small pig abattoirs of Hanoi, Vietnam. Ann N Y Sci. 1081:269-272.

Le H. N. (2005) Contamination by E. Coli, Salmonella and Aerobe of animal origin foodstuff at the market in Hue city. Journal of agriculture and rural development. 2(5):34-36.

Ngo V. B., and Truong Q. (2008) A survey on bacterial contamination in suckling pig and meat in several slaughterhouses in Hai Phong. J. Sci. & Devel. 6(1):21-25.

Nguyen V. K., Pham K. D. (2009). Effect of some compound feeds on pig growth, carcass quality, heavy metal and antibiotic residues in pork. J. Sci. & Devel. 7(4):476-483.

Pham K. D., Degand G., Douny C., Pierret G., Delahaut P., Vu D. T., Granier B., Louise S. M. (2013). Preliminary evaluation of antimicrobial residue levels in marketed pork and chicken meat in the red river delta region of Vietnam. Food and Public Health. 3(6):267-276.

Pham K. D., Saegerman C., Douny C., V. D. T., Ha X. B., Dang V. B., Pham H. N., Louise S. M. (2013). First survey on the use of antibiotics in pig and poultry production in the red river delta region of Vietnam. Food and Public Health. 3(5):247-256.

PO-02-51

Effect of Floor Bedding Materials on Egg Production and Behavioral Characteristics of Laying Hens

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ABSTRACT

The objective of this study was to investigate the effect of floor bedding materials (sand vs. rice hull) on egg production and behavioral characteristic of laying hens. Totally 800 brown laying hens were used for 6 weeks feeding study. The birds were randomly allocated to 2 treatments according the bedding materials (sand vs. rice hull) with 4 replications per treatment. Egg production performance parameters (HDEP, egg weight, egg mass and feed conversion) were measured every week. Behavior characteristics (standing, sitting, walking, drinking, feeding, dust bathing, pecking, flapping and aggressive pecking) were recorded three consecutive days every three week. HDEP was no significantly different between sand and rice hull groups. Number of drinking activity per bird during given amount of time was higher in rice hull group than that in sand group. However, the number of feeding activity per bird of sand group was higher than birds of rice hull group. There were no significant differences on other behavioral characteristics (standing, sitting, walking, dust bathing, pecking, flapping and aggressive pecking). This study implied that types of bedding materials did not affect to egg production performance, but affect both feeding and drinking behaviors.

INTRODUCTION

Laying hens are known that they still kept behavioral characteristics similar with red jungle fowl, the ancestor of domesticated modern chicken throughout the last 8,000 years. Typical examples of these behavioral characteristics were perching, nesting and dusting bath. Some behavioral characteristics are related to the laying performance. It means that specific facilities could be helpful for stimulating the manifestations of desired behavioral characteristics for layer. If behavioral characteristics were restricted, the production or quality of egg would be negatively affected.

In addition, the consumer concerns on the welfare housing system for laying hen had been increased especially with the ban of the conventional cage in EU. This welfare housing systems were designed not only to improve egg production but also to reflect natural behavioral characteristics. Most established standards requre the welfare housing systems to be furnished with facilities such as dusting bath place, perch and nesting box. Floor housing system has been classified as the widely using welfare housing system for laying hen and also easily conform the most welfare standard. Bedding materials of floor housing system has been divided as artificial materials type (slat) and natural materials type (rice hull, sand, wood saw and etc.). Bedding materials could be significant environmental factor for egg production. However, not much information is available how bedding materials affect egg production and behavioral characteristics. Therefore, the objective of this study was to investigate the effect of floor bedding materials (sand *vs* rice hull) on egg production and behavioral characteristic of laying hens.

MATERIAL AND METHOD

This experiment was conducted in floor of the research farm and analysis laboratory at KNU.

Totally 800 brown laying hens were used for 6 weeks feeding study. The birds were randomly allocated to 2 treatments according the bedding materials (sand vs. rice hull) with 4 replications per treatment. There were slope slat, perches, nesting box, feeder and water supply line in floor. Others environmental factors such as temperature and humidity were controlled by automatic ventilation system. 14L and 10D cycle was used in this experiment. Experimental diet were formulated to meet NRC (1994) requirement. All eggs were collected daily at the same time. And residual feed were collected weekly for calculating feed intake. Egg performance parameters were HDEP, egg weight, egg mass, feed intake and FCR. Haugh unit was calculated by using tripod micrometer to measure albumen height and followed the equation proposed by Haugh (Stadelman, et al., 1995). Yolk color was measured by using Roche fan. Shell thickness was measured using thickness meter. Shell hardness was measured using hardness tester. Behavior characteristics (standing, sitting, walking, drinking, feeding, dust bathing, pecking, flapping and aggressive pecking) were recorded three consecutive days every three week and analyzed using

sample scanning method. All the data collected were analyzed using T-test procedure of IBM SPSS 22.0 version.

RESULTS AND DISCUSSION

Rice hull group showed that feed conversion ratio (FCR) were improved (P<0.05) comparing to sand group (Table 1). HDEP, egg weight, egg mass were not significantly differed between rice hull and sand groups. However, HDEP and egg mass in rice hull group were tended to be higher than those in sand group.

Shell thickness of egg laid by birds in sand group was higher (P<0.05) than that in rice hull group (Table 2). There were no significant differences between rice hull and sand groups in haugh unit, yolk color score and shell strength. However, yolk color in rice hull groups had a tendency to be higher than that in sand group.

There were no significant differences between rice hull and sand group in sanding, sitting, walking, dust bathing, pecking, flapping and aggressive pecking behaviors (Table 3). However, feeding and drinking activities in sand group were observed more frequently (P<0.05) than those in rice hull group.

CONCLUSION AND IMPLICATION

This study showed that rice hull and sand as bedding materials in floor did not impart remarkable impact on egg production. However, FCR was improved when rice hull was used as bedding material. Shell thickness in sand group was higher than that in rice hull group. Pecking was slightly higher in sand group. It was presumed that birds in sand groups would be able to take certain amount of soils by pecking. Intake of sand would lead to a feeling of physical fullness for birds, therefore require more nutrients. This could be the reason how only the feeding activity would be increased in sand group compared to rice hull group.

Keywords: Floor, Bedding materials, Egg, Behavior

Table 1. Effect of dietary supplemented LAB species on diarrhea score in weaned piglets

	None	*L. acidophilus*	*L. plantarum*	*L. rhamnosus*	*B. bifidum*	*B. breve*	*S.thermophilus*	Culture media
Diarrhea score	3.25	6.50	4.25	3.75	3.25	5.25	6.25	3.50

Table 2. Effect of dietary supplemented LAB species on the morphological parameters of intestines in weaned piglets

	None	*L. plantartum*	*L. fermentum*	*L. reuteri*	*B. bifidum*	*B. lactis*	*B. longum*	Culture media	SEM	P-value
Villus height (*u*m)										
Duodenum	490.95^{a}	394.75^{bc}	323.83^{c}	395.39^{bc}	405.05^{b}	389.50^{bc}	405.42^{b}	417.77^{b}	10.209	0.006
Jejunum	339.90	313.89	342.32	359.87	341.26	332.77	334.45	332.86	4.382	0.390
Ilium	325.69	286.54	320.13	307.72	315.73	314.83	299.70	303.88	4.634	0.585
Crypt depth (*u*m)										
Duodenum	271.25^{ab}	305.66^{a}	231.33^{bc}	247.37^{b}	253.58^{b}	250.46^{b}	246.31^{b}	198.18^{c}	6.130	0.000
Jejunum	179.65^{b}	208.46^{ab}	182.16^{b}	187.49^{b}	199.08^{b}	213.15^{ab}	244.80^{a}	222.42^{ab}	5.424	0.025
Ilium	211.21	195.84	185.61	198.41	198.23	194.43	208.21	198.87	3.642	0.784
Villus height: crypt depth										
Duodenum	1.84^{ab}	1.29^{d}	1.41^{cd}	1.61^{bcd}	1.63^{bcd}	1.56^{bcd}	1.65^{bc}	2.14^{a}	0.050	0.000
Jejunum	1.99	1.57	1.89	1.99	1.77	1.56	1.39	1.54	0.059	0.051
Ilium	1.55	1.50	1.74	1.57	1.61	1.63	1.45	1.54	0.029	0.321

Table 3 Effect of dietary supplemented LAB species on microbial population in large intestinal content

	None	*L. acidophilus*	*L. plantarum*	*L. rhamnosus*	*B. bifidum*	*B. breve*	*S. thermophilus*	Culture
***Lactobacillus spp.* (log10 CFU/g)**	8.48	8.54	8.30	8.34	8.51	8.49	8.48	8.31
***E.coli* (log10 CFU/g)**	6.14	6.11	6.41	6.52	6.57	6.04	6.16	6.40
***Salmonella spp.* (log10 CFU/g)**	ND	ND	ND	ND	ND	ND	ND	ND

REFERENCES

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption. *The American Journal of Clinical Nutrition*, *55*(1), 299S-308S.

Hampson, D. J. (1986). Alterations in piglet small intestinal structure at weaning. *Research in veterinary science*, *40*(1), 32-40.

Heo, J. M., Opapeju, F. O., Pluske, J. R., Kim, J. C., Hampson, D. J., & Nyachoti, C. M. (2013). Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhea without using in-feed antimicrobial compounds. *Journal of animal physiology and animal nutrition*, *97*(2), 207-237.

Liong, M. T. (2007). Probiotics: a critical review of their potential role as antihypertensives, immune modulators, hypocholesterolemics, and perimenopausal treatments. *Nutrition reviews*, *65*(7), 316-328.

Pluske, J. R., Hampson, D. J., & Williams, I. H. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review.*Livestock production science*, *51*(1), 215-236.

Pié, S., Lallès, J. P., Blazy, F., Laffitte, J., Sève, B., & Oswald, I. P. (2004). Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *The Journal of nutrition*, *134*(3), 641-647.

Stadelman, W. J., Newkirk, D., & Newby, L. (1995). Egg science and technology. CRC Press.

Van Beers-Schreurs, H. M. G., Vellenga, L., Wensing, T., & Breukink, H. J. (1992). The pathogenesis of the post-weaning syndrome in weaned piglets; a review. *Veterinary Quarterly*, *14*(1), 29-34.

Wu, Y., Jiang, Z., Zheng, C., Wang, L., Zhu, C., Yang, X. & Ma, X. (2015). Effects of protein sources and levels in antibiotic-free diets on diarrhea, intestinal morphology, and expression of tight junctions in weaned piglets.*Animal Nutrition*, *1*(3), 170-176.

Wijtten, P. J., van der Meulen, J., & Verstegen, M. W. (2011). Intestinal barrier function and absorption in pigs after weaning: a review. *British journal of nutrition*, *105*(07), 967-981.

PO-02-59 Effect of cow brush on the Hair cortisol and basic behavior of the fattening Hanwoo steers

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Objective

Hair cortisol in animals may provide reliable approach to define stress condition. This study was conducted to investigate the hair cortisol and basic behavior of fattening steers.

Methodology

Twenty-eight steers (7 heads/pen, aged 24-month) with body weight of an approximate 619.8 kg. Steers were randomly assigned in a 2x2 factorial design by type of control and treatment. In control groups cow brush was not implemented, whereas cow brush was installed at right side of the center on the treatment groups. Hair cortisol collected new hair which grown during the experimental period (last cut). After collection in tubes plasma was obtained by wrapping in aluminum foil, unwrapping and washing with isopropanol and methanol, drying, grinding, and then applying hair cortisol assay using kit according to manufacturers recommendation (Salimetrics, high sensitivity cortisol kit, no. 1-3002, State College, USA, 16803). Behavior data observed the feeding, locomotion, lying, rubbing, and fighting behaviors of the individuals in the group. The behavior of the Hanwoo steers was observed from the shoot images, it took 12 hours during a day (06:00 AM-6:00 PM) from the four installed CCTV cameras (IR LED Camera; APD-7070V), with the behavioral changes noted every two minutes. Statistical analysis was carried out using T-test procedure of SAS (version 9.0; SAS institute Inc., Cary, NC).

Result

The hair cortisol in control groups showed higher ($p<0.05$) than treatment groups. No differences ($p>0.05$) were found in feeding and locomotion time between control and treatment groups. Lying time was higher ($P>0.05$) in control groups than treatment groups. Rubbing and fighting were counted high in the treatment groups than control groups with a significantly difference ($p<0.05$).

Conclusion

The using of a cow brush could give a positive effect on the steers as reducing stress by liberation from the itching and brush something aside on the there's hair.

PO-02-61

EFFECTS OF EXERCISE ON THE HAEMATOLOGY PARAMETERS OF PONY

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Abstract

The objective of this study was to investigate the effect of exercise on haematology parameters of peripheral blood in pony. Seven female ponies were conducted in three exercise programs, namely 3 hr walking (refers as light exercise program), 30 min trotting with 10 min walking (refers as medium intensity exercise program), and 25 min trotting with 5 min cantering and 10 min walking (refers as high intensity exercise program), during August 2015 and July 2016. A total of 79 pre- and post-exercise blood sample sets were taken and assessed using automatic hematology analyzer. Traits analyzed included white blood cell (WBC), lymphocyte (LYM), monocyte (MONO), granulocyte (GRA), hemoglobin (HGB), red blood cell (RBC), red blood cell distribution width (RDWa), platelet (PLT), mean platelet volume (MPV), and mean corpuscular parameters, including volume (MCV), hemoglobin (MCH), and hemoglobin concentration (MCHC). Data were analyzed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC. USA). There was no significant difference in trait studied before and after the light exercise program. However, WBC, MONO, HGB and RBC were increased after the medium and high intensity exercise. Also, no significant difference between pre- and post- exercise was observed in WBC, MONO, GRA, MCV, MCH, MCHC, RDW, PLT, and MPV among program comparisons. LYMs were significantly increase after post-exercise in medium and high intensity exercise programs. However, it was not the case in light exercise program. Similar results were also observed in WBC, RBC and HGB. Medium and high intensity exercise could significantly increase WBC. Also, significant increase in RBC of pony after high intensity exercise. In summary, light or medium intensity exercise would bring benefit to pony in terms of physiological parameters but not high intensity one.

Introduction

Animal welfare becomes an important issue recently. Horse has served for leisure activities, sport, and working purposes for many years. However, abuse of horses commonly occurred due to the improper services, e.g. overloading during exercise or other activities. Literatures often indicated that the immunity response could be reduced after high-intensity or long lasting exercise in horse (Raidal *et al*., 1999; Krumrych, 2009; Luehr, 2010; Cywińska *et al.,* 2012). Most horses in Taiwan are raised in recreation spots and serve as riding animals for leisure purpose. Therefore, the daily loading of animal will not be expected to be even, i.e., animals probably need work more heavy during weekend than weekdays, which might cause problem in terms of physiological function of horse. Therefore, the objective of this study is to investigate the effects of exercise on hematology parameters in pony.

Materials and Methods

Seven female ponies were used for three intensity exercise programs, namely 3 hr walking (refers as light exercise program), 30 min trotting with 10 min walking (refers as medium intensity exercise program), and 25 min trotting with 5 min cantering and 10 min walking (refers as high intensity exercise program), during August 2015 and July 2016. Each pony was blood sampled before and after exercise. A total of 79 pre- and post-exercise blood sample sets were taken and assessed using automatic hematology analyzer. Traits analyzed included white blood cell (WBC), lymphocyte (LYM), monocyte (MONO), granulocyte (GRA), hemoglobin (HGB), red blood cell (RBC), red blood cell distribution width (RDWa), platelet (PLT), mean platelet volume (MPV), and mean corpuscular parameters, including volume (MCV), hemoglobin (MCH), and hemoglobin concentration (MCHC). Data were analyzed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC. USA), and GLM procedure was employed for exercise program comparison and significant test.

Results and Discussion

There was no significant difference in trait studied before and after the light exercise program. However, WBC, MONO, HGB and RBC were increased after the medium and high intensity exercise. Also, no significant difference between pre- and post- exercise was observed in WBC, MONO, GRA, MCV, MCH, MCHC, RDW, PLT, and MPV among program comparisons. LYMs were significantly increase after post-exercise in medium and high intensity

exercise programs. However, it was not the case in light exercise program. Similar results were also observed in WBC, RBC and HGB. Medium and high intensity exercise could significantly increase WBC. Also, significant increase in RBC of pony after high intensity exercise. The reason for increased of haematology levels might be due to the mobilized of immunocompetent cells to circulation system when exercise caused an acute bout effect. Furthermore, stress hormones such as catecholamines and cortisol could also stimulate the release of RBC from the bone marrow. Therefore, both the neutrophils and all lymphocyte subpopulations were recruited into the blood circulation. In summary, light intensity exercise program would bring benefit to pony in terms of haematology parameters, and high intensity program showed the adverse effects.

Table 1 Pre- and post- in haematology parameter estimates of pony

Trait	Ecercise Intrnsity								
	Light			Medium			Hight		
	Before	After	F-test	Before	After	F-test	Before	After	F-test
WBC, 10^9/L	7.51	8.12	NS	8.46	8.6	**	7.72	8.46	*
LYM, 10^9/L	2.13	2.28	NS	2	2.5	**	2.11	2.71	*
MONO, 10^9/L	0.57	0.62	NS	0.64	0.68	NS	0.59	0.77	NS
GRA, 10^9/L	4.8	5.22	NS	5.8	5.42	NS	5.03	4.99	NS
HGB, g/dL	11.02	11.12	NS	10.77	11.56	*	10.61	12.43	**
RBC, 10^{12}/L	6.29	6.34	NS	6.25	6.71	*	6.06	7.15	**
MCV, fL	46.33	46.01	NS	46.2	45.94	NS	46.87	46.67	NS
MCH, pg	17.56	17.57	NS	17.29	17.3	NS	17.56	17.42	NS
MCHC, 10^{12}/L	37.92	38.21	NS	37.46	37.67	NS	37.51	37.36	NS
RDW_P, %	24.76	24.73	NS	24.7	24.8	NS	24.68	24.86	NS
RDWa, fL	35.45	35.07	NS	35.22	35.25	NS	35.94	36.23	NS
PLT, 10^9/L	92.69	103.48	NS	148.39	129.87	NS	161.95	122.95	NS
MPV, fL	6.35	6.36	NS	6.42	6.44	NS	6.46	6.42	NS

WBC: White blood cell; LYM: Lymphocyte; MONO: Monocyte; GRA: Granulocyte; HGB: Hemoglobin; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW%: Red blood cell distribution width % ; RDWa: Red blood cell distribution width; PLT: Platelet; MPV: Mean platelet volume.

NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$.

Table 2 Least square estimate (± SE) of haematology parameter in pony under different exercise programs

Trait	Ecercise Intrnsity			P-value
	Light	Medium	Hight	
WBC, 10^9/L	0.27 ± 0.44	0.18 ± 0.21	0.51 ± 0.34	0.7200
LYM, 10^9/L	-0.07 ± 0.19^a	0.51 ± 0.091^b	0.57 ± 0.15^b	0.0181
MONO, 10^9/L	0.05 ± 0.61	0.03 ± 0.03	0.93 ± 0.05	0.5747
GRA, 10^9/L	0.30 ± 0.44	-0.34 ± 0.21	-0.15 ± 0.34	0.4279
HGB, g/dL	-0.14 ± 0.44^a	0.66 ± 0.21^{ab}	1.45 ± 0.34^b	0.0184
RBC, 10^{12}/L	-0.11 ± 0.26^a	0.38 ± 0.12^a	0.89 ± 0.20^b	0.0092
MCV, fL	-0.23 ± 0.29	-0.27 ± 0.14	-0.28 ± 0.22	0.9929
MCH, pg	-0.01 ± 0.13	0.00 ± 0.06	-0.15 ± 0.10	0.4239
MCHC, 10^{12}/L	0.25 ± 0.28	0.21 ± 0.13	-0.11 ± 0.21	0.4061
RDW_P, %	-0.19 ± 0.22	0.18 ± 0.11	0.31 ± 0.17	0.2045
RDWa, fL	-0.59 ± 0.43	0.07 ± 0.20	0.04 ± 0.32	0.2172
PLT, 10^9/L	30.01± 30.92	-16.16 ± 14.85	-32.98 ± 23.71	0.2674
MPV, fL	-0.26 ± 0.16	-0.04 ± 0.06	0.18 ± 0.14	0.6330

WBC: White blood cell; LYM: Lymphocyte; MONO: Monocyte; GRA: Granulocyte; HGB: Hemoglobin; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW%: Red blood cell distribution width % ; RDWa: Red blood cell distribution width; PLT: Platelet; MPV: Mean platelet volume.

[a, b] Value with differences superscript within row differ ($P < 0.05$).

References

Cywiñska, A., E Szarska, R. Górecka, L. Witkowski, Z. Wyszyñska, M. Hecold, A. Bereznowski, A. Schollenberger, and A. Winnicka1. 2012. Lymphocyte proliferation activity after limited (Light class) and long (CEI) distance endurance rides in horses. Cent. Eur. J. Immunol. 37:326–331.

Escribano, B. M., F.M. Castejón, R. Vivo, S. Agüera, E.I. Agüera, and M.D. Rubio. 2005. Nonspecific immune response of peripheral blood neutrophils in two horse breeds (Anglo-Arabian and Spanish-Arabian): response to exercise. Comp. Immunol. Microb. 28:145–154.

Escribano, B. M., E. Agüera, R. Vivo, R. Santisteban, F, Castejón, and M. Rubio. 2002. Benefits of moderate training to the nonspecific immune response of colts. Equine Vet. J. 34:1821–85.

Jensen-Waern, M., A. Lindbergt, A. Johannisson, G, Grondahl, J. A. Lindgren, and B. Essen-Gustavsson. 1999. The effects of an endurance ride on metabolism, and neutrophil function. Equine Vet. J. 30:605–609.

Luehr, J. 2010. The effect of introduction to training on some haematological and immunological parameters in the thoroughbred horse. Master thesis, University of Limerick., Ireland.

Krumrych, W. 2006. Variability of clinical and haematological indices in the course of training exercise in jumping horses. Bull Vet. Inst. Pulawy. 50:391–396.

Liburt, N. R., A. A. Adams, A. Betancourt, D. W. Horohov, and K. H. Mckeever. 2010. Equine Vet. J. 42:280–288.

Nesse, L., and A. Blom. 2002. Effects of racing on lymphocyte proliferation in horses. AJVR. 63:1–3.

Raidal, S .L., D.N. Love, G. D. Bailey and R. J. Rose. 1999. Effect of single bouts of moderate and high intensity exercise and training on equine peripheral blood neutrophil function. Res. Vet. Sci. 68:141–146.

PO-02-65 The effect of various management strategies upon milk 25-hydroxyvitamin D concentration and milk composition in dairy cows

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Objective

The vitamin D (VD), a hormonal precursor, is needed for producing 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which is calcium (Ca)-regulating hormone. In mammals, VD is synthesized by the skin in response to food intake and ultraviolet rays from the sun. VD supplied from feed and sun is quickly transport and stored in the liver. VD in the liver is converted to 25-hydroxyvitamin D [25(OH)D] by VD-25-hydroxylase and released into the blood. The 25(OH)D is circulated and converted to 1,25$(OH)_2$D in the kidney. The 1,25$(OH)_2$D is involved in the homeostasis of Ca and phosphorus (P), and immune function (Reinhardt and Hustmyer, 1987; Daynes et al., 1995). Dairy cattle produces plenty of milk containing high concentration of Ca, these Ca synthesis mechanism is very important. However, recently dairy cows are housed in a cowshed for the main part of the day, and as a consequence, they predominantly rely upon food intake to synthesize VD (Nutrient Requirements of Dairy Cattle, 2002). However, the *in vivo* contribution of sunlight-induced VD synthesis under grazing conditions remains unclear. The aim of this study was to determine the effect of various management strategies upon 25(OH)D concentration and milk composition in dairy cows.

Methodology

Animal and Experimental Design

Dairy cows at Farm A were allowed to graze during the daytime from February to October ('Grazing') and from November to March were placed in a paddock during the daytime ('Non-Grazing'). The cows were housed in tie-stalls on a rubber mat from 1400 - 0900 h throughout all seasons. The grazing area was 4 ha and used for rotational grazing. Species identified in pasture forage were orchardgrass (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*), hybrid ryegrass (*Lolium x hybridum Hausskn*), and white clover (*Trifolium repens*). During the non-grazing periods, cows were fed hay (2 kg/cow) in a paddock. They were fed an individually calculated ratio of corn silage as the major forage component and soybean meal as the major concentrate component determined on the basis of individual milk yield twice a day at 0630 and 1400 h. They were milked twice daily in tie-stalls. Dairy cows at Farm B were placed in a paddock all the time except for during milking and when fed hay of orchardgrass (*Dactylis glomerata*), Alfalfa (*Medicago sativa*), and Timothy (*Phleum pratense*), and concentrate. They were milked twice a day at 0700 and 1800 h in tie-stalls. Dairy cows at Farm C were managed in freestall barn and fed TMR. They were milked thrice a day at 0430, 1200 and 2000 h in rotary milking parlour.

Sample collection

Bulk milk samples were collected from three farms once a month. Each sample was collected into a polistyrene conical tube (BD Falcon, NY, USA) and immediately frozen at -20℃ until analyses for 25(OH)D, immunoglobulins (IgG, IgA, and IgM), b-carotene, and vitamin E concentration and milk composition. Concentrations of milk fat, protein, lactose, non-fat solids, and somatic cell count were also analyzed by Dairy Tochigi Agricultural Cooperatives.

Analysis of the milk samples

25(OH)D. Concentrations of 25(OH)D in milk were determined using ELISA (25(OH)-vitamin D xpress ELISA Kit, Immundiagnostik AG, Bensheim, Germany) according to the guidelines of the manufacturer.

Immunoglobulins. Milk samples for immunoglobulin analysis were dissolved and centrifuged at 3,000 rpm at 25℃ for 15 min. The cream layer was removed, and the pH was adjusted to 4.6 using HCl. Whey was obtained as

the sample for immunoglobulins when the casein was removed. Concentrations of IgG, IgA, and IgM in whey were determined using ELISA (Bovine IgG ELISA Quantitation Set; Bovine IgA ELISA Quantitation Set; Bovine IgM ELISA Quantitation Set, Bethy Laboratories, Inc., TX, USA) according to the guidelines of the manufacturer.

b-carotene and vitamin E. Concentration of b-carotene and vitamin E in milk samples were determined using a reverse-phase high performance liquid chromatography (HPLC) method using an InertSustain C18 column (4.6 i.d. × 250mm, GL Sciences, Tokyo, Japan). The mobile phase was detected using methanol tetrahydrofuran (9:1 v/v) and flow rate was controlled at 2 ml/min. b-carotene and vitamin E were determined by retention time and absorption spectrum obtained by a photodiode array detector.

Performance. Individual milk yields were recorded automatically at each milking event using a computerized recording system (Strangko, Skjern, Denmark). Concentrations of milk fat, protein, lactose, solids-non-fat (SNF), and somatic cell count (SCC) were analyzed for each milk sample using CombiFoss (Foss Electric, Hillerød, Denmark) at Dairy Tochigi Agricultural Cooperatives. Milk urea nitrogen (MUN) concentration was calculated by using the milk urea value.

Statistical Analysis. All data were analyzed with the JMP® 12 (SAS Institute Inc., Cary, NC, USA). Data were analyzed using principal component analysis (PCA) and multivariate analysis.

Result and Discussion

There was no significant difference in 25(OH)D concentration or milk composition from cows held under the 3 farms over a one year period (Figure 1). Furthermore, there was no significant difference in 25(OH)D concentration when compared between the 'Grazing' and 'Non-Grazing' groups at Farm A. Milk 25(OH)D concentration and composition remained consistent throughout the experimental period regardless of management strategy. Moreover, the comparison of the all data from 3 farms were conducted using a PCA. In the first 2 dimensions, PCA explained 59.7 % of the variation with principal components (PC) I and PC II accounting for 44.5 and 15.2 %, respectively. There was a different characteristic by the individual farms (Figure 2). Concentration of 25(OH)D had a positive correlation with PC I score (R = 0.52), and a negative correlation with PC II score (R = -0.52). Moreover, PC I score had a positive correlation with fat (R = 0.92), protein (R = 0.85), lactose (R = 0.65), SNF (R = 0.86), IgG (R = 0.11), b-carotene (R = 0.06), and vitamin E (R = 0.81). PC II had a positive correlation with fat (R = 0.02), lactose (R = 0.04), IgG (R = 0.69), IgM (R = 0.77), b-carotene (R = 0.67), and vitamin E (R = 0.21). The individual milk yields in Farm A during Grazing or Non-Grazing, Farm B, and Farm C, is 18.3 ± 0.75, 15.0 ± 0.81, 26.2 ± 0.72, and 29.4 ± 0.72 kg, respectively. It is known that milk composition reduces following increased milk yield (Pedernera at al., 2008; Løvendahl et al., 2011). Therefore, the PC I would indicate the low stress against lactation energy. Milk from grazing dairy cows contains higher levels of b-carotene and vitamin E than milk from non-grazing dairy cows and has a characteristic nutritional composition, flavor, and color (Koehn, 1943; Mitchell et al, 1944; Parrish et al., 1949). The b-carotene and vitamin E content of dairy cow diets are highly variable, but fresh forage (e.g., pasture) has relatively high concentrations of b-carotene (Johansson et al., 2014). Therefore, the PC II would indicate the level of the intake of fresh feed such as pasture.

Moreover, there were significant positive correlation between 25(OH)D and fat ($R = 0.41$, $P < 0.05$), protein ($R = 0.47$, $P < 0.01$), lactose ($R = 0.36$, $P < 0.05$), and SNF ($R = 0.51$, $P < 0.01$). On the other hand, there were significant negative correlation between 25(OH)D and individual milk yields ($R = -0.39$, $P < 0.05$), IgA ($R = 0.38$, $P < 0.05$), and IgM ($R = -0.42$, $P < 0.05$). The 25(OH)D dissolve in the milk fat globule because VD is a fat-soluble vitamin, resulted in being positive correlation between both compositions this relationship among milk composition were similar to previous report (Pedernera at al., 2008; Løvendahl et al., 2011). In the immunoglobulins, $1,25(OH)_2D$, that is produced from 25(OH)D, has been shown to promote the humoral immunity such as immunoglobulins and inhibit the cell-mediated immunity simultaneously (Daynes et al., 1995). However, $1,25(OH)_2D$ in the kidney is adjusted strictly and homeostasis. Sun-cured hay also may provide enough vitamin D to prevent symptoms of vitamin D deficiency (Thomas and Moore, 1951). Therefore, 3 different management systems may have no effect on immunoglobulins due to sufficient vitamin D from feed and sunlight.

Conclusion

These results suggest that the various management strategies affect milk 25(OH)D concentration and milk composition in dairy cows.

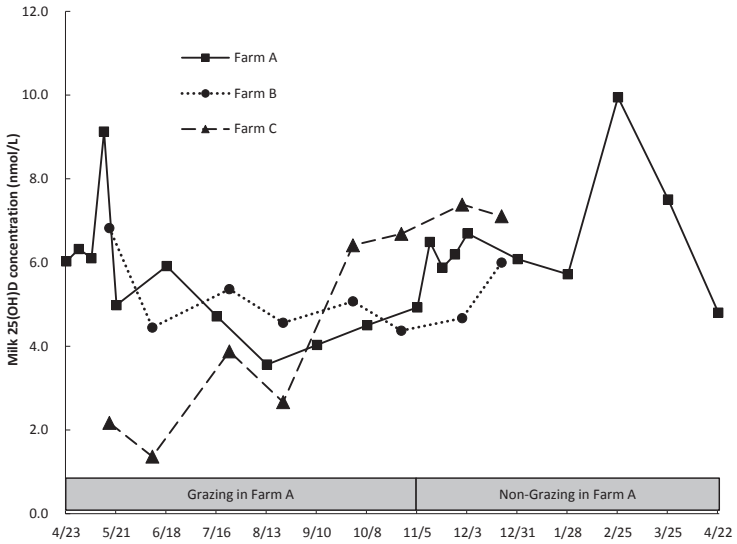

Figure 1. The effect of various management strategies upon milk 25-hydroxyvitamin D concentration and milk composition in dairy cows.

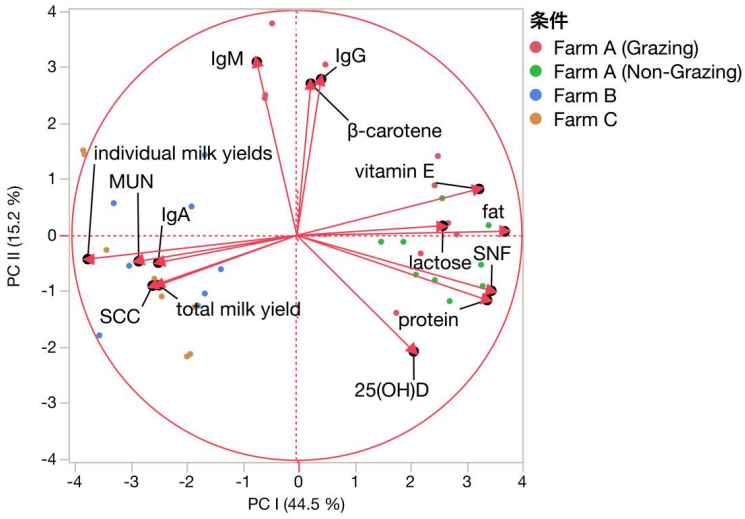

Figure 2. Principal component analysis of the various management strategies and milk composition data.

Reference

Daynes RA, Araneo BA, Hennebold J, Enioutina E, Mu HH. Steroids as regulators of the mammalian immune response. J Invest Dermatol. 1995;105(1 Suppl):14S-19S.

Johansson B, Persson Waller K, Jensen SK, Lindqvist H, Nadeau E. Status of vitamins E and A and β-carotene and health in organic dairy cows fed a diet without synthetic vitamins. J Dairy Sci. 2014 Mar;97(3):1682-92.

Koehn, CJ. Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama. J Dairy Sci. 1943;26(8):673-681.

Løvendahl P, Chagunda MG. Covariance among milking frequency, milk yield, and milk composition from automatically milked cows. J Dairy Sci. 2011;94(11):5381-92.

Mitchell JH, Wise GH. The Comparative Effects of Continual and Rotational Systems of Grazing on the Carotene Content of Permanent Pasture Herbage and of the Milk Produced Therefrom. J Dairy Sci. 1944;27(3):189-196.

NRC. 2001. Nutrient Requirements of Dairy Cattle. Natl. Acad. Sci., Washington, DC.

Parrish DB, Martin WH, Atkeson FW, Hughes JS. The Vitamin A and Carotene Content of Market Butter Produced in Kansas. J Dairy Sci. 1946;29(2):91-99.

Pedernera M, García SC, Horagadoga A, Barchia I, Fulkerson WJ. Energy balance and reproduction on dairy cows fed to achieve low or high milk production on a pasture-based system. J Dairy Sci. 2008;91(10):3896-907.

Reinhardt TA, Hustmyer FG. Role of vitamin D in the immune system. J Dairy Sci. 1987;70(5):952-62. Review.

Thomas JW, Moore LA. Factors Affecting the Antirachitic Activity of Alfalfa and Its Ability to Prevent Rickets in Young Calves. J Dairy Sci. 1951;34(9):916-928.

PO-02-73

Palm kernel expeller and its extract modify caecal microbial population of broiler chickens differently

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Introduction

Microbial community in gastrointestinal tract (GIT) plays an important role in overall health and function of the host. Recent study on the diversity of chicken gut microbiota succession in caeca of broiler chicken fed with commercial feed showed that the population of beneficial microbes was comparatively lower than the potentially pathogenic bacteria, rendering the need of gut modulation to improve the gut health of the chicken (Shaufi *et al*., 2015). However, the overuse of antibiotics escalate the emergence of antibiotic resistant pathogens, leading to the ban on the use of antibiotics in broiler production. One alternative is to replace antibiotics with prebiotic dietary fibers such as manna-oligosaccharides (MOS).

MOS is a major oligosaccharides that can be extracted from palm kernel expeller (PKE), a major by-products of the palm oil industry in Malaysia. Earlier study by Allen et al. (1997) showed that inclusion of PKE in broiler's diet decreased *Salmonella* colonization in broilers while a latter study by Jahromi *et al.* (2016) shows that MOS extracted from PKE have positive effect on broilers gut health, particularly by improving the growth of *Lactobacillus*. Thus, in this study, we evaluated the changes in caecal microbial population of broiler chicken fed with either raw PKE (Raw-PKE) or PKE-extract (Oligo-PKE) by using quantitative real-time PCR (qPCR).

Material and Methods

The PKE in this study were obtained from Kangar, Malaysia. These PKE were used as raw ingredients in the experimental feed and as raw ingredients for the production of Oligo-PKE. Oligo-PKE were produced according to method described by Chen et al., 2015, with slight modification. Briefly, PKE were subjected to high temperature auto-hydrolysis, followed by methanol-chloroform (2:1) extraction of oligosaccharides. Samples were kept in -20°C for further use.

108 one-day-old broiler chicks (Cobb 500) were randomly assigned into 3 dietary groups (i) basal diet (Control), (ii) basal diet containing 20% raw PKE diet (Raw-PKE), and (iii) basal diet + 1% PKE-extract (Oligo-PKE). At day-35, caecal contents of all birds were collected and the microbial populations were determined according to the method described by Chen et al. (2015). Briefly, the DNA was extracted from caecal samples by using DNA Stool Mini kit (Qiagen), concentration of each extracted DNA were normalized 100 ng/µl. The populations of total bacteria, *Bifidobacterium*, *Lactobacillus*, *E. coli*, Enterococcaceae, and Enterobacteriaceae were determined by q-PCR (BioRad, USA). Each assay was performed in duplicate and the cycle threshold (C_T) was calculated and compared to a standard curve made by serial dilution of plasmid DNA of each microbial group.

Results and Discussion

This study was carried out to examine whether supplementing broilers with different form of PKE will modify the caecal bacterial population of the broilers. Overall, the data (Figure 1) showed that there was no significant different in total bacteria population measured in each treatment group. Significantly higher population of beneficial bacteria (*Lactobacillus*, *Bifidobacterium*, and Enterococcaceae) were recorded in birds fed with Oligo-PKE group as compared to Control and Raw-PKE group. However, these populations were found to be lower in birds in Raw-PKE group as compared to Control group. In addition, the population of Enterobacteriaceae was significantly higher in birds fed with Raw-PKE diet as compared to Control ($P<0.05$), whereas there were no significant different in Oligo-PKE group as compared to Control group.

Enterococcaceae represent a group of gram-positive bacteria in which some are lactic acid producers. Lactic acid production will reduce the pH of the gut, thus preventing growth of pathogens. However, in this study, pathogenic bacteria population were higher in both treatment group as compared to Control. This shows that MOS extracted from PKE cannot inhibits the growth of pathogens but have a positive effect in supporting growth of beneficial bacteria instead. On the other hand, Enterobacteriaceae consist of common gram-negative bacteria which are

consider pathogenic to the host. In this study, there is no significant difference observed in *E. coli* population in each treatment group. The higher population of Enterobacteriaceae observed in Raw-PKE group may indicated the present of other pathogenic bacteria other than *E.coli*. This could be environmental pathogens present initially in raw PKE which were then introduced to broilers fed with PKE. Most of these environmental pathogens would have been killed in the process of Oligo-PKE preparation. Nevertheless, inclusion of PKE in broiler's diet have shown to inhibit colonization of *Salmonella* (Allen et al., 1997). This shows that PKE may be affective in inhibiting growth of selective pathogens only.

In conclusion, the use of PKE and PKE-extract alters the caecal microbial populations of broiler chickens. In order for PKE to be used as prebiotics, the oligosaccharides must be extracted from PKE and be used as a supplement, rather than direct inclusion of raw PKE into broiler feed.

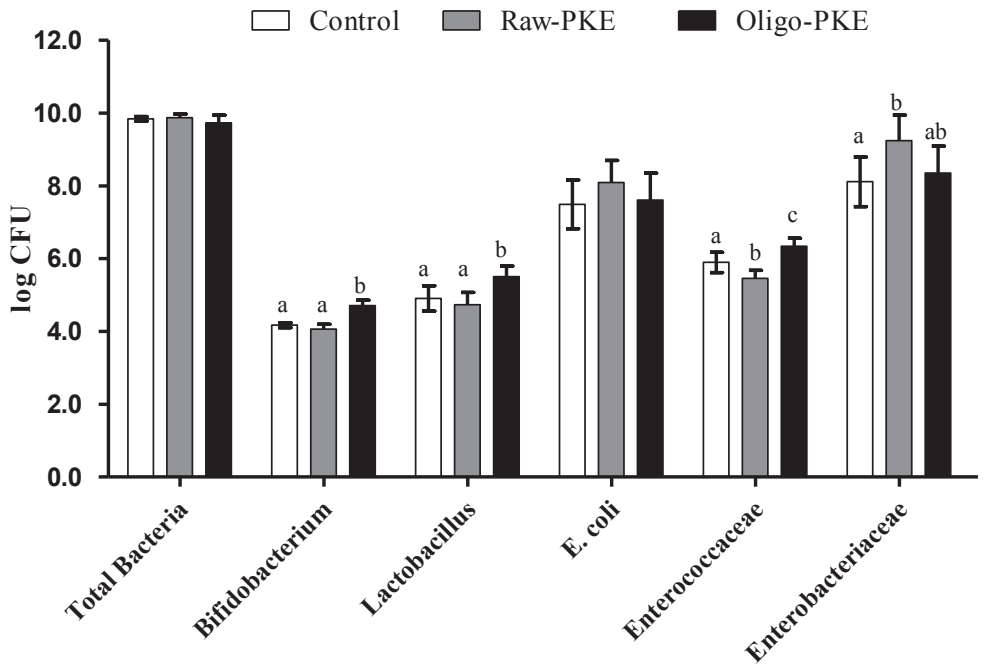

Figure 1: Caecal microbial population of broiler fed with basal diet (Control), Raw-PKE and Oligo-PKE obtained on day-35 post feeding.

References

Allen, V.M., Fernandez, F. and Hinton, M.H. (1997). Evaluation of the influence of supplementing the diet with mannose or palm kernelmeal on*Salmonella* colonisation in poultry. *British Poultry Science* 38(5): 485-488.

Chen, W. L., Liang, J. B., Jahromi, M. F., Abdullah, N., Ho, Y. W., andTufarelli, V. (2014). Enzyme treatment enhances release of prebiotic oligosaccharides from palm kernel expeller. *BioResources*, 10(1), 196-209.

Jahromi, M. F., Liang, J. B., Abdullah, N., Goh, Y. M., Ebrahimi, R., andShokryazdan, P. (2015). Extraction and Characterization of Oligosaccharides from Palm Kernel Cake as Prebiotic. *BioResources*, 11(1), 674-695.

Shaufi, M. A. M., Sieo, C. C., Chong, C. W., Gan, H. M., and Ho, Y. W. (2015). Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. *Gut pathogens*, *7*(1), 1.

PO-02-74

DETECTION OF THE CONNECTIN/TITIN 20-kDa FRAGMENT INCREASED IN CHICKEN SARCOPLASM DURING POSTMORTEM AGING

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ABSTRACT

Meat palatability is affected by various factors such as tenderness, flavor and juiciness, especially tenderness is one of the most important factors of meat quality. Nowadays, meats, which we eat usually, become tender in prolonged storage after suffering rigor-shortened toughness during postmortem aging. However, an objective and standard method evaluating meat aging has not been established yet. In our previous studies, it was revealed that connectin/titin near the Z-line of sarcomere was digested to the 20-kDa fragment and the fragment increased in sarcoplasm during postmortem aging. Also the monoclonal antibody against the 20-kDa fragment was prepared and the GST fused recombinant protein which cloned a part of the fragment was expressed in *E. coli*. The aim of this study is to detect binding of the antibody to the fragment in order to develop a suitable method evaluating meat aging. The binding of the antibody to the fragment was examined by Western blotting. Moreover the binding of the antibody to the GST fused recombinant protein investigated by both Western blotting and ELISA. As the results, it shows that the antibody binds to both the 20-kDa fragment of sarcoplasm and the GST fused recombinant protein. Furthermore, the GST fused recombinant protein was determined quantitatively by ELISA. Therefore, it is concluded that monoclonal antibody is useful for detecting the 20-kDa fragment of chicken sarcoplasm during postmortem aging.

INTRODUCTION

Connectin/titin is a huge molecule of 3000-kDa to connect with the M-line from the Z-line of a sarcomere and contributes to maintenance of sarcomere structure（Maruyama, K. , 1997）. So far, there are many reports on the degradation of connectin/titin as a factor of meat tenderization during postmortem aging. Structural protein changes occurred in myofibrils such as myofibrillar fragmentation and the splitting of α-connectin into β-connectin fragments were observed in aged muscles (Koohmaraie, M. ,1992 ; Takahashi, K, et al., 1992). We have shown that the connectin/titin 20-kDa fragment increased in sarcoplasm with postmortem aging of chicken breast muscle. (Yamanoue, M, et al., 2010) Increase in the fragment of sarcoplasm seemed to correlate with changes in myofibrillar structure around Z-line during postmortem aging. It was considered that antibody against connectin/titin 20-kDa fragment was capable of a candidate as a detection probe to clarify the relevance between increase in the fragment of sarcoplasm and meat tenderization. So, the aim of this study is to detect binding of the antibody to the fragment by both Western blotting and ELISA in order to develop a method evaluating meat tenderization.

MATERIALS AND METHODS

Preparation of muscle samples: Chicken breast muscle was taken immediately after slaughter and treated antiseptically by dipping in 1mM NaN_3. The samples, after wrapped with polyethylene film and aluminum foil, were stored at 4°C. Five grams of muscle pieces at 0, 12, 48 and 72 hours postmortem were weighed respectively and homogenized in a solution containing 5 mM EDTA, 10 mM Tris-HCl (pH7.0) and 1% protease inhibitor cocktail (Nacalai tesque) at 10,000 rpm for 1 min. After centrifugation at 3,000 rpm for 10 min at 4°C, supernatant was collected and then stored at -80°C until future use by gel electrophoresis.

Preparation of recombinant connectin/titin fragment: Two parts of the connectin/titin 20-kDa fragment (G-CF and G-NFL fragment) were cloned from chicken cDNA library and plasmid vectors pGEX6P of the GST fused G-CF and G-NFL fragments were constructed in our laboratory. Both of recombinant fragments were overexpressed in *E. coli* BL21 strain, and then were purified by using affinity column chromatography of glutathione-sepharose.

Preparation of monoclonal antibody from hybridoma cell culture: Monoclonal antibodies (mAbs), namely 1-3C and 16-4B4, were developed in our laboratory according to the conventional method (Yamanoue, M et al., 2010). Thaw freezing hybridoma cell lines of 1-3C and 16-4B4 which secrete monoclonal antibodies against the 20-kDa fragment were incubated in RPMI-1640 medium containing 5% FBS at 37°C with 5% CO_2. Supernatants after

centrifuged cultured cells at 1,500 rpm for 5 min were collected and the antibodies were partially purified by ammonium sulfate fractionation. After that, the monoclonal antibodies were transferred to PBS by dialysis.
Western blotting: The GST-NFL fragment and sarcoplasmic proteins prepared from 0 to 72 hours postmortem were separated by SDS-PAGE (Laemmli, U. K., 1970) and were electrotransferred from polyacrylamide gel to PVDF membranes (Tawbin, H.et al., 1979). Membranes were blocked with 7.5 % non-fat dry milk in TBS-T solution for 1h, and then incubated with either mAb 1-3C or 16-4B4 for 1h. After washing with TBS-T, the membranes ware incubated for 1h with goat anti-mouse IgG antibody conjugated with horse radish peroxidase (Sigma), then incubated with immnostar LD (wako) and detected by using Typhoon 9400 (GE Healthcare) imaging system.
Direct ELISA: Direct ELISA was conducted to detect quantitatively the GST fused recombinant fragment mimicking the connectin/titin fragment of chicken sarcoplasm during postmortem aging (O.E. Tsitsilonis.et al., 2002). ELISA 96-well plates were coated with the G-CF fragment, then blocked with PBS containing 0.5% bovine serum albumin (BSA). After washing with PBS-T, either mAb 1-3C or mAb 16-4B4 was incubated to bind to the G-CF fragment for 1 h. After washing with PBS-T, the plates were incubated with goat anti-mouse IgG secondary antibody conjugated with horse radish peroxidase (Sigma). Detection was completed by the addition of the enzyme substrate, o-phenylenediamine (OPD) and H_2O_2, followed by adding 2N H_2SO_4 to stop color development. The antibody binding was quantified by measurement of spectrophotometric absorption at 492 nm.

RESULTS AND DISCUSSION

Figure 1 shows the results of western blotting to detect the G-NFL fragment and the connectin/titin 20-kDa fragment of chicken sarcoplasm with monoclonal antibody (mAb 1-3C). It was indicated that monoclonal antibody binds to both the 20-kDa and the G-NFL fragments. The same results were obtained when using mAb 16-4B4. (data not shown)

Figure 2 shows that the G-CF fragment was determined quantitatively by ELISA. Both antibodies bind to the G-CF fragment. Absorbance from 0 to 100 ng antibodies increased rapidly to reach plateau above 100 ng of antibodies. The binding ability of mAb 16-4B4 was stronger than that of mAb 1-3C. Thus, monoclonal antibodies are useful for detecting the connectin/titin fragment.

Results of western blotting to detect the 20-kDa fragment of sarcoplasm are shown in Figure 3. Both antibodies bind to the 20-kDa fragment of sarcoplasm and the binding increased gradually during postmortem aging of chicken muscle. The bands around 37-kDa are non-specific band, which were directly bound by the goat anti-mouse IgG secondary antibody. Also, the band of 28-kDa is estimated to be composed of phosphoglycerate mutase 1, triosephosphate isomerase or carbonic anhydrase by mass spectrography. (data not shown) The reason why mAbs bind to those proteins is unclear.

CONCLUSION

In this study, we succeeded in detection of increase in the connectin/titin 20-kDa fragment of chicken sarcoplasm by using mAbs and suggest that the connectin/titin 20-kDa fragment is a candidate for useful index of meat tenderization during postmortem aging.

Keywords：connectin/titin, monoclonal antibody, ELISA

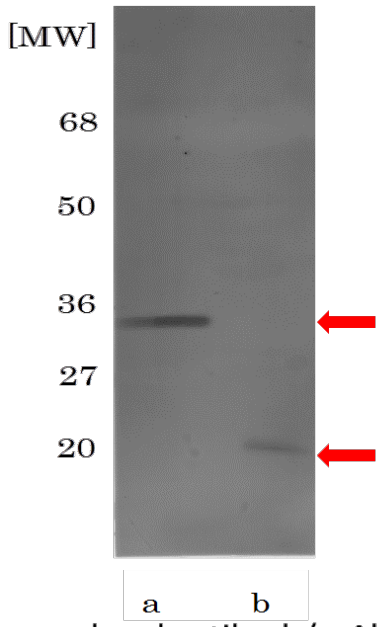

Fig 1. Detection of monoclonal antibody(mAb 1-3C) binding to both chicken sarcoplasm and GST fused recombinant protein . Red arrows indicate the G-NFL fragment and the 20-kDa fragment.
(a) G-NFL fragment , (b) Chicken sarcoplasm at 72 hours

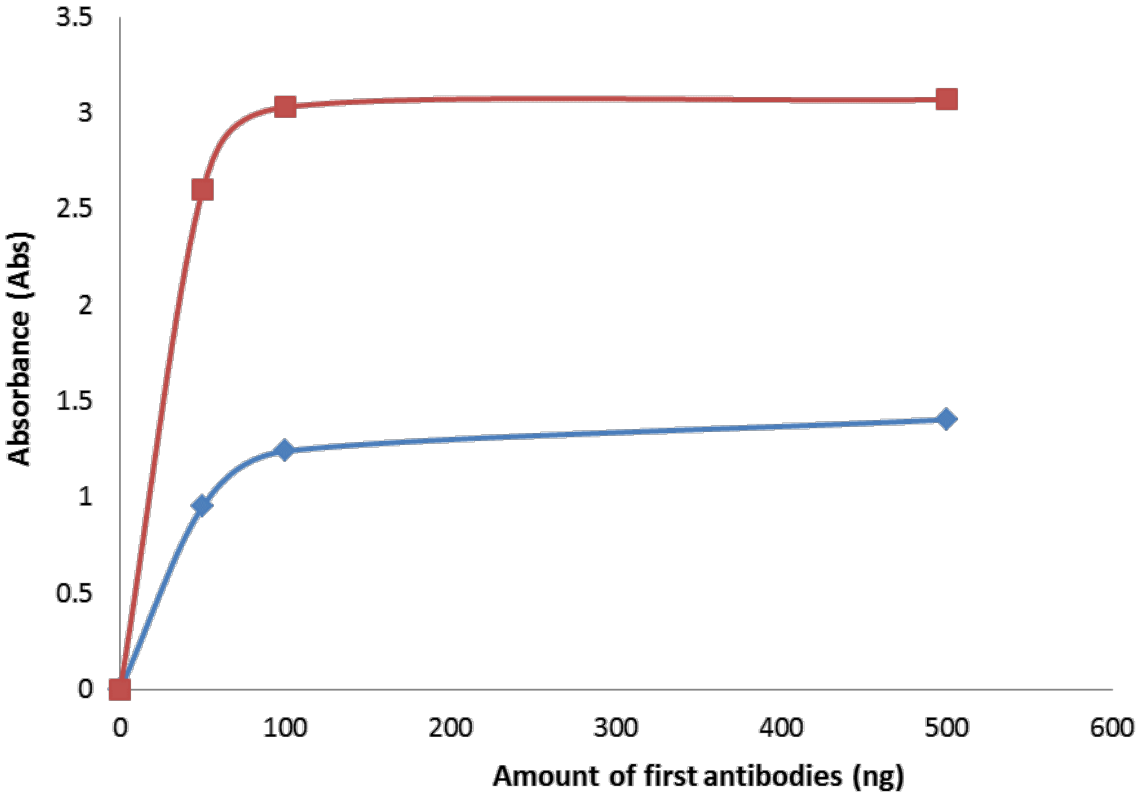

Fig 2. Binding of monoclonal antibodies by direct ELISA using the G-CF fragment as an antigen.

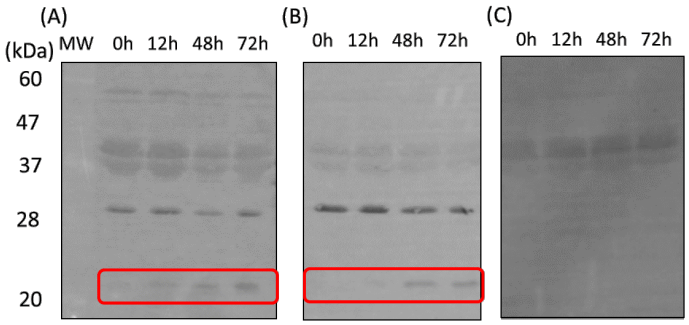

Fig 3. Increase of the 20-kDa fragment of sarcoplasm during aging time. Red suquares indicate the connectin/titin 20-kDa fragment. (A) mAb 16-4B4, (B) mAb 1-3C, (C) control (only secondary antibody)

REFERENCES

・Koohmaraie. M. (1992). The role of Ca^{2+}-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. *Biochimie*, 74, 239-245.

・Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259): 680-685.

・ Maruyama, K. (1997). Connectin / titin, giant elastic protein of muscle., */FASEB J/.*, 11, 341-345

・ O.E. Tsitsilonis, S. Stoeva, H. Echner, A. Balafas, L. Margomenou, H.L. Katsoulas, et al. (2002).A skeletal muscle troponin T specific ELISA based on the use of an antibody against the soluble troponin T (16–31) fragment. J Immunological Methods, 268, pp. 141–148

・Takahashi, K, Hattori, A, Tatsumi, R & Takai, K(1992). Calcium-induced splitting of connectin filaments into ß-connectin and a 1,200-kDa subfragment. J Biochem.111(6):778-82.

・Tawbin, H., Staehelin, T. & Gordon, J. (1979).Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350-4354

・Yamanoue, M , Kubo, T, Matsunaga, K, Ueda, S & Takagi, M (2010). Expression and antibody production of connectin fragment increased in sarcoplasm during postmortem aging of chicken muscle. Proc. 57th ICoMST , P072

PO-02-81

A Whole Genome Association Analysis for serum and milk β -hydroxybutiric acid and milk acetone in Holstein cows

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ABSTRACT

The Ketosis disease influences both productive and reproductive performances of dairy cattle, and concentrations of acetone, β-hydroxybutiric acid in blood (**BHBA**) and milk (**MBHBA**) of dairy cows can be considered as indicators for clinical or subclinical ketosis. A genome-wide association study (**GWAS**) was conducted to identify QTLs responsible for levels of ketone bodies in serum and milk in Holstein cows. Samples for ACE, BHBA and MBHBA were obtained from 112 cows with between 5 and 20 days in milk. Those samples were genotyped using the Illumina BovineSNP50K BeadChip panel. After a basic genotyping pruning for low genotyping, SNP missingness, minor allele frequency and HWE test, a total of 43,519 SNPs retained which spanned over a 2,526 Mb distance. A single marker regression analysis was done using **PLINK 1.07** software package, identified any associations between a SNP and a trait in question. A total 42 SNPs were significant at genome-wide P<0.05, in which 29 SNPs were found for ACE only. The BHBA and MBHBA mostly shared similar QTL locations; thus, both of them were possibly governed by similar set of genes located on BTA7 (MIR2462), BTA11 (AHSA2, ETAA1, and C1D), BTA13 (RUM1, SPATA2, and CHMP4C), BTA15 (SLC5A12 and AMBRA1), BTA18 (CDH8 and CDH1), BTA22 (AZI2), and BTA 23(FBXL2, LRRC1, ENPP5, and RCAN2). The INRA-736 SNP (close to AMBRA1) was significant for all three traits, however. Most SNPs for ACE was located on BTA9 (12) and BTA16 (7). This study indicates that the ketosis in Holstein might be influenced by certain genomic regions and a selection on these traits using them could reduce the ketosis incidence. A study including a higher sample size would be more effective to verify the current results as well as for possible identification of newer associations.

INTRODUCTION

Ketosis is frequently occurs in dairy cows especially in early lactation period when they have a negative energy metabolic balance and with the accumulation of the excessive levels of ketone bodies like acetone, BHBA and acetoacetate in the body. They are synthesized when fatty acids are broken down excessively in a liver. Therefore the BHBA concentrations can be used for an indicator of ketosis. Other studies has been suggested a threshold for BHBA to define ketosis ranging from 1.0 to 1.4 mmol/L but in this study 1.2 mmol/L is chosed for a threshold of ketosis. Ketosis causes anorexia, decrease milk production and body weight which finally bring out an economic loss.

Over the last decades, a technology which analyzes single nucleotide polymorphism (**SNP**) has been developed by the SNP microarray chip so it is possible to determine almost genotypes of SNPs more effectively compared with past. With the advent of genome-wide panels for SNP, SNPs have been widely used for the detection and localization of QTL, and have proved powerful and useful in identification of casual mutations associated with traits (Li Jiang, et al., 2010). Therefore a GWAS is commonly used in dairy cattle industry to SNP and certain traits like disease or production performance especially related with economically significant traits. The aim of this study is to identify QTLs responsible for levels of acetone, BHBA and fat percent and protein percent as milk production traits from Holstein cows.

MATERIALS AND METHODS

Samples for BHBA in milk and blood, acetone, fat percentage and protein percentage from 92 healthy and 30 subclinical ketotic female cows between 5 and 20 days in milk. Blood samples were tested with the Precision Xtra meter to obtain BHBA concentrations. Milk samples were analyzed by FTIR spectroscopy using a CombiFoss™ FT + system (Foss Analytical A/S, Denmark) with previously developed calibration equation.

Those samples were genotyped using the Illumina BovineSNP50K Bead Chip panel containing 54,609 SNPs with a median spacing of 37.4 kb. The SNPs that located in sex chromosomes were deleted before pruning. Because they have large-scale discordance between SNP genotypes and animal sex indicating poor SNP calling (Brian K Merdith,

et al., 2012). For quality control firstly, the SNP with a minor allelic frequency of <1% and missing genotypes its SNP >10% were eliminated. Secondly, if there were more than 10% missing for individuals and p-value of chi-square test for Hardy-Weinberg equilibrium >10?? were removed. BHBA and acetone have been proved as indirect detectors for ketosis therefore in this study the analysis was conducted with not only normal but also ketotic cows. On the other hand, milk fat percent and milk protein percent are one of the most popular milk production traits so only ketotic cows were carried out for figure out the candidate QTLs.

After filtering 41570 SNPs and 112 phenotype records were progressed for BHBA and acetone, also 42246 SNPs and 29 phenotype records were proceeded for fat percent and protein percent. A single marker regression analysis was done using **PLINK 1.07** software package, identified any associations between a SNP and a trait in question.

RESULT

Ketosis indicators

A total 42 SNPs have a significant association with BHBA, MBHBA and Acetone at genome-wide $p<0.05$, in which 13 SNPs were found for BHBA and MBHB as shown in table2. There are 30 SNPs were found for acetone only as described in table3. And all traits are sharing INRA-736 with similar significant level.

Milk composition

Using single marker regression analysis, BTA4 (CALCR) and BTA28 (COA6) has significant association with each fat percent and protein percent of ketotic cows.

CONCLUSION

This study indicates that the ketosis in Holstein might be influenced by certain genomic regions and a selection on these traits using them could reduce the ketosis incidence. Identification of SNP could be usefully adapted as a marker. A larger dataset is needed to confirm SNPs effect on ketosis traits.

Table 1. Statistics for traits

Traits	Mean	SD	Min.	Max	N
BHBA	0.987	0.592	0.3	3.6	82
MBHBA	0.092	0.078	0	0.52	82
ACETONE	0.114	0.146	0	0.96	82
Fat %	5.118	1.116	3.38	8.46	30
Protein %	3.718	0.848	2.67	5.6	30

Table2. Selected SNPs of significant (p<0.05) with BHBA and MBHBA

Chr	SNP	Gene	Distance(bp)	P-value	
				BHBA	MBHBA
7	*BTB-01019887*	*MIR2462*	1305350	0.001893	0.001892
11	*ARS-BFGL-NGS-114897*	*ETAA1*	95690	0.02527	0.02525
11	*Hapmap39736-BTA-26188*	*AHSA2*	788343	0.04016	0.04013
13	*ARS-BFGL-NGS-38412*	*RUM1*	38125	0.006855	0.006849
14	*Hapmap35917-SCAFFOLD20*	*CHMP4C*	465624	0.02527	0.02525
15	*ARS-BFGL-NGS-76542*	*SLC5A12*	introninc	0.00496	0.004956
15	*INRA-736*	*AMBRA1*	introninc	0.03435	0.03432
18	*BTA-113658-no-rs*	*CDH8*	248806	0.001893	0.001892
18	*BTA-43000-no-rs*	*CDH1*	introninc	0.007906	0.0079
22	*ARS-BFGL-NGS-31517*	*AZI2*	69169	0.02527	0.02525
22	*Hapmap42000-BTA-89464*	*FBXL2*	introninc	0.01952	0.0195
23	*ARS-BFGL-BAC-29489*	*RCAN2*	14114	0.001893	0.001892
23	*Hapmap51467-BTA-57073*	*LRRC1*	42149	0.01952	0.0195

Table3. Selected SNPs of significant (p<0.05) with Acetone

Chr	SNP	Gene	Distance(bp)	P-value
1	*ARS-BFGL-NGS-85061*	*TBC1D5*	68877	2.E-02
2	*BTB-01230813*	*LDLRAP1*	107948	2.E-02
3	*ARS-BFGL-NGS-27852*	*INADL*	intronic	4.E-02
9	*ARS-BFGL-NGS-113787*	*NT5DC1*	718004	1.E-02
9	*ARS-BFGL-NGS-35694*	*PDE7B*	176229	3.E-02
9	*ARS-BFGL-NGS-5833*	*MMS22L*	402581	3.E-03
9	*ARS-BFGL-NGS-77406*	*ARHGAP18*	59707	2.E-02
9	*BTA-01195-rs29012162*	*PDE7B*	147189	2.E-02
9	*BTA-103750-no-rs*	*MYO6*	intronic	3.E-03
9	*BTA-84179-no-rs*	*TAAR6*	19855	3.E-02
9	*BTA-84286-no-rs*	*MYB*	7353	2.E-02
9	*Hapmap48558-BTA-10101*	*PDE7B*	350906	3.E-02
9	*Hapmap49774-BTA-84178*	*TAAR6*	1185	3.E-02
9	*Hapmap51348-BTA-90742*	*MYB*	328146	2.E-02
9	*Hapmap53034-rs2901142*	*ARHGAP18*	81742	3.E-02
12	*ARS-BFGL-NGS-32656*	*FAM155A*	intronic	3.E-02
12	*BTA-29126-no-rs*	*METTL21E*	1073243	3.E-02
15	*ARS-BFGL-NGS-68340*			2.E-02
15	*Hapmap43113-BTA-96564*	*FDX1*	91070	4.E-02
15	*INRA-736*	*AMBRA1*	intronic	4.E-02
16	*ARS-BFGL-NGS-107190*	*R3HDM2*	908476	2.E-02
16	*ARS-BFGL-NGS-1753*	*BRINP3*	808247	4.E-03
16	*ARS-BFGL-NGS-65916*	*CNIH3*	52294	1.E-02
16	*BTA-06182-rs29020546*	*SERTAD4*	117715	4.E-02
16	*BTA-118128-no-rs*	*RGS18*	118854	1.E-02
16	*BTB-01324160*	*LRRN2*	246432	2.E-02
16	*BTB-01540999*	*R3HDM2*	1544179	3.E-02
20	*BTA-50044-no-rs*	*PLK2*	407180	2.E-02

References

S.Wijga , J. W. M. Bastiaansen, et al. (2012) “Genome associations with somatic cell score in first-lactation Holstein cows.” Jornal of dairy science 95: 899-908.

Jiang L, Liu J, Sun D, Ma P, Ding X, et al. (2010) ”Genome Wide Association Studies for Milk Production Traits in Chinese Holstein Population” PLoS ONE 5(10): e13661. doi:10.1371/journal.pone.0013661

PO-03-1

Genetic Diversity of Swamp Buffalo in Uttaradit Province, Thailand

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ABSTRACT

Buffaloes have long been an important animal and an integral part of the Thai agricultural system. However, the number of buffaloes has been continuously decreasing in Thailand. Knowledge of phylogeny and genetic characterization of native breeds is the first step in conservation programs. In this study, the genetic diversity of Thai swamp buffalo, randomly selected from nine different district in Uttaradit province, Thailand were studied using mitochondrial DNA cytochrome b gene. From 27 buffaloes represented by 1,200 bp fragments of mtDNA (cytochrome b) were successfully amplified. Analysis of cytochrome b sequences revealed all sequence in this study were swamp buffalo and separated from river buffalo clade. This result showed that swamp buffaloes in Uttaradit no have flourishing of genetic diversity. This study would contribute a comprehensive understanding of the genetic diversity of buffaloes in Uttaradit province and provide insightful knowledge of DNA application for biodiversity management in breeding improvement and buffalo conservation.

Introduction

The Asiatic water buffalo (*Bubalus bubalis*) have been divided into two types, the river buffalo (2n=50) and the swamp buffalo (2n=48) based on morphological, behavioral and geographical distribution (Macgregor, 1941). In Thailand, most buffalo are classified as the swamp type and are usually dark gray and have lone, gently curved horns. Buffalo are native to Thailand and are well adapted to poor conditions (Sraphet *et al.*, 2008).

Overall buffalo numbers are increasing worldwide at 1.3 percent annually, the numbers are reducing dramatically in Thailand (Triwitayakorn et al., 2006). As a consequence, the Thai swamp buffalo are not only eligible for, but also require conservation as well as sustainable utilization in the production system. Detailed knowledge of genetic variation within and among different breeds is very important for understanding and improving traits of economic importance (Sangwan, 2012).

The molecular markers and DNA sequencing have taken as good tools to classify the taxonomy and phylogenetic relationship of species. Mitochondrial DNA (mtDNA) is the genetic material that exists outside the nucleus in eukaryotic cells. Cytochrome b gene in mtDNA has a moderate evolutionary rate and a clear evolutionary pattern that is suitable for the studies on the phylogenetic evolution at the intra- and interspecific levels. (Astorga and Galleguillor, 1998)

In studies of the genetic diversity of the Asian swamp buffalo samples collected from different regions of Uttaradit province in order to analyze genetic variation within and among populations. In this study, we used mitochondrial DNA cytochrome b gene that have previously been used in genetic diversity studies of buffalo.

Materials and Methods

Sample collection

A total of Thai swamp buffalo were randomly selected from nine amphoe in Uttaradit (Mueang (M), Lablae (LL), Thapla (TP), Tron (T), Faktha (FT), Ban Khok (BK), Thong Saen Khan (TK), Pichai (PC) and Nampat (NP)). Blood sample were collected from each animal and genomic DNA was extracted from blood samples according to Sambrook and Russell (2001).

Jugular vein blood samples were randomly obtained from twenty seven swamp buffalo from nine amphoe in Uttaradit. DNA was extracted using PURE Gene™ DNA purification Kit. As recommended by the manufacturer.

Amplification of cytochrome b fragments and sequencing

The conserved primer pair, forward primer (cyt b) 5' ATGACCAACATCCGAAAATCC 3' and reverse primer (cyt b) 5' TCTGGTTTACAAGACCAGTGT 3' were used to amplify the 1,200 base fragment of the cytochrome b region of the buffaloes. The amplification reaction was carried out in a 25 µl reaction mixture consisting of 1.25 unit Tag polymerase, 1x buffer, 1 M each forward and reverse primer, 0.2 mM dNTPs and 50 ng of DNA. The PCR cycle profile was 94 °C for 2 min before the first cycle, then 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min for 35 cycles. The reaction products (5 µl each) were used for electrophoresis with and appropriate size marker on

1.5% agarose in 1x-Tris acetate buffer (TAE). After electrophoresis the gels were stained with ethidium bromide and were examined to verify amplification of the cytochrome b fragment. The PCR product were purified using QIAquick PCR purification kit (Qiagen, Inc.) and the resulting purified products were used in the sequencing.
Sequence analysis and alignment were carried out using NCBI-BLASTN and ClustalW multiple sequence alignment programs. The Mega4 program was used for computing the alignment and the phylogenetic tree.
Polymerase chain reaction (PCR) was performed in a total volume of 20 µl containing 50 ng of genomic DNA, 10 pmole each forward and reverse primers, 200 µM dNTP, 1x PCR buffer, 1.5 mM $MgCl_2$ and 1.5 u Taq polymerase. PCR was accomplished by 1 min 94 °C, 1 min at primer annealing temperature, and 1 min at 72 °C for 30 cycles. The PCR products were separated on 1% agarose gel and were visualized by gel document. To estimate size of the PCR products, 100 bp DNA standard ladder was loaded in parallel with samples.

Results

In this study, twenty seven Thai swamp buffalo from nine district in Uttaradit province were selected for analysis of this relationship and genetic variation. Blood samples of individuals were collected and used for genomic DNA isolation.
In this study, the 1,200 bases of the mtDNA cytochrome b region of swamp buffalo were amplified using polymerase reaction. The reaction products were run on 1% agarose gel and each of them gave only one sharp band in the correct size (Figure 1). Sample from twenty seven swamp buffalo were sequenced in both directions (forward and reverse). The sequence of the fragment was corrected using Blast software. The multiple sequence alignment results between these twenty seven buffalo and the published results in GenBank database (Figure 2). Buffalo populations are generally most closely related to populations are geographically near.
This present study shows the utility of applying mitochondrial DNA cytochrome b gene for the analysis of diversity and genetic relationships of the Thai swamp buffalo in Uttaradit province and is the first report of genetic relationship of these populations in Uttaradit.
However, the results of the study in the Thai buffalo contribute the knowledge of genetic information of native Thai buffalo. Due to the crisis of a sharp decline in the number of the Thai swamp buffalo, our data will be useful for the further planning by the Department of Livestock Development, Thailand to establish an effective breeding program and conservation plan for this species.

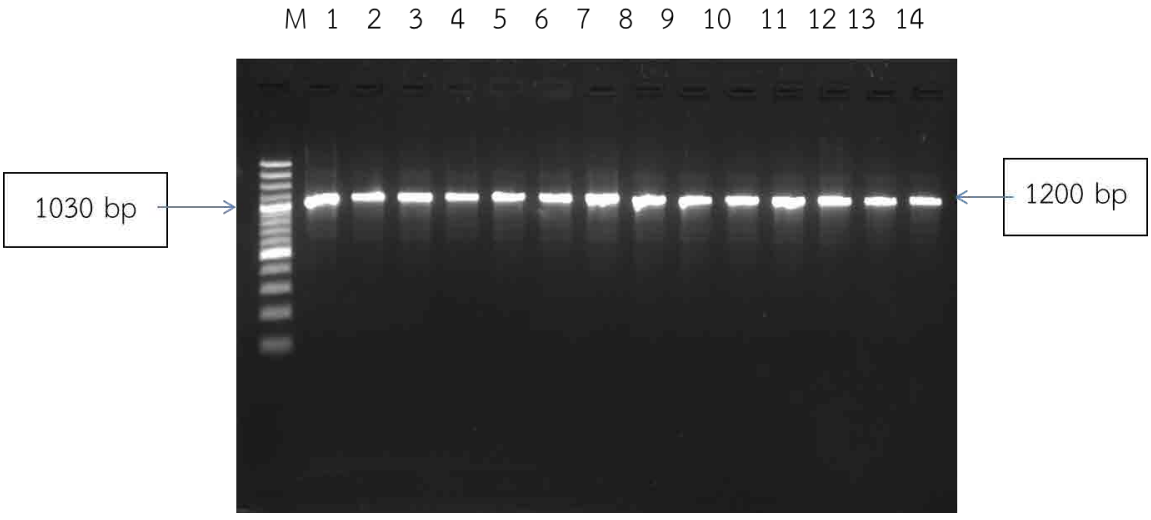

Figure 1 1,200 bp of cytochrome b genes are amplified by PCR and detect by 1.5% agarose gel electrophoresis. Lane M contains DNA marker and lane 1-14 are samples from buffalo.

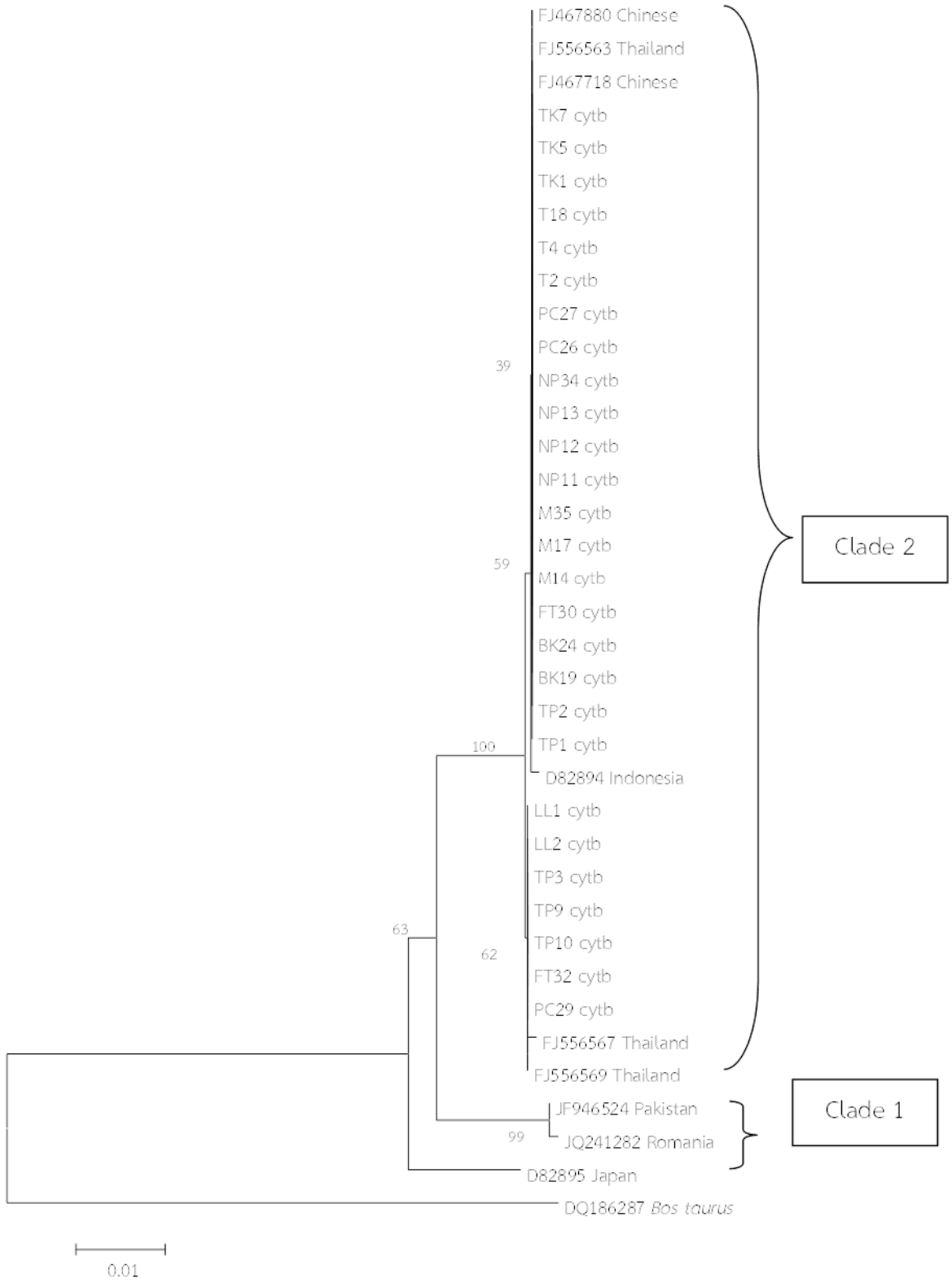

Figure 2 Neighbour-joining tree for cytochrome b of Thai native buffalo, with the bovine sequence as an outgroup. Numbers on the nodes are percentage bootstrap values from 1000 replications, and a scale bar for branch lengths is shown.

The neighbor-joining tree (Fig.2) shows two major clusters, one of clades found in swamp buffalo and one found in river buffalo.

Conclusion and Discussion

This phylogeny, together with the presence of only one clade in Uttaradit province, suggests that the species may have originated in this area of mainland south-east Asia, and subsequently spread north to China, and west to the Indian subcontinent, where the river type evolved. This suggested evolution of the river type from an ancestral swamp-like animal is further supported by the morphological similarity of the swamp type to the wild Asian buffalo (*Bubalus arnee*) (Barker *et al*., 1997). This research showed that swamp buffaloes in Uttaradit no have flourishing of genetic diversity. This study would contribute a comprehensive understanding of the genetic diversity of buffaloes in Uttaradit province and provide insightful knowledge of DNA application for biodiversity management in breeding improvement and buffalo conservation.

Keywords : Genetic diversity, Swamp buffalo, Uttaradit, Thailand

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References

Astorga, M. and R. Galleguillor. 1998. Genetic divergence of jack mackerel of genus Trachurus from northwestern and southeastern pacific. Rev. Bio. Mar. Oceanogr. 33:155-161.

Barker, J.S.F., S.S. Moore, D.J.S. Hetzel, D. Evans, S.G. Tan and K. Byrne. 1997. Genetic diversity of Asian water buffalo (Bubalus bubalis): microsatellite variation and comparison with protein coding loci. Anim. Genet. 28: 103-115.

Sambrook, J. and D.W. Russel. 2001. Molecular cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, New York.

Sangwan M.L. 2012. Analysis of genetic diversity of Indian buffalo breeds by DNA markers. Journal of buffalo science. 1: 91-101.

Sraphet, S., B. Moolmuang, A. Na-Chiangmai, S. Panyim, D.R. Smith and K. Triwitayakorn. 2008. Use of Cattle Microsatellite Markers to Assess Genetic Diversity of Thai Swamp Buffalo (*Bubalus bubalis*). Asian-Aust. J. Anim. Sci. Vol. 21, No. 2: 177 – 180.

Triwitayakorn, K., B. Moolmuang, S. Sraphet, S. Panyim, A. Na-Chiangmai1 and D. R. Smith. 2006. Analysis of Genetic Diversity of the Thai Swamp Buffalo *(Bubalus bubalis*) Using Cattle Microsatellite DNA Markers. Anim. Sci. Vol 19, No. 5: 617-621.

PO-03-4

Phenotypic Characterization of Native Chicken Ecotypes in Lower Northern, Thailand

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Introduction

Thai native chickens have been originated from four types of South East Asia jungle fowls. They are Red jungle fowls, Ceylon jungle fowls, Grey jungle fowls and Green jungle fowls (Sawai et al., 2010). Naturally, native chickens are not enclosure by housing but they are let free grazing around trees and the space under farmer's house. Thus, the native chicken development is depending much on the nature and their owner's life styles. Their death usually caused by natural disaster and infectious diseases but those stronger can survive to reproduce and increase number of their population. The process of natural selection in Thai native chickens continues until today. For this reason, raising the native chickens is categorized as one of the cultural heritages of local people and also one of variety biotechnologies that fit the living style of farmers in rural areas in Thailand. Living in harmony among native chickens, farmers are in multi-relationship and need to cooperate with each other. (H.R.H. Princess Maha Chakri Sirindhorn and H.I.H. Prince Akishinonomiya Fumihito, 2009). During period from 2005 to 2007, bird flu was spread all over the world and Phitsanulok was not an exception. The high mortality rate native chickens influenced strongly both of quantitative and qualitative characteristics of the native chickens (Duangjinda et al., 2012) as well as to income of farmers in the regions. The basic characters of certain breeds have been developed and might have been improved continuously. Biological diversity has been discovered afterwards. Those external factors could lead to the changes of native chickens characters, which exposed through phenotypes from older generation to new generation. All the information about phenotypic characterization of native chickens could be helpful to conservation, development of native chickens as well as planning and supporting to chicken farmers in the region. It will be more meaningful in future when especially ASEAN Economic Community is developing.

Materials and Methods

The study was conducted in Bang Rakam, Mueang, Wang Thong, Bang Krathum and Phrom Phiram Districts of Phitsanulok Province, Thailand. A total of 440 native chicken populations were collected from August 2013 to November 2015. The adult chickens were characterized under field conditions for phenotypic characterization traits following FAO standard descriptors (FAO, 2012). The phenotypic characters studied were classified into qualitative and quantitative traits; 7 qualitative traits namely comb type (Hin, Single, Wong-duan, Bae, Ghog-chaba, Pea, Strawberry, Cushion, and Walnut comb), plumage neck colour, plumage back colour were defined as golden-yellow, partridge, grey, cuckoo, red, white, black, and orange, long curving tails colour (partridge, grey, cuckoo, white, and black), back tails colour (partridge, grey, cuckoo, white, black, and orange), plumage wing colour (golden-yellow, partridge, dark green, grey, cuckoo, red, white, black, and orange) and shank colour (yellow, white, black, green, and grey) of native chicken, and 5 quantitative traits; body weight (kg), body height (cm), body length (cm), wing length (cm) and shank length (cm) of native chicken. Factor in this study was 5 districts ecotypes (Bang Rakam, Mueang, Wang Thong, Bang Krathum and Phrom Phiram) of Phitsanulok province.

The dataset was analysed in order to determine factors affecting body weight, body height, body length, wing length and shank length of native chicken. All considered factors (Bang Rakam, Mueang, Wang Thong, Bang Krathum and Phrom Phiram Districts) were tested for their effect on the variation of the studied traits using GLM procedures in SAS software (SAS, 2004). Least square means of the studied traits were estimated by the considering factors, and then were compared using a t-test in all model except for comb type, plumage neck colour, plumage back colour, long tails colour, back tails colour, plumage wing colour and shank colour of native chicken, which used a chi-square test, at an $a = 0.05$.

Results and discussion

Results of qualitative phenotypic characteristics of native chicken (comb type, plumage neck colour, plumage back colour, long curving tails colour, back tails colour, plumage wing colour and shank colour) showed significant differences among the 5 districts ecotypes ($p<0.0001$; Table 1). Native chickens in 5 districts ecotypes had the

highest Hin comb (58.17%) followed with Pea comb (8.66%), Wong-duan (8.17%), Ghog-chaba (6.68%), Strawberry (5.45%), single (4.46%), Bae (3.71%), Cushion (2.72%), and Walnut comb (1.98%). The shank colour had the highest yellow (39.94%) followed by white (39.04%), grey (10.81%), green (6.31%), and back (3.90%), respectively. These resulted were in agreement with the study of Punrapee et al. (2000a; 200b); Suphawadee (2014) and Nguyen Hoang Thinh et al. (2015) who found that Hin comb and yellow shank had the highest. Moreover, diversity of comb in male native chickens known by the popularity of location. There are 11 types of comb in Pichit Province such as Hin, Au, Ja or Jak, Bye Sree, Tum, Teo, Bae, Dhog-GonKai, Dhog-Ghaba, Wong-duan, and Nok-Takrum (Punrapee, et al., 2000a).

According to results of neck plumage colour, partridge was the highest (35.54%) followed by golden-yellow (18.22%), cuckoo (9.79%), black (9.34%), red (8.20%), orange (7.97%), grey (6.15%), and white colour was the lowest (4.78%). Similarly in back plumage colour, partridge took majority with 37.95%, followed by golden-yellow (17.73%), cuckoo (12.05%), red (11.14%), black (7.95%), grey (5.68%), orange (4.55%), and white colour (2.95%). Long curving tails colour had the highest partridge (39.24%) followed by black (17.73%), white (12.05%), cuckoo (5.20%), and grey colour (2.36%). However, black colour was the highest ratio for back tails colour with 40.43%, followed by partridge (34.21%), cuckoo (9.09%), white (7.89%), grey (6.22%), and orange colour (2.15%). Wing plumage colour had the highest partridge (28.77%) followed by black (15.75%), red (14.38%), golden-yellow (14.16%), cuckoo (10.96%), orange (5.94%), grey (5.48%), white (3.20%) and dark green colour (1.37%). From the results of qualitative phenotypic characteristics indicated neck plumage, back plumage, long curving tails, back tails, and wing plumage colour variations for the different at 5 districts ecotypes of native chicken. The results are in agreement with Punrapee, et al. (2000a); Suphawadee (2014) and Suphawadee et al. (2016) who reported that the original breeding becomes hard to find today since farmers are not interested in any pure breed. Free grazing chicken could lead to the variation of breeds and genes and diversification of hybrid chickens. However, it was contrast for the fighting cock raisers when they do not care much about the colour of features and other phenotypic characterizations than origination of their chickens.

According to the results of body weight, wing length and shank length of native chickens, there were significantly differences among chickens from different ecotypes ($p<0.0001$) except for height and length of body (Table 2). Phrom Phiram chickens showed the highest body weight (2.72 ± 0.06 kg), followed by chickens from Wang Thong (2.66 ± 0.06 kg), Bang Krathum (2.61 ± 0.06 kg), Mueang (2.39 ± 0.03 kg), and Bang Rakam (2.30 ± 0.04 kg). However, chickens from Bang Rakam had higher wing and shank length than those from Mueang (40.28 ± 0.35 vs 39.23 ± 0.31 cm and 9.07 ± 0.11 vs 8.75 ± 0.10 cm respectively). These values were similar to those from native chicken in Pichit Province (Punrapee, et al., 2000a, 2000b, 2000c). These results implied that in order to development of production, conservation and preservation of native chickens, farmers need to be promoted and supported from government and private organizations in sustainable manner of each ecotypes.

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Table 1 Qualitative phenotype characteristics of 5 native chicken ecotypes of Phitsanulok Province, Thailand

Phenotype characteristics	Districts					Total (%)
	Bang Rakam (%)	Mueang (%)	Wang Thong (%)	Bang Krathum (%)	Phrom Phiram (%)	
Comb type						
Hin	33.65	33.33	100	100	100	58.17
Single	13.46	2.67	-	-	-	4.46
Wong-duan	29.81	1.33	-	-	-	8.17
Bae	1.92	8.67	-	-	-	3.71
Ghog-chaba	21.15	3.33	-	-	-	6.68
Pea	-	23.33	-	-	-	8.66
Strawberry	-	14.67	-	-	-	5.45
Cushion	-	7.33	-	-	-	2.72
Walnut	-	5.33	-	-	-	1.98
Neck plumage colour						
Golden-yellow	15.09	18.03	8.00	46.00	8.00	18.22
Partridge	13.21	29.51	50.00	38.00	88.00	35.54
Grey	2.83	4.37	18.00	14.00	-	6.15
Cuckoo	3.77	14.75	22.00	2.00	-	9.79
Red	15.09	9.29	2.00	-	4.00	8.20
White	9.43	6.01	-	-	-	4.78
Black	30.19	4.92	-	-	-	9.34
Orange	10.38	13.11	-	-	-	7.97
Back plumage colour						
Golden-yellow	13.08	18.03	8.00	46.00	8.00	17.73
Partridge	17.76	32.79	50.00	38.00	88.00	37.95
Grey	1.87	3.83	18.00	14.00	-	5.68
Cuckoo	5.61	19.13	22.00	2.00	-	12.05
Red	23.36	11.48	2.00	-	4.00	11.14
White	6.54	3.28	-	-	-	2.95
Black	28.04	2.73	-	-	-	7.95
Orange	3.74	8.74	-	-	-	4.55
Long curving tails colour						
Partridge	69.52	30.41	-	-	85.42	39.24
Grey	0.95	5.26	-	-	-	2.36
Cuckoo	3.81	10.53	-	-	-	5.20
White	24.76	9.36	40.82	60.00	2.08	21.99
Black	0.95	44.44	59.18	40.00	12.50	31.21
Back tails colour						
Partridge	66.35	44.05	-	-	-	34.21
Grey	0.96	5.36	18.37	14.29	-	6.22
Cuckoo	8.65	10.71	22.45	-	-	9.09
White	16.35	8.33	-	2.04	2.08	7.89
Black	3.85	28.57	59.18	83.67	97.92	40.43
Orange	3.85	2.98	-	-	-	2.15
Wing plumage colour						
Golden-yellow	9.35	12.09	8.00	44.00	8.16	14.16

Partridge	27.10	15.38	50.00	2.00	87.76	28.77
Dark green	-	3.30	-	-	-	1.37
Grey	1.87	3.30	18.00	14.00	-	5.48
Cuckoo	5.61	16.48	22.00	2.00	-	10.96
Red	21.50	20.33	2.00	-	4.08	14.38
White	8.41	2.75	-	-	-	3.20
Black	21.50	14.84	-	38.00	-	15.75
Orange	4.67	11.54	-	-	-	5.94
shank colour						
Yellow	65.00	46.90	-	-	-	39.94
White	15.00	18.62	100	100	100	39.04
Black	1.00	8.28	-	-	-	3.90
Green	4.00	11.72	-	-	-	6.31
Grey	15.00	14.48	-	-	-	10.81

Table 2 Individual average body weight, body height, body length, wing length and shank length of native chicken ecotypes

Traits	**Districts**					**Sig**
	Bang Rakam	**Mueang**	**Wang Thong**	**Bang Krathum**	**Phrom Phiram**	
Body weight, kg	2.30±0.04[b]	2.39±0.03[b]	2.66±0.06[a]	2.61±0.06[a]	2.72±0.06[a]	0.0001
Body height,cm	54.59±0.63	54.76±0.57	-	-	-	0.8412
Body length,cm	21.56±0.23	21.44±0.21	-	-	-	0.6870
Wing length,cm	40.28±0.35[a]	39.23±0.31[b]	-	-	-	0.0247
Shank length,cm	9.07±0.11[a]	8.75±0.10[b]	-	-	-	0.0307

[a,b]Different superscripts within each row are significantly different (P<0.05)

References

Duangjinda M., K. Choprakarn, S. Suwanlee, P. Amnueysit and O. Thieme. (2012). Impact of the avian influenza outbreak on indigenous chicken genetic resources in Thailand. World's Poultry Science. 68: 503 512 p.

FAO. 2012. Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines No. 11. Rome. Italy.

H.R.H. Princess Maha Chakri Sirindhorn and H.I.H. Prince Akishino no miya Fumihito. 2009 .Chickens and Humans in Thailand: Their Multiple Relationships and Domestication. The Siam Society under Royal Patronage, Bangkok. 450 p.

Nguyen Hoang Thinh, Hoang Anh Tuan, Pham Kim Dang, and Bui Huu Doau. 2015. Multi-spurred chicken breed and livelihood of ethnic people in North Vietnam: characterization and prospects. Khon Kaen Agr. J. 43 suppl. 2: 25-30.

Punrapee Amnueysit, Komsan Amnueysit, and Sanit Toumsakul. 2000a. Diversity of Thai Cocks at Amphur Muang Pichit Province 1) Breeds of Cocks. Proceedings of 38th Kasetsart University Annual Conference. pp 282-291.

Punrapee Amnueysit, Komsan Amnueysit, Sanit Toumsakul, and Boontum Potikoung. 2000b. Diversity of Thai Cocks at Amphur Muang Pichit Province 2) Luang Group. Proceedings of 38th Kasetsart University Annual Conference. 292-300 pp.

Punrapee Amnueysit, Komsan Amnueysit, Sanit Toumsakul, and Boontum Potikoung. 2000c. Diversity of Thai Cocks at Amphur Muang Pichit Province 3) Pradee Group. Proceedings of 38th Kasetsart University Annual Conference. 301-309 pp.

SAS. 2004. SAS 9.13 Help and documentation. SAS Institute Inc., Cary, NC, USA

Sawai, H., H. L. Kim, K. Kuno, S. Suzuki, H. Gotoh, M. Takada, N. Takahata, Y. Satta, and F. Akishinonomiya. 2010. The origin and genetic variation of domestic chickens with special reference to jungle fowls Gallus g. gallus and G. varius. PLoS One. 5:e10639.

Suphawadee Yaemkong. 2014. Diversity of phenotypic characteristics of Thai Indigenous Chicken in Phitsanulok Province. Rajabhat Journal of Science, Humanities & Social Sciences. 15 (2): 63-73.

Suphawadee Yaemkong, Punnares Rattanapradit, Norakamol Laorodphan, Prapasiri Jaipong, Pathompong Buarapa, Watcharin Singchuang. (2016). Factor of Farmers Affecting on Phenotypic Characteristics of Native Chickens in Phitsanulok Province. Khon Kaen Agr. J. 44 suppl. 2: 890-898.

PO-03-9

Pre and Post Weaning Growth Performance of 50% Bore-Native and 50% Anglonubian-Native Crossbred Goat

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ABSTRACT

The objective of this study was to investigate the influence of parental genotypes on body weight traits using data from a crossbreeding experiment between the local goats and their crossbreds with the Anglo-Nubian and Boer goats from Australia and P.R. China. The 50% Anglo-Nubian x 50% Native (AN) and 50% Boer x 50% Native (BN) crossbred goat kids raised at Yala Livestock Breeding and Research Centre. Data was collected on 48 goats of different genotypes (AN and BN), lines (Australia and P.R. China) and sex (male and female) groups. The animal kept in a semi-intensive system with concentrated feed at 1% of body weight. The results revealed that the birth weight of AN was higher ($P < 0.05$) than BN but the body weight at 3, 6 and 9-month old did not significant ($P > 0.05$). While that compared between gender, AN of Australian male was higher body weight at 3 and 6-month old (13.92 and 19.17 kg, respectively). BN of Australian male was higher body weight at 9-month old (26.67 kg). For the study of body measurement (heart girth, body length and wither height) at 3 and 6-month old, AN of Australian line was higher than other groups (50.75, 51.25, 49.67 and 57.50, 57.46, 55.83 cm, respectively). While that at 9-month old BN was higher (66.17, 63.67, 62.75 cm). Male goats generally grow faster and are heavier with superior body conformational measurements than female goats.

INTRODUCTION

Goat population in Thailand more than 60 percent are fed in Southern part. They are predominantly raised by smallholders, particularly of the Muslim community. The goat breed is mainly a native goat that suitable environment but small size and low growth rate. The Department of Livestock Development imported Anglo-Nubian and Boer goats from People's Republic of China (PRC) and Australia in 1983 and 1996, respectively to improve growth rate, carcass quality, and production for the royal model farm project and establish Thai meat goat strain in the south. Boer goats will be ideal for providing meat. Have strong legs, good in finding food but yield a little milk. Anglo Nubian goats will be considered a dairy or dual-purpose breed. The body structure is big and tall. Native goats can live in the widespread of rain area and resistant parasites. This study was to investigate the influence of parental genotypes on body weight traits using data from a crossbreeding experiment between the native goats and their crossbreds with The 50% Anglo-Nubian x 50% Native (AN) and 50% Boer x 50% Native (BN) crossbred goat kids raised. In order to help farmers get better returns. Goat meat can sell for a higher price, to promote goat farming career and to support industry of halal food. This study is aim to research about Pre and Post Weaning Growth Performance of 50% Bore-Native and 50% Anglo-Nubian x Native crossbred goat to compare the sire from Australia and China for improving productivity native goat in southern part of Thailand.

MATERIALS AND METHODS

Animals and their management

Animal testing and management at Yala Livestock Breeding and Research Centre. Fourty-eight crossbred kids were used: 50% Boer x 50% Native crossbred kids (12 Males, 12 females) and 50% Anglo-Nubian x 50% Native crossbred kids (12 Males, 12 females) The experiment was randomized in blocks (RCBD) with sex blocks divided into four groups as follows.

Group 1: 50% Australian Bore x 50% Native crossbred kids;
Group 2: 50% Chinese Bore x 50%Native crossbred kids;
Group 3: 50% Australian Anglo-Nubian x 50% Native crossbred kids;
Group 4: 50% Chinese Anglo3Nubian x 50% Native crossbred kids;

The animals were given concentrate feed (18% crude protein) before weaning and (16% crude protein) after weaning 1% of body weight.

Data collection

Weighing of body weight and measuring body traits (heart girth, body length and wither height) at age of birth, 3, 6 and 9 months.

Statistical analysis

Data were analyzed using the General Linear Model procedures of SAS. Significant differences were further analysed using Duncan's new multiple range test and the least significant difference test for sensory evaluation data.

RESULTS AND DISCUSSION

Body weight at various ages

Table 1 The birth weight of Anglo-Nubian x Native crossbred goat of both Australian and Chinese were higher than Bore x Native crossbred goat (P<0.05). but the body weight at 3, 6 and 9-month old did not significant. While Thumrong and Team (2006) reported that Anglo-Nubian x Native crossbred goat weight at the age of 3, 6 and 9 months were 10.9, 13.9 and 18.1 kg respectively. Milton *et al*. (1991) reported that Anglo-Nubian x Thai Native crossbred goats weight at the age of birth, 3, 6 and 12 months were 2.0, 11.2, 16.1 and 26.7 kg respectively which have lower weight than this study.

Table 2. Comparative analysis between gender found that AN of Australian male was higher body weight at 3 and 6-month old. BN of Australian male was higher body weight at 9-month old. In accordance with the McGregor and Attwood (2002) reported that large goat such as Bore and Anglo-Nubian is growing faster than a small pedigree goat. Such as native goat normally, male kids had higher growth rate than female.

CONCLUSIONS

Anglo-Nubian x Native goat at birth until the age of 6 months has body weight and size ratio more than Bore x Native goat. Bore x Native goat will gain more weigh and has larger size at age of 9 months. And goat genotype from Australian had more growth rate than both in Anglo-Nubian and Boer. Male goat will larger than female in all ages and genotypes.

Table 1 Least square means (LSM) and standard errors (SE) for body weight stratified by genotypes and lines (kg)

Breed	Line	Birth weight	3-month weight	6-month weight	9-month weight
AN	Australia	2.31±0.10^{a}	12.25±0.72	17.58±0.72	23.04±0.89
AN	China	2.08±0.10^{a}	12.42±0.72	18.58±0.72	24.23±0.93
BN	Australia	1.76±0.10^{b}	11.45±0.72	18.12±0.72	26.00±0.89
BN	China	1.61±0.10^{b}	11.33±0.72	17.54±0.72	24.17±0.89

AN = 50% Anglo-Nubian x 50% Native; BN = 50% Boer x 50% Native;
$^{a, b}$ Means with different superscripts within column differ significantly.

Table 2 LSM and SE for body weight stratified by genotypes, lines and sex (kg)

Breed	Line	Sex	Birth weight	3-month weight	6-month weight	9-month weight
AN	Australia	F	2.42±0.14^{a}	10.58±0.97^{b}	16.00±0.97^{b}	21.42±1.24^{b}
AN	Australia	M	2.20±0.14^{a}	13.92±0.97^{a}	19.17±0.97^{a}	24.67±1.24^{ab}
AN	China	F	2.10±0.14^{a}	12.67±0.97^{ab}	18.17±0.97^{ab}	23.33±1.24^{ab}
AN	China	M	2.07±0.14^{a}	12.17±0.97^{ab}	19.00±0.97^{a}	25.30±1.24^{a}
BN	Australia	F	1.57±0.14^{b}	11.08±0.97^{b}	17.33±0.97^{ab}	25.53±1.24^{a}
BN	Australia	M	1.95±0.14^{a}	11.83±0.97^{ab}	18.92±0.97^{a}	26.67±1.24^{a}
BN	China	F	1.67±0.14^{b}	10.42±0.97^{b}	16.83±0.97^{ab}	23.83±1.24^{ab}
BN	China	M	1.55±0.14^{b}	12.25±0.97^{ab}	18.25±0.97^{ab}	24.50±1.24^{ab}

AN = 50% Anglo-Nubian x 50% Native; BN = 50% Boer x 50% Native; F = female; M = male;
$^{a, b}$ Means with different superscripts within column differ significantly.

Body Traits at various ages

For the study of body measurement at 3 and 6-month old, AN of Australian line was higher than other groups. While that at 9-month old BN was higher. Male goats generally grow faster and are heavier with superior body conformational measurements than female goats. However, should measure a mature goat. In general, a goat, a mature female, aged 37-48 months, while males age 24-36 months (Katongole et al, 1994).

Table 3 LSM ± SE for body measurement stratified by genotypes and lines (cm)

Traits	Breed	Line	Birth	3-month old	6-month old	9-month old
GIR	AN	Australia	29.33±0.10^{a}	50.75±1.22	57.50±1.32	61.83±1.31^{b}
GIR	AN	China	29.00±0.10^{a}	50.08±1.22	57.00±1.32	63.64±1.3^{ab}
GIR	BN	Australia	27.75±0.10^{b}	49.83±1.22	57.83±1.32	66.17±1.31^{a}
GIR	BN	China	27.25±0.10^{b}	47.67±1.22	57.67±1.32	67.75±1.31^{a}
STP	AN	Australia	29.38±0.58^{a}	51.25±1.06^{a}	57.46±1.46^{a}	61.58±1.29^{ab}
STP	AN	China	28.92±0.58^{ab}	49.17±1.06^{ab}	55.25±1.46^{a}	60.00±1.35^{b}
STP	BN	Australia	27.50±0.58^{ab}	47.33±1.06^{b}	54.00±1.46^{a}	63.67±1.29^{a}
STP	BN	China	27.17±0.58^{b}	46.42±1.06^{b}	55.00±1.46^{a}	63.29±1.29^{a}
WH	AN	Australia	27.83±0.48^{ab}	49.67±1.24^{a}	55.83±1.31	59.08±1.26^{b}
WH	AN	China	28.33±0.48^{a}	48.67±1.24^{a}	55.83±1.31	62.18±1.32^{ab}
WH	BN	Australia	26.58±0.48^{b}	44.33±1.24^{a}	52.50±1.31	62.75±1.26^{a}
WH	BN	China	27.42±0.48^{ab}	44.75±1.24^{b}	53.50±1.31	62.25±1.26^{ab}

GIR = heart girth; STP = body length (shoulder to pin); WH = wither height;
AN = 50% Anglo-Nubian x 50% Native; BN = 50% Boer x 50% Native;
$^{a, b}$ Means with different superscripts within column differ significantly.

Measuring body composition goat to compare between genders within each pedigrees, the body measuring has Heart girth Body length and Height are shown in Table 4.

Table 4 LSM ± SE for body measurement stratified by genotypes, lines and sex (cm)

Traits	Breed	Line	Sex	Birth	3-month old	6-month old	9-month old
GIR	AN	Australia	F	29.67±0.63^{a}	49.50±1.63^{b}	54.83±1.81^{b}	59.83±1.76^{b}
GIR	AN	Australia	M	29.00±0.63^{a}	52.50±1.63^{a}	60.17±1.81^{a}	63.83±1.76^{ab}
GIR	AN	China	F	29.00±0.63^{a}	50.50±1.63^{a}	56.33±1.81^{ab}	61.33±1.76^{b}
GIR	AN	China	M	29.67±0.63^{a}	49.67±1.63^{a}	57.67±1.81^{ab}	66.40±1.93^{a}
GIR	BN	Australia	F	27.00±0.63^{b}	49.50±1.63^{a}	58.17±1.81^{ab}	67.50±1.76^{a}
GIR	BN	Australia	M	28.50±0.63^{ab}	50.17±1.63^{a}	57.50±1.81^{ab}	64.83±1.76^{ab}
GIR	BN	China	F	27.50±0.63^{b}	44.50±1.63^{b}	55.67±1.81^{ab}	66.67±1.76^{a}
GIR	BN	China	M	27.00±0.63^{b}	50.83±1.63^{a}	59.67±1.81^{ab}	68.83±1.76^{a}
STP	AN	Australia	F	28.83±0.75^{a}	48.83±1.36^{b}	55.83±1.63^{ab}	60.67±1.82^{ab}
STP	AN	Australia	M	29.92±0.75^{a}	53.67±1.36^{a}	59.08±1.63^{a}	62.50±1.82^{ab}
STP	AN	China	F	28.83±0.75^{a}	49.33±1.36^{b}	53.67±1.63^{b}	57.83±1.82^{b}
STP	AN	China	M	29.00±0.75^{a}	49.00±1.36^{b}	56.83±1.63^{ab}	62.60±1.82^{ab}
STP	BN	Australia	F	25.83±0.75^{b}	47.67±1.36^{bc}	53.33±1.63^{b}	64.67±1.82^{a}
STP	BN	Australia	M	29.17±0.75^{a}	47.00±1.36^{bc}	54.67±1.63^{ab}	62.67±1.82^{ab}
STP	BN	China	F	27.83±0.75^{ab}	43.83±1.36^{c}	53.33±1.63^{b}	63.17±1.82^{a}
STP	BN	China	M	26.50±0.75^{b}	49.00±1.36^{b}	56.67±1.63^{ab}	64.67±1.82^{a}
WH	AN	Australia	F	27.17±0.69^{ab}	47.67±1.60^{a}	55.17±1.81^{ab}	57.83±1.72^{b}
WH	AN	Australia	M	28.50±0.69^{a}	51.67±1.60^{a}	56.50±1.81^{a}	60.33±1.72^{ab}
WH	AN	China	F	28.33±0.69^{a}	48.67±1.60^{a}	55.67±1.81^{ab}	60.83±1.72^{ab}
WH	AN	China	M	28.33±0.69^{a}	48.67±1.60^{a}	56.00±1.81^{a}	63.80±1.88^{a}
WH	BN	Australia	F	26.00±0.69^{b}	45.50±1.60^{b}	52.50±1.81^{ab}	64.17±1.72^{a}
WH	BN	Australia	M	27.17±0.69^{ab}	43.17±1.60^{b}	52.50±1.81^{ab}	61.33±1.72^{ab}
WH	BN	China	F	27.67±0.69^{ab}	41.50±1.60^{b}	50.50±1.81^{b}	60.17±1.72^{ab}
WH	BN	China	M	27.17±0.69^{ab}	48.00±1.60^{a}	56.67±1.81^{a}	64.83±1.72^{a}

$^{a, b, c}$ Means with different superscripts within column differ significantly.

Keywords: Growth Performance, Body measurement, Meat Goat, 50% Crossbred

REFERENCES

Thongchumroon, T., T. Thommalee, S. Dechaphan and S. Kochapakdee. 2546. Post-weaning growth of Thai native, Anglo-Nubian and Thai native x Anglo-Nubian crossbred goat raised at Yala Livestock Breeding and Research Center. Action Plan Research and development of small animal,2546. Department of livestock development. pp. 127-133

Katongole, J.B.D., B. Sebolai and M. J. Madimabe . 1994. Morphological characterisation of the Tswana goat. *In* Proceedings of the Third Biennial Conference of the African Small Ruminant Research Network. UICC, Kampala, Uganda 5-9 December 1994

McGregor, B. and M. Attwood. 2002. Meat and offal yields of goats. http://www.dpi.vic.gov.au

Milton, J.T.B., S. Saithanoo. and P. Praditrungwatana. 1991. Goat management in the Asian humid tropics. In: Goat Production in the Asian Humid Tropics. S. Saithanoo and B.W. Norton (Eds.). *Proc. of a Seminar in Hat Yai, Thailand*, ISBN 974-605-062-1.

PO-03-10

Genetic influence of HNF4 α gene SNP on the growth performance in Korean native chickens.

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Introduction

The study about genes involved in chicken lipid metabolism and growth could provide ways correlated genomic selection in poultry breeding using molecular markers. The HNF4 α (hepatocyte nuclear factor 4, alpha) is a transcription factor related to the lipid and insulin metabolism in humans (Hatzis and Talianidis, 2001; Mosialou et al., 2010; Yin et al., 2010; Hayhurst et al., 2001). Navas et al. (1999) described that some mutations in the human *HNF4 α* gene lead to diabetes. The *HNF4a* gene encoded this factor, is mapped on chromosome 15 composed of 10 exons. It is possible candidate gene for fat deposition and improvement of growth in chicken. The A543G single nucleotide polymorphism (SNP) in *HNF4 α* gene was also known that it was associated with broiler bone traits and body weight traits (Silva et al., 2012). The objective of this study was to investigate the association between the SNP in *HNF4 α* gene and growth trait for improving the performance in Korean native chickens (KNCs).

Methodology

A total of 764 DNA samples of KNCs were collected from the livestock farm of Gyeongnam National University of Science and Technology in Korea. The body weight measured from birth to 40 weeks of age for the analysis. The PCR-RFLF method was designed to genotype the *HNF4 α* gene in chickens. The amplified fragment had 752 bp located between 11483-12235 bp of the gene. The information of primer set and annealing temperature for polymerase chain reaction (PCR) was shown in Table 1. After treatment of restriction enzyme (*Xho I*) to the products, three different fragment patterns (AA, AG, GG) were observed and analyzed the association between these genotypes and growth traits in KNCs.

Result and conclusion

The frequencies of three genotypes were 0.47 (AA), 0.43 (AG) and 0.10 (GG), respectively (Table 2). The SNP of *HNF4 α* was highly significantly ($p<0.001$) associated with all-round growth in KNCs (Table 3). The HNF4a gene was chosen as a candidate gene by its relation to the transactivation involved in the lipid transport and metabolism in human (Yin et al., 2010; Hayhurst et al., 2001). In current study, Silva et al. (2012) reported that the A543G within *HNF4a* gene has an additive effect on the carcass traits (abdominal fat, wings yield and weight sticks yield) in broilers. KNC strain is domestic animal in Korea bred for dual purpose and their genome has the difference in other commercial breeds. The results from the present suggest a potential of the A543G SNP within *HNF4 α* gene for marker-assisted selection in KNCs.

Table 1. Sequences of primer set and annealing temperature for the PCR.

	Primer sequences	Annealing Tm (℃)
Forward	5'-ATT GCC CAG GCC TTC ATA AGG GTA-3'	60
Reverse	5'-AAA TAG AGA CTC GTC ACG GGT GCA-3'	

Table 2. Allele and genotype frequencies for the A543G SNP within *HNF4α* gene in KNC.

	Allele frequency		Genotype frequency		
A543G	A	G	AA	AG	GG
	0.68	0.32	0.47	0.43	0.1

Table 3. Association of the A543G SNP within *HNF4α* gene with body weight in KNC.

p-value	**Birth weight**	**BW 2**	**BW 4**	**BW 6**
AA	45.29 ± 0.23	193.59 ± 1.39	447.9 ± 3.00	787.96 ± 6.14
AG	43.41 ± 0.27	169.68 ± 1.14	389.38 ± 2.91	674.72 ± 5.87
GG	40.47 ± 0.45	131.9 ± 1.5	282.41 ± 3.39	484.79 ± 5.05
p-value	<0.001	<0.001	<0.001	<0.001
	BW 8	**BW 10**	**BW 12**	**BW 14**
AA	1087.27 ± 8.37	1385.11 ± 9.97	1780.73 ± 12.82	2007.69 ± 15.33
AG	930.45 ± 7.93	1218.82 ± 10.00	1539.66 ± 11.62	1741.01 ± 13.13
GG	672.3 ± 7.22	928.38 ± 9.56	1151.95 ± 11.98	1284.3 ± 13.12
p-value	<0.001	<0.001	<0.001	<0.001
	BW16	**BW 18**	**BW 20**	**BW 24**
AA	2166.06 ± 16.96	2352.37 ± 18.28	2522.85 ± 20.91	2888.78 ± 22.2
AG	1915.35 ± 16.00	2058.38 ± 16.77	2172.28 ± 18.61	2437.82 ± 17.81
GG	1434.38 ± 14.68	1537.71 ± 16.72	1590.1 ± 20.17	1890.43 ± 23.55
p-value	<0.001	<0.001	<0.001	<0.001
	BW 28	**BW 32**	**BW 36**	**BW 40**
AA	2941.59 ± 21.46	2980.89 ± 23.27	2955.3 ± 26.59	3315.12 ± 32.42
AG	2475.25 ± 15.85	2499.9 ± 16.73	2496.68 ± 17.72	2751.04 ± 21.92
GG	2007.59 ± 23.18	1966.06 ± 33.23	1960.31 ± 23.98	2142.57 ± 22.01
p-value	<0.001	<0.001	<0.001	<0.001

[1)]BW means body weight at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, 32, 36, and 40 weeks of the age.

References

1. Hatzis, P., and Talianidis, I. (2001). Regulatory mechanisms controlling human hepatocyte nuclear factor 4α gene expression. Molecular and Cellular Biology, 21(21), 7320-7330.

2. Hayhurst, G. P., Lee, Y. H., Lambert, G., Ward, J. M., & Gonzalez, F. J. (2001). Hepatocyte nuclear factor 4α (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Molecular and cellular biology, 21(4), 1393-1403.

3. Mosialou, I., Zannis, V. I., and Kardassis, D. (2010). Regulation of human apolipoprotein m gene expression by orphan and ligand-dependent nuclear receptors. Journal of Biological Chemistry, 285(40), 30719-30730.

4. Silva, V. H., Pandolfi, J. R. C., Godoy, T. F., Peixoto, J. O., Tessmann, A. L., and Ledur, M. C. (2012). A SNP in the hepatocyte nuclear factor 4 gene associated with carcass traits in males from a paternal broiler line. World´s Poultry Science Journal, Supplement 1, 51-53.

5. Yin, L., Ma, H., Ge, X., Edwards, P. A., and Zhang, Y. (2011). Hepatic hepatocyte nuclear factor 4α is essential for maintaining triglyceride and cholesterol homeostasis. Arteriosclerosis, thrombosis, and vascular biology, 31(2), 328-336.

PO-03-11 Genetic variations in the Ovocalyxin-32 gene are associated with egg production traits in Korean native chickens

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Introduction

The Ovocalyxin-32 (*OCX-32*) is present at high levels in the uterine fluid during the final phase of eggshell formation. The OCX-32 gene (GeneBank accession no. NM_204535) located on thehromosome 9 was identified as a potential candidate gene influencing eggshell traits (Takahashi et al., 2009). Recently, single nucleotide polymorphism (SNP) of *OCX-32* gene has been reported that association with egg production traits (shell color, albumen height, early egg weight, puncture score and yolk weight) in poultry (Fulton et al.,2012). Therefore, this study was performed to find novel SNPs and to analyze the association between genetic variations of *OCX-32* gene and egg production traits in the four Korean native chicken (KNC) breeds (Ogol, black, gray, and white).

Methodology

The genomic DNA samples were extracted from bloods of KNC populations (n = 120) offered from National Institute of Animal Science in Korea. The twenty-one variations (sixteen SNPs and five INDELs) in the intron 1 of *OCX-32* gene were analyzed using sequencing method. The primer set (forward primer: 5' TGTTTCTGATGAAGAGCCAGA3', reverse primer: 5' CTTTGCCACTCTGTAGGCTGT3') were designed for condition of polymerase chain reaction (PCR). After PCR, the products were performed sequencing using BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA) and ABI PRISM 3730XL Analyzer (Applied Biosystems, CA). Statistical analysis of variance was performed by using the SAS Ver. 9.2 Package with PROC GLM model.

Result and conclusion

A total of 747 SNPs within this gene are reported in the NCBI SNP database. In the intron region performed sequencing in *OCX-32* gene, the 21 SNPs have been discovered. Of these 21 SNPs, the 9 variants (1179T>C, 1183A>G, 1337A>G, 1347A>T, 1500A>C, 913delA, insCTT, insTTC, 1360delT) were novel (Fig 1).

The genotype frequencies of new SNPs and INDELs in 4 strains of KNC shown in Tables 1 and 2, respectively. The results of association analysis between 5 novel SNPs within *OCX-32* gene and egg production traits are shown in the Table 3 and the effects of 4 INDELs newly discovered on performances in KNCs are shown in Table 4. The association study revealed strain-specific significant effect on ratio of egg production (EPR), egg weight (EW) and day at the first laying (AFL).

Five variations newly identified (1179T>A, 1183A>G, 1347A>T, 1500A>C, insTTC) showed the significant association ($p<0.05$) with egg production traits (egg production ratio, age at first egg, egg weight) in KNCs.

Numerous reports in this gene suggest that the *OCX-32* gene is potential gene for molecular marker associated with egg production and so can be used for breed selection in laying chickens (Yang et al., 2007; Uemoto et al., 2009; Takahashi et al., 2009; Lee et al., 2014). Fulton et al. (2012) reported 29 polymorphic sites within exons 2-6 of the *OCX-32* gene and their associations with shell color, albumen height, early egg weight, puncture score and yolk weight in commercially utilized lines (White Leghorn, White Plymouth Rock and Rhode Island Red). Lee et al. (2014) suggested that two SNPs (c. 494A>G and c.267T>G) located intron 5 have an effects on the egg production traits in KNCs.These results suggest that variations in *OCX-32* gene, newly discovered in present study, may be useful as genetic markers for egg production traits in the Korean native chicken breeding.

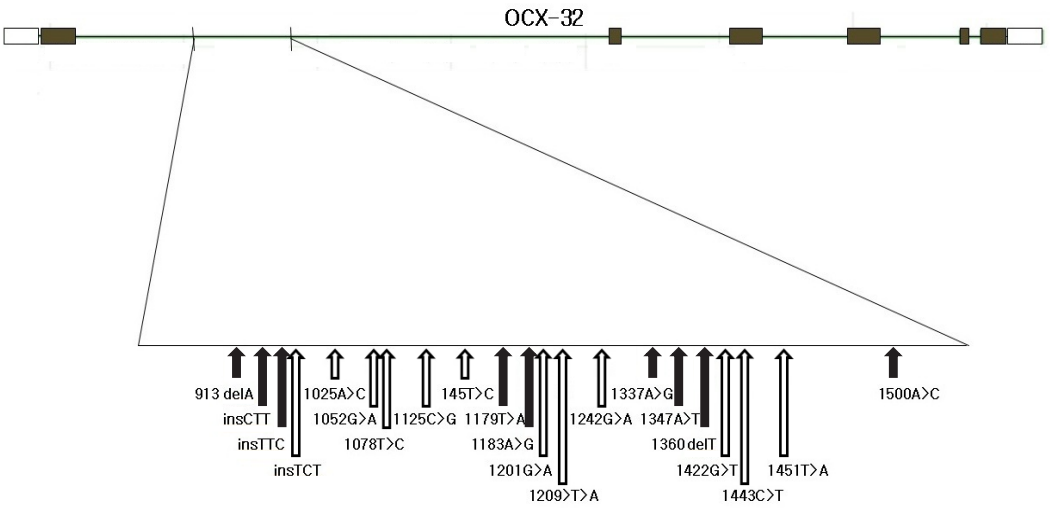

Fig. 1. Identification of sequence polymorphism in *OCX-32* (GeneBank accession no. NM_204535). Coding exons are marked by shaded blocks and 5` and 3` UTR by white blocks. The positons indicated at black arrow is Novel variations.

Table 1. Genotype frequencies of novel SNPs on OCX-32 gene in Korean native chickens

SNP	genotype	strain			
		KOC[1]	KBC[2]	KGC[3]	KWC[4]
1179T>A	TT	0.50	0.52	0	0
	TA	0	0.04	0	0
	AA	0.50	0.44	1.00	1.00
1183A>G	AA	0.61	0.67	0	0
	GG	0.39	0.33	1.00	1.00
1337A>G	AA	1.00	0.81	1.00	1.00
	AG	0	0.04	0	0
	GG	0	0.15	0	0
1347A>T	AA	0.89	0.96	1.00	1.00
	TT	0.11	0.04	0	0
1500A>C	AA	0.90	0.90	1.00	1.00
	AC	0	0.10	0	0
	CC	0.10	0	0	0

[1]KOC = Korean Ogol chicken [2]KBC = Korean black chicken

[3]KGC = Korean gray chicken [4]KWC = Korean white chicken

Table 2. Genotype frequencies of novel INDELs on OCX-32 gene in Korean native chickens

SNP	genotype	strain			
		KOC[1]	KBC[2]	KGC[3]	KWC[4]
913 del A	no del	0.63	0.49	0	0
	del A	0.37	0.51	1.00	1.00
ins CTT	no ins	0.89	0.96	0.90	0.70
	+CTT	0.11	0.04	0.10	0.30
ins TTC	no ins	0.89	0.89	1.00	0.70
	+TTC	0.11	0.11	0	0.30
1360 del T	no del	0.50	0.69	0	0
	del T	0.50	0.31	1.00	1.00

[1]KOC = Korean Ogol chicken [2]KBC = Korean black chicken

[3]KGC = Korean gray chicken [4]KWC = Korean white chicken

Table 3. Association between novel SNPs of *OCX-32* gene and egg production traits in Korean native chickens

		Strain											
		KOC[1]			KBC[2]			KGC[3]			KWC[4]		
SNP	geno type	EPR[5] (%)	AFE[6] (days)	EW[7] (g)	EPR (%)	AFE (days)	EW (g)	EPR (%)	AFE (days)	EW (g)	EPR (%)	AFE (days)	EW (g)
1179 T>A	TT	72.4±1.8[a]	152.0±2.9	53.4±0.6[a]	65.8±2.6	135.9±2.0	54.7±0.6	-	-	-	-	-	-
	TA	-	-	-	78.0	143.0	58.0	-	-	-	-	-	-
	AA	81.1±2.6[b]	145.9±4.0	50.4±1.3[b]	65.8±2.6	135.9±3.1	57.1±1.2	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
1183 A>G	AA	72.4±1.8[a]	152.0±2.9	53.4±0.6[a]	64.7±2.5	136.1±1.7	55.0±0.6	-	-	-	-	-	-
	GG	81.1±2.6[b]	145.9±4.0	50.4±1.3[b]	72.2±5.2	136.2±4.0	57.2±1.4	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
1337 A>G	AA	74.8±1.7	149.8±2.2	52.5±0.7	64.7±2.5	136.1±1.7	55.0±0.6	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
	AG	-	-	-	73.0	133.0	60.2	-	-	-	-	-	-
	GG	-	-	-	64.7±2.5	136.6±4.5	56.7±1.5	-	-	-	-	-	-
1347 A>T	AA	77.5±1.5[a]	148.2±2.4	52.0±0.7	67.5±2.5	136.4±1.7	55.7±0.7	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
	TT	61.6±3.7[b]	161.6±3.7	54.1±1.2	59.6	129.0	57.0	-	-	-	-	-	-
1500 A>C	AA	76.5±1.6[a]	148.1±2.2[a]	52.1±0.7	68.0±2.4[a]	136.2±1.8	55.8±0.6	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
	AC	-	-	-	52.2±15.3[b]	138.3±2.6	55.4±2.7	-	-	-	-	-	-
	CC	58.2±2.8[b]	169.5±0.5[b]	54.7±1.9	-	-	-	-	-	-	-	-	-

[1]KOC = Korean Ogol chicken [2]KBC = Korean black chicken [3]KGC = Korean gray chicken [4]KWC = Korean white chicken

[5]EPR = egg production ratio [6]AFE = age at first egg [7]EW = egg weight

[abc]means within a row with no common superscript differ significantly ($p<0.05$).

Table 4. Association between novel INDELs of *OCX-32* gene and egg production traits in Korean native chickens

SNP	geno type	Strain											
		KOC[1]			KBC[2]			KGC[3]			KWC[4]		
		EPR[5] (%)	AFE[6] (days)	EW[7] (g)	EPR (%)	AFE (days)	EW (g)	EPR (%)	AFE (days)	EW (g)	EPR (%)	AFE (days)	EW (g)
913 del A	no del	74.3±1.5	149.7±2.7	53.2±0.7	65.8±2.4	136.2±1.8	55.0±0.6	-	-	-	-	-	-
	del A	75.4±3.2	150.0±3.6	51.7±1.1	72.2±3.6	136.2±2.8	57.2±0.9	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6
ins CTT	no ins	74.85±1.8	150.8±2.5	52.61±0.6	66.5±2.4	136.0±1.8	55.9±0.6	50.0	154.0	46.0	64.4±4.7	154.4±2.1	49.4±0.6
	+CTT	75.2±8.4	143.7±5.3	50.9±3.0	86.3	139.0	52.6	64.4±3.1	147.9±4.0	48.7±0.7	-	-	-
ins TTC	no ins	75.8±1.9	151.0±2.5	52.3±0.7	65.8±2.6	136.8±1.8	55.8±0.6	62.9±2.9	148.5±3.6	48.4±0.6	68.2±5.6	159.1±1.9[a]	49.6±0.6
	+TTC	76.1±2.2	138.0±3.5	51.3±2.4	78.2±1.0	130.7±4.7	55.9±2.8	-	-	-	55.6±8.0	143.3±3.4[b]	48.6±0.8
1360 del T	no del	74.3±1.4	149.7±2.7	53.2±0.7	65.8±2.4	136.2±1.8	55.0±0.6	-	-	-	-	-	-
	del T	75.4±3.2	150.0±3.6	51.7±1.1	72.2±3.6	136.2±2.8	57.2±0.9	62.9±2.9	148.5±3.6	48.4±0.6	64.4±4.7	154.4±2.1	49.4±0.6

[1]KOC = Korean Ogol chicken [2]KBC = Korean black chicken [3]KGC = Korean gray chicken [4]KWC = Korean white chicken

[5]EPR = egg production ratio [6]AFE = age at first egg [7]EW = egg weight

[ab]Means within a row with no common superscript differ significantly ($p<0.05$).

References

1. Fulton JE, Soller M, Lund R, Arango J, Lipkin E 2012 Variation in the ovocalyxin-32 gene in commercial egg-laying chickens and its relationship with egg production and egg quality traits. Anlm Genet 43:102-113.
2. Lee JY, Choi SY, Kim CD, Hong YH, Jeong DK, Lee SJ 2014 Effects of c.494A>C and c.267T>G SNPs in *OCX-32* Gene of Korean Native Chicken on Egg Production Traits. PoultSci 41:191-196.
3. Takahashi H, Yang D, Sasaki O, Furukawa T, Nirasawa K 2009 Mapping of quantitative trait loci affecting eggshell quality on chromosome 9 in an F2 intercross between two chicken lines divergently selected for eggshell strength. Anim Genet 40:779-782.
4. Uemoto Y, Suzuki C, Sato S, Sato S, Ohtake T, Sasaki O, Takahashi H, Kobayashi E 2009 Polymorphism of the ovocalyxin-32 gene and its association with egg production traits in the chicken. Poultry Sci 88:2512-2517.
5. Yang KT, Lin CY, Liou JS, Fan YH, Chiou SH, Huang CW, Wu CP, Lin EC, Chen CF, Lee YP, Lee WC, Ding ST, Cheng WTK, Huang MC 2007 Differentially expressed transcripts in shell glands from low and high egg production strains of chickens using cDNA microarrays. AnimReprodSci 101:113-124.

PO-03-15

Effects of Napier grass and Thapra Stylo silages with different levels of concentrate on milk production of dairy cows

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The effect of Napier grass mixed with Thapra stylo silages (50:50, dry matter basis) with 2 levels (6 and 10 kg/h/d) of concentrate feeding on feed intake, digestibility, milk production and milk composition were determined in 10 dairy cows in randomized complete block design (RCBD). Cows were allotted at random to receive *ad libitum* of Napier grass -Thapra stylo silage with different concentrate levels: 1) Napier grass -Thapra stylo silage + 6 of concentrate and 2) Napier grass- Thapra stylo silage + 10 of concentrate.

Total silage intake was not different among silages. Total voluntary feed intake increased (P< 0.05) in cow fed with higher concentrate feeding. The dry matter, crude protein, acid detergent fiber and neutral detergent fiber digestibility and were not significant different in cow fed with different concentrate levels. Milk production and milk composition were similar at the two levels of concentrate feeding.

Introduction

Ensiling is well-known method to preserve the moist crops by controlling anaerobic fermentation (Yahaya et al., 2004). The success of the ensiling can be achieved when the number of lactic acid bacteria (LAB) dominates and activity of clostridia restricts during the fermentation (Bureenok et al., 2005). The tropical silages are poorly fermentation due to the high content of fiber fraction and low amount of water soluble carbohydrates (WSC), leading to increase buffering capacity of silages. Moreover, the low quality of tropical silages is often caused by the low nutritive value of the herbage ensiled. In the tropical countries, the grass silage has low quality of CP content (at approximately 3-5%), while the legume silage has high CP content (10-15%). The one way for higher quality grass silage in tropical areas is ensiled with legumes (Tjandraatmadja et al., 1994). The combinations between grasses and legumes have been reported to improve feed intake and nutrient digestion (McDonald, 1991). There is limited research of feeding cows with grass-legume mixed silages in this area. This study was aimed to study the effect of Napier grass (*Pennisetum purpureum*) and Thapra stylo (*Stylosanthes guianensis* CIAT184) silages with different levels of concentrates on voluntary feed intake, nutrient digestibly and milk production in dairy cows.

Materials and Methods

Silage preparation

FJLB was prepared from Napier grass (*Pennisetum purpureum*) and Thapra stylo (*Stylosanthes guianensis CIAT 184*) before making silage (Bureenok et al., 2005). Napier grass and Thapra stylo were harvested at 45 and 60 d after planting, respectively, and chopped into 2- to 3-cm lengths. The chopped crops were mixed and treated with 1% FJLB (FJLB) which prepared from these crops, then packed tightly in 100-kg plastic drums and stored at room temperature (27–30°C) until feeding experiment start.

Animals, Feeding

Ten Holstein Friesian crossbred cows were individually housed in metabolic cages. The cows were randomly allocated in a RCBD to receive 1 of 2 *ad libitum* diets: T1) Napier grass -Thapra stylo silage + 6 of concentrate and T2) Napier grass- Thapra stylo silage + 10 of concentrate. The 74-d experimental period consisted of a 14 d of adaptation period and 60 d of sampling. Feed was offered twice daily at 08:00 and 15:00 h, and the refused portions were weighed daily before the morning feeding. BW was measured before the morning feeding at the beginning and end of each experimental period. The daily dry matter (DM) intake per unit of metabolic BW was calculated with the mean value of initial BW and final BW of each period. During the last 5 d of each period, the feces samples were collected daily for each cow in the morning before feeding.

Chemical Analyses

Silage samples from the center of each plastic drum in each treatment were collected. Subsamples (50 g) were macerated with 150 ml of distilled water and stored in a refrigerator at 4°C for 12 h. The extract was filtered

using No.5 filter paper (Whatman, England). The pH of silage was determined with a pH meter (Lab 860, Schott). The DM content of the silages and feces were determined by oven drying at 70°C for 48 h. The dried sample was milled to pass through a 1.0 mm sieve. The nitrogen was determined by the Kjeldahl procedure (AOAC, 1995). The NDF and ADF concentrations were determined by methods described by Van Soest et al. (1991). Diet and fecal samples were analyzed for acid-insoluble ash (AIA) using a modification of procedure by Van Keulen and Young (1977). Digestibility by AIA was calculated as the ratio of acid-insoluble ash in feed and feces.

After milking for both a.m. and p.m., milk samples were analyzed for fat using the Gerber method. The Kjeldahl method for nitrogen analysis in milk protein (6.38×N), solids not fat was analyzed according to AOAC method (1995).

Statistical analyses

Statistical analyses were performed using thegeneral linear models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). All data were analyzed using the procedures of SAS for a RCBD.

Results and Discussions

The chemical composition of silage and concentrate was shown in Table 1. The pH of Napier grass silage in this experimental were lower than Thapra stylo silages and in the range of the normally pH (around 3.8-4.2) (McDonald et al., 1991). The chemical analysis of silage confirms that the value of legumes as a source of protein, with higher than in the grass silages. The total dry matter intake ($BW^{0.75}$) was the highest ($P< 0.05$) in cow fed with T2 (Table 2). The CP, NDF and ADF digestibility was not significantly different in cow fed with these diets (Table 3). Dewhurst et al. (2003) also found that feeding of grass-legume silages with 4 and 8 kg of concentrate were not different in nutrient digestibility.

Milk production in cow was not significantly different among diets (Table 4). Milk production and milk composition were similar at the two levels of concentrate feeding. This may cause by the dairy cows in this experiment were mid-lactation that the milk composition were not significantly different (Abd El-Razek et al., 1982).

Keywords: Napier grass, Thapra stylo legumes, silage, nutrients digestibility, milk production

Table 1. Chemical composition of silages and concentrate.

	Napier	Thapra	Concentrate
pH	4.09	4.51	-
DM (%)	33.75	33.57	91.25
CP (%DM)	5.31	11.71	18.33
NDF (%DM)	75.78	67.78	21.74
ADF (%DM)	56.24	50.63	13.46
Ash (%DM)	8.94	12.40	8.30

DM = dry matter, EE = ether extract, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 2. Effect of silages and concentrate level on voluntary feed intake (VFI)

	T1		T2		SEM	P - value
Silage Intake						
%Body weight (BW)	1.11		1.10		0.07	0.9104
g/kgBW$^{0.75}$	54.44		52.92		2.86	0.7971
Concentrate Intake						
%Body weight (BW)	0.96	b	1.70	a	0.10	0.0053
g/kgBW$^{0.75}$	46.86	b	82.00	a	3.80	0.0017
Total Intake						
kg/day	12.01	b	15.28	a	0.19	<.0001
%Body weight (BW)	2.07	b	2.80	a	0.14	0.027
g/kgBW$^{0.75}$	101.30	b	134.92	a	4.95	0.0094

T1 = Concentrate feeding level at 6 kg/h/d; T2 = Concentrate feeding level 10 kg/h/d.
Values in the same row followed by different letters are significantly different ($p<0.05$).
SI = silage intake, DM = dry matter, BW = body weight, BW$^{0.75}$ = metabolic body weight VFI = voluntary feed intake.

Table 3. Effects of silages and concentrate level on digestibility (%)

	T1	T2	SEM	P-value
Dry matter	72.41	79.80	2.19	0.1301
Organic matter	74.24	81.40	2.47	0.1017
Crude protein	72.34	80.76	2.67	0.0983
Acid detergent fiber	58.86	63.82	3.90	0.6205
Neutral detergent fiber	65.37	68.31	3.15	0.6536

T1 = Concentrate feeding level at 6 kg/h/d; T2 = Concentrate feeding level 10 kg/h/d.

Table 4. Effects of silages and concentrate level on milk production and milk compositions

	T1	T2	SEM	P-value
Milk yield (kg/d)	10.13	9.76	0.89	0.8476
4%FCM (kg/d)	8.95	9.31	0.62	0.7920
Milk fat (%)	3.25	3.88	0.22	0.2087
Milk protein (%)	2.86	3.20	0.12	0.2227
Solid not fat (%)	7.53	8.03	0.19	0.2586
Total solid (%)	10.78	11.91	0.36	0.1795

T1 = Concentrate feeding level at 6 kg/h/d; T2 = Concentrate feeding level 10 kg/h/d.
FCM = fat corrected milk, 4% FCM = kg. Milk * (0.4 + (0.15*%fat)

References

Abd El-Razek, A.B., A. Hassan, M.H. Salem and B. Badawy. 1982. Effect of subtropical climate on the lactational performance of Holstein cows at different stage of lactation. Ind. J. Dairy Sci. 35: 285-290.

AOAC, 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington. VA, USA.

Bureenok, S., T. Namihira, M. Tamaki, S. Mizumachi, Y. Kawamoto and T. Nakada. 2005. Fermentative quality of guineagrass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive. Asian-Aust. J. Anim. Sci. 18: 807-811.

Dewhurst, R.J., W.J. Fisher, J.K.S. Tweed and R.J. Wilkins. 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86: 2598-2611.

Japan Grassland Farming Forage Seed Association.1994. Guide Book for Quality Evaluation of forage. Tokyo, Japan. (Jpn). pp. 82-87.

McDonald, P., A.R. Henderson, and S.J. E. Heron. 1991. The biochemistry of silage 2nd Ed. Chalombe Publications, Marlow, England.

SAS. 1985. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Tjandraatmadja, M., B. Norton and I. Macrae. 1994. Ensilage of tropical grasses mixed with legumes and molasses. World J. Microb.Biotech.10: 82-87.

Van Keulen, J. and Young, B.A. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Science 44:282-287, 1977.

Van Soest, P.J., J.B. Robertson and B.A Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

PO-03-17 Additive effects on fermentation quality of fermented juice (from oil palm fronds) of epiphytic lactic bacteria

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INTRODUCTION

Oil palm fronds (OPFs); consisting of the petiole and many long leaflets, are by-products of the oil palm (*Elais Guineenis*); a commercial crops widely planted in the south of Thailand. As agricultural wastes, OPFs are available when the palms are pruned during the harvesting of fresh fruit bunches (FFBs) for oil palm production. Fronds are left rotting between the rows of palm trees in order to conserve nutrient in the soil. Considering the nutritive value of OPFs, the chemical analysis shows the crude protein (CP) content at 4.7%, crude fiber (CF) at 38.5%, lignocellulose (ADF) at 55.6%, ash at 3.2% and 2.1% fat (Abu Hassan et al., 1996). Due to OPFs being a major biomass throughout the year, these becomes a promising source of roughage feed for ruminants (Abu Hassan et al., 1996; Alimon and Bejo, 1995; Oshio et al., 1990). Although, OPFs has a potential source as feedstuffs, their utilization are limited because of their high lignin and low CP contents resulting in lower digestibility (Zain et al., 2014).

Commonly, forage plants can be conserved through the fermentation process of silage making. Tropical crops usually have low contents of water soluble carbohydrates (WSCs), high buffering capacity and low lactic acid bacteria (LAB) (Catchpoole and Henzell, 1971) and are difficult to ensile resulting in entirely low fermentation quality and digestibility (Niimi and Kawamura, 1998). To improve the quality and digestibility of tropical plants for animal feeding in the future, thus several researches focused on silage fermentation have been done. Productive silage requires epiphytic LAB and WSCs to produce lactic acid (LA) for rapid decrease in pH (McDonald et al., 1991). During silage making process, fermentation under anaerobic conditions is dominated by microbial activity controlled by these following factors: a) type of microorganisms, b) available contents of WSCs as substrate for the microbial growth and c) moisture contents (Kung, 2014). With reference to Ohmomo et al. (2002) report, in early stage of fermentation, *Lactococus* and *Lactobacillus* species; anaerobic bacteria, grow together with aerobic microorganisms like yeasts and molds because of the existence of O_2 in plant materials. When O_2 is excluded, an anaerobic environment is developed leading to the fermentation. During fermentation stage, homofermentative LAB could utilize WSCs to produce LA resulting in the pH reduction between 3.8 and 5.0 (Weinberg and Muck, 1996). The decline in pH under anaerobic conditions inhibits the growth of other microorganisms generating undesirable fermentation (Scudamore and Livesey, 1998).

Ohshima et al. (1996) studied the effect of glucose addition in comparison with addition of previously fermented juice (PFJ) on alfafa fermentation. It was found that the addition of fermented juice of epiphytic LAB (FJLB) was more effective to improve the fermentation quality than the sugar content. Therefore, increasing in the numbers of LAB is very crucial for fermentation quality because homolactic fermentation contributes a 100% of theoretical dry matters (DM) recovery and 99% for energy (Kung, 2014). This is a reason why modifications of fermented plant juices are recommended. Many researches focused on applying additives into fermented juices in order to improve fermentation quality. Tamada et al. (1999) reported that addition of sugars into FJLB could enhance the initial LAB growth under anaerobic conditions. With regard to the LAB activity, Muller and Lier (1994) confirmed that LAB could be able to convert glucose to lactic acid efficiently. Agreeing with this result, the FJLB prepared with glucose consisted of the potential LAB species in high numbers (Bureenok et al., 2005). Nevertheless, information in the literature was studied on fermented juice mostly in silage. There is no reported data correlating with fermented juice of epiphytic lactic bacteria from OPFs. Hence, in this experiment, we aimed to investigate the effect of applying the various sucrose contents in FJLB prepared from OPFs on fermentation quality in comparison with that of non-sucrose treated one.

METHODOLOGY

Preparation of FJLBs: Natural oil palm fronds (OPFs) were cut from an experimental field at King Mongkut's

Institute of Technology Ladkrabang Prince of Chumphon Campus (Chumphon province, Thailand) and immediately chopped into 1-2 cm pieces. Approximately 25 g of the OPFs was taken and macerated with 50 ml of sterilized distilled water by using a home blender. This was then filtered through a double layer of cheesecloth (Bureenok et al., 2005). The filtrate was divided into 4 parts. One was no sucrose added (FJLB0) and the other 3 parts were treated with 1% , 3% and 5% (w/v) sucrose as FJLB1S, FJLB3S and FJLB5S, respectively and mixed well. All FJLBs materials were anaerobically incubated for 2 days at 30°C.

Chemical analysis of FJLBs: Before and after incubation, the pH values of the FJLBs were measured by using a pH meter. Total sugar contents were investigated using the Sulfuric Acid-UV method and colorimetrically measured at 315 nm (Albalasmeh et al., 2013).

Microbiological analysis of FJLBs: The fermented juice of OPFs was diluted serially to a dilution factor of 10^{-6} and spread on MRS agar plates. Viable cell counts were determined after 2 days of incubation at 37°C. The numbers of LAB in all FJLBs are expressed as the colony-forming units per ml (Kozaki et al., 1992).

Statistical analysis: All experiments were carried out with three replicates per treatment. Data obtained from all treatments were studied using analysis of variance (ANOVA) in a completely randomized design (CRD) and the significance of the difference among treatment means was calculated by the Duncan's new multiple range test (DMRT) (SAS, 1988).

RESULTS

The chemical and microbiological analyses of all FJLBs for the 2-days anaerobic fermentation are summarized in Table 1. The predominant changes observed in pH, LAB counts and total sugar contents, occurred within 2 days of incubation period. These changes give similar results as reported by Movsavi et al. (2011).

The effect of sucrose additions on pH changes throughout lactic fermentation of OPFs juices is shown in Figure 1. The pH of all fermented juices decreased during fermentation process. In case of non-sucrose OPFs fermented juice (FJLB0), the pH value was higher from the beginning to the end of fermentation process in comparison with the other sucrose-treated materials. The initial pH of FJLB0 was 5.17 and slightly reduced until it reached to 4.7 at 36-hours fermentation. No further pH declined until the end of experiment. On the contrary, the pH of sucrose-added FJLBs decreased rapidly and maintained almost the same value from 42 hours to 48 hours and the values were significantly ($p<0.05$) lower than that of the non-sucrose FJLB. Obviously, the decrease of pH value was approximately 1.6, 2.1 and 1.9 times lower in the FJLB treated with 1%, 3% and 5% sucrose, respectively, compared with sucrose-free juice. This could be explained that the epiphytic LAB are presented in the fermented juice of OPFs and convert sucrose into lactic acid during the fermentation process, resulting in the pH reduction (Weinberg and Muck, 1996). In addition, the lowest pH value was found in FJLB treated with 3% of sucrose at 4.25.

In common, the epiphytic LAB counts were low at the beginning and increased rapidly within 2 days of anaerobic incubation (Figure 2). The LAB numbers of fresh OPFs juice was 1.83 log cfu/ml and lower than the sucrose-treated FJLBs because of a low level of water soluble carbohydrates (WSCs) contents of fresh OPFs. The addition of various sucrose concentrations had an effect on the enhancing growth of microorganisms in fermented juice of OPFs. Namely, the numbers of LAB counts of sucrose-treated FJLBs had significantly larger value than non-additive one ($p<0.05$) in any period, corresponding with their lower pH values. This could be insisted the presence of epiphytic LAB in the fermented juice of OPFs and influenced on the conversion of sucrose into lactic acid in the fermentation process. As expected, the pH of fermented juices is declined. From this work, it revealed that the sucrose addition into fermented juice of OPFs caused the pH reduction and increasing of numbers of LAB counts simultaneously. However, after 2-days incubation, the increase of the numbers of LAB in FJLB3S and FJLB5S was approximately 1.2 times higher compared with FJLB0. There was not outstanding change in the LAB numbers for FJLB3S and FJLB5S, even though Ebrahimi et al. (2014) previously reported that the insufficient WSCs contents in OPFs should be hightened to promote LAB fermentation. This might be assumed that the excess level of sucrose could utilize the growth of the other microorganisms, instead of LAB growth.

Total sugar (TS) contents varied with time of fermentation and the FJLB materials as illustrated in Figure 3. All TS contents were significantly different ($p<0.05$). From the initial of time, TS contents in all treatments were different depending on sucrose concentrations. During incubation period, sucrose was consumed by the LAB resulting in a lower TS contents. In comparison with non-additives fermented juice, TS contents of sucrose-added OPFs

juices reduced dramatically. These confirmed the activity on sugar conversion of LAB to lactic acid and correlated with the declined pH values. The consumption rate of sucrose of FJLB3S was 4.7 times higher and 4.9 times higher in FJLB5S compared with FJLB0.In view of TS contents of 3% and 5% sucrose-added juices, the change in consumption rate was just 6.2 mg/ml and negligible. The TS contents of FJLB5S after finish fermentation remained quite higher than the others evidently; whereas LAB counts at 48 hours of FJLB5S showed negligible different compared with FJLB3S. This could be realized that the addition of sucrose with higher than 3% w/v had no impact on enhancing the LAB growth and could not improve fermentation quality. Consequently, FJLB from OPFs prepared with 3% sucrose was more potential than the other sucrose treatments and non-additive one.

CONCLUSION

This experiment revealed that applying sucrose as additives into fermented juice of OPFs can enhance LAB population and cause the decline of pH value. According to the results observed in this work, FJLB from OPFs prepared with 3% w/v of sucrose (FJLB3S) exhibited the most suitable alternative to use as a silage additive in the future.

ACKNOWLEDGEMENT

The authors acknowledge King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus, Chumphon Province, Thailand for the facilities and financial supports in this research.

Table 1 The pH values, numbers of LAB counts and total sugar contents of fermented juice of epiphytic lactic bacteria (FJLB) from OPFs with and without sucrose after 2-days incubation.

Parameters	Experimental treatments			
	FJLB0	FJLB1S	FJLB3S	FJLB5S
pH	4.76[a]	4.50[b]	4.25[c]	4.27[c]
LAB (Log cfu/ml)	8.12[c]	9.19[b]	9.46[a]	9.65[a]
Total sugar contents (mg/ml)	2.49[d]	39.33[c]	135.32[b]	197.85[a]

[a,b,c,d] Mean values in the same row with different superscript are significantly different ($p<0.05$).
FJLB = Fermented juice of epiphytic lactic acid bacteria; LAB = Lactic acid bacteria.

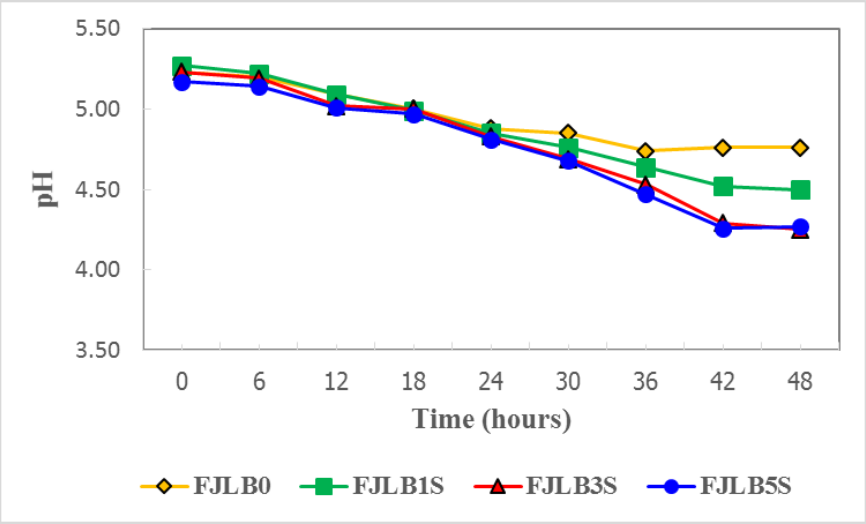

Figure 1 Changes in pH values of FJLBs from OPFs during the fermentation period.

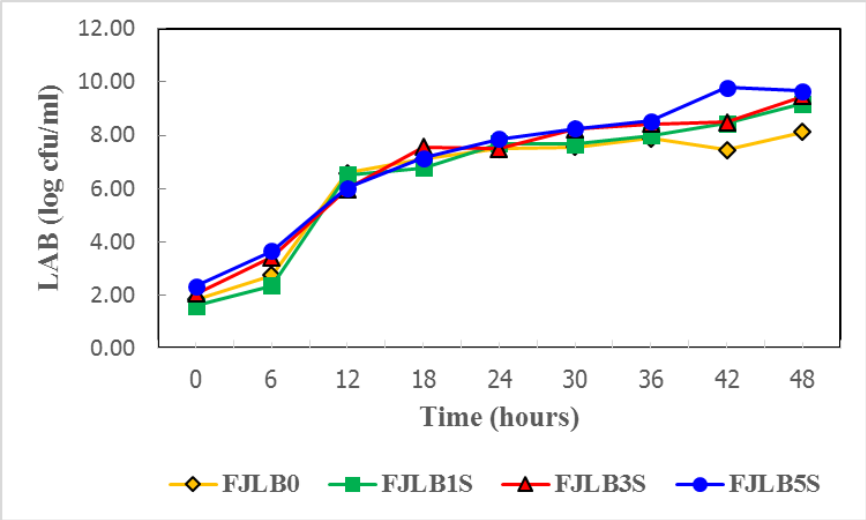

Figure 2 Changes in LAB counts of FJLBs from OPFs during the fermentation period.

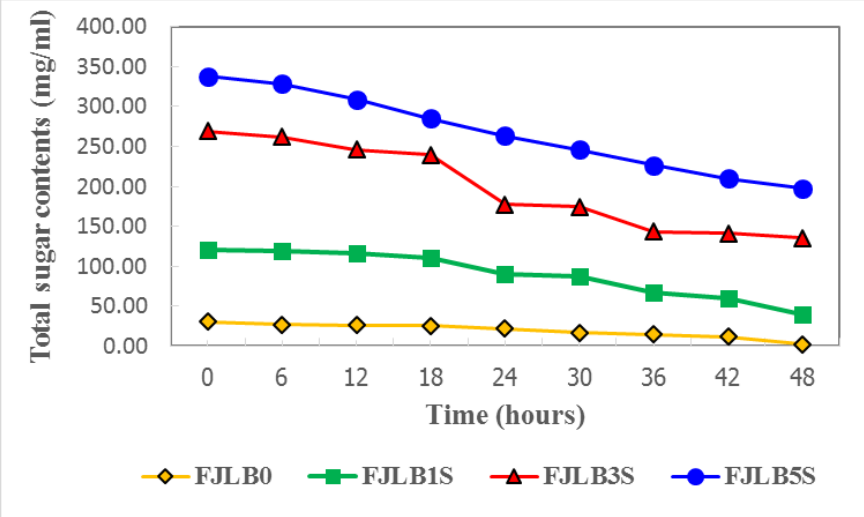

Figure 3 Changes in the TS contents of FJLBs from OPFs during the fermentation period.

REFERENCES

Abu Hassan, O., Ishida, M., Mohd. Shukri, I. and Ahmed Tajuddin Z. 1996. Oil-palm fronds as a roughage feed source for ruminants in Malaysia. Extension bulletins / ASPAC; Food and Fertilizer Technology Center. pp. 8.

Albalasmeh, A. A., Berhe, A. A. and Ghezzehei, T. A. 2013. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydr. Polym. 97: 253-261.

Alimon, A. R. and Bejo, M. H. 1995. Feeding systems based on oil palm by-products in Malaysia. In: Proceedings of 1st International Symposium on the Integration of Livestock to Oil Palm Production. MSAP/FAO and UPM, June 25-27, 1995, Kurla Lumpur, Malaysia, pp. 33-37.

Bureenok, S., Namihira, T., Kawamoto, Y. and Nakada, T. 2005. Additives effects of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of guineagrass (*Panicum maximum* Jacq.) silage. Grassl. Sci. 51: 243-248.

Catchpoole, V. R. and Henzell, E. F. 1971. Silage and silage-making from tropical herbage species. Herb. Abst. 41: 213-221.

Ebrahimi, M., Rajion, M. A., Goh, Y. M., Farjam, A. S., Sazili, A.Q. and Schonewille, J.T. 2014. The effects of adding lactic acid bacteria and cellulose in oil palm (*Elais Guineenis* Jacq.) frond silages on fermentation quality, chemical composition and *In Vitro* digestibility. Ital. J. Anim. Sci. 13(3): 557-561.

Kozaki, M., Uchimura, T. and Okada, S. 1992. Experimental Manual of Lactic Acid Bacteria. Asakurashoten, Tokyu, Japan. 6-16.

Kung, Jr., L. 2014. A Review on Silage Additives and Enzymes. Department of Animal and Food Sciences University of Delaware Newark, DE 19717-1303.

McDonald, P., Henderson, A.R. and Heron, S.J.E. 1991. The Biochemistry of Silage, 2nd ed. Chalcombe Publ., Cambrian Printers, Ltd., Marlow, Bucks, Aberystwyth, Wales, UK.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Emam-Djomeh, Z. and Kiani, H. 2011. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World J. Microbiol. Biotechnol. 27: 123-128.

Muller, M. and Lier, D. 1994. Fermentation by epiphytic lactic acid bacteria. J. Appl. Bacteriol. 76: 406-411.

Niimi, M. and Kawamura, O. 1998. Degradation of cell wall constituents of guineagrass (*Panicum maximum* Jacq.) during ensiling. Grassl. Sci. 43: 413-417.

Ohmomo, S., Tanaka, O., Kitamoto, H. K. and Cai, Y. 2002. Silage and microbial performance, old story but new problems. Jpn. Agric. Res. Q. 36: 59-71.

Ohshima, M., Proydak, N. and Nishino, N. 1996. Relationships among coagulation methods, yields and nutritive values of LPC. In: Proceedings of 5th International Conference on Leaf Protein Research. LEAFPRO-96, Vol. 1, June 17-21, 1996. Don State Technical University, Rostov-on-Don, Russia. pp. 61-68.

Oshio, S., Abu Hassan, O., Takiwara, A., Mohd Jaafar, D., Abe, A., Dahlan, I. and Nakanishi, N. 1990. Processing and utilization of oil palm by-products for ruminants. MARDI/TARC@JIRCAS Collaborative Study Report, p. 110.

SAS. 1998. User's Guide: Statistics. Version 9.2 Edition. SAS Institute Inc., Cary, NC, USA.

Scudamore, K.A. and Livesey, C.T. 1998. Occurrence and significance of mycotoxins in forage crops and silage: a review. J. Sci. Food Agri. 77: 1-17.

Tamada, J., Yokota, H., Ohshima, M. and Tamaki, M. 1999. Effect of additives, storage temperature and regional difference of ensiling on the fermentation quality of Napier grass (*Penisetum purpureum* Schum.) silage. Asian-Aust. J. Anim. Sci. 12: 28-35.

Weinberg, Z.G. and Muck, R.E. 1996. New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol. Rev. 19: 53-68.

Zain, M., Rahman, J. and Khasrad. 2014. Effect of palm oil by products on *In Vitro* fermentation and nutrient digestibility. Anim. Nutr. Feed Techn. 14: 175-181.

PO-03-18

Influence of age on oil palm fronds quality as roughage for ruminants

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INTRODUCTION

Roughages sources are important for ruminant especially in arid and semi-arid areas. Oil palm trees are the most important plantation crop in south of Thailand. Oil palm has an economic lifetime of 25–30 years and pruning at least two fronds every harvest time. The utilization of agricultural by-products was necessary in the shortage areas of roughage for ruminant. Age of at plant also influences chemical composition of oil palm fronds and utilization as roughage resources for animal. Oil palm fronds are normally available to ruminant feed in the area but difference age of plant had an effect on nutritional value of feed ingredients (Pinos-Rodriguez et al., 2008). Protein, nitrogen free extract, ether extract tend to decrease with advancing age, whereas crude fiber and lignin increase (Ball et al., 2001). Thus, effect of oil palm age on chemical composition in oil palm fronds was important to investigate for efficiency to provide a clearly ruminant feed. The objective of this research is study on quality of oil palm fronds from difference age of oil palm tree as ruminant roughage.

MATERIALS AND METHODS

Sampling can be conducted from oil palms (*Elaeis guineensis,* Jacq.) grow in Pathiu district area, Chumphon province, Thailand. The oil palm frond were randomly collected from pruning oil palm tree at least two fronds. The oil palm tree are varying age of study between 4-24 years and divided into eleven age groups (4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 years) of oil palm tree. The samples were collected from oil palm frond each age and cut 1-2 cm and randomly collected of 200 grams for dried in a hot air oven at 60°C for two days and calculated dry matter of samples. The chemical analysis were carried out following method AOAC (1990) that contain: crude protein (CP: nitrogen content x 6.25), ether extract (EE), ash were determined after grinded 1 mm by hammer mill machine. Additionally, grinded samples also to be analyzed for NDF and ADF according to Van Soest et al. (1991). The data were analyzed using the procedures of SPSS 16 for a completely randomized design (CRD) and compared mean by Duncan's Multiple Rang Test (DMRT).

RESULTS AND DISCUSSION

Comparing the chemical compositions of oil palm fronds used in this study were significantly difference ($P<0.05$) between each age of oil palm tree shown in Table 1. The data showed that ash values were significantly different ($P<0.05$) and ash level increased with increasing age of the oil palm. Age of oil palm was impacted ($P<0.05$) on EE of oil palm fronds. The young oil palm fronds (4-10 years) showed a small amount of EE level ($P<0.05$) while increase EE difference on 12-16 years. Moreover, crude protein of oil palm fronds was significantly decrease ($P<0.05$) follow increasing age of oil palm, which crude protein tend to decrease with advancing age. However, increasing age of oil palm was not significantly different on neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the oil palm fronds ($P>0.05$). Low NDF is usually desired as maturity of the plant at harvest increases, cell wall content of the plant increases and NDF increases (Ball et al., 2001). Whereas, the lower the ADF value relates to high the digestibility of fiber and the high the level of non-fiber carbohydrate (Patnum et al., 2008). The older age of oil palm had low CP, high ash and EE but not difference in NDF and ADF value shown in Figure 1.

CONCLUSION

The oil palm fronds in this study were significantly difference ($P<0.05$) between each age of oil palm tree. The ash level increased with increasing age of the oil palm and the older age of oil palm had low CP but high ash and EE. The young oil palm fronds (4-10 years) showed a small amount of EE level while increase EE difference on 12-16 years. The oil palm fronds was significantly decrease crude protein ($P<0.05$) follow increasing age of oil palm. In conclusion, although age had decrease CP but it had no effect on quality of oil palm fronds due to fiber content not different which is suitable to use as roughage for ruminants.

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Table1.The chemical composition of oil palm fronds silage with different FJLB level

Components / Age	Ash	EE	CP	NDF	ADF
	% DM				
4 years	6.57^{cde}	0.81^{cd}	5.21^{cd}	69.25	48.92
6 years	6.34^{de}	0.90^{cd}	7.72^{bc}	69.94	50.52
8 years	6.38^{c}	0.93^{cd}	8.98^{a}	65.96	55.38
10 years	6.82^{bcde}	0.80^{d}	7.83^{b}	65.83	52.15
12 years	7.40^{b}	1.36^{ab}	8.36^{ab}	68.67	49.22
14 years	6.96^{bcd}	1.58^{a}	7.61^{bc}	67.14	54.22
16 years	7.32^{b}	1.47^{a}	8.15^{ab}	67.58	54.30
18 years	6.82^{bcde}	1.26^{ab}	5.49^{d}	67.55	54.62
20 years	7.04^{cd}	1.21^{b}	4.36^{e}	69.70	53.36
22 years	8.28^{a}	1.05^{c}	6.75^{c}	68.85	51.70
24 years	8.63^{a}	1.34^{ab}	5.74^{d}	70.72	56.20

Notes: CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber

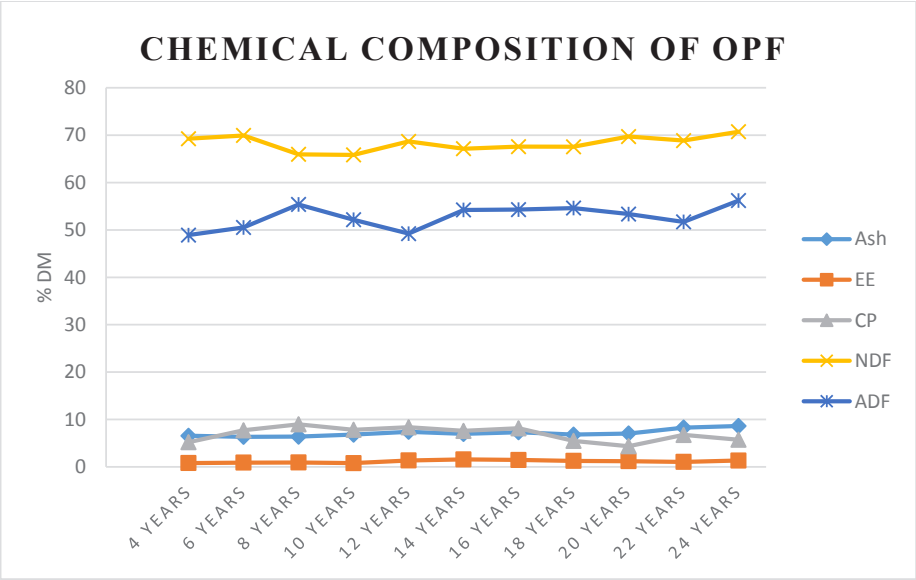

Figure 1 The chemical composition of oil palm oil fronds (OPF) each ages

REFERENCES

AOAC. 1990. Official Methods of Analysis, Vol.1, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, USA. 69-90.

Ball, D.M., M. Collins, G.D. Lacefield, N.P. Martin, D.A. Mertens, K.E. Olson, D.H. Putnam, D.J. Undersander and M.W. Wolf. 2001. Understanding forage quality. American Farm Bureau Federation Publication 1-01, Park Ridge, IL

Patnum, D.H., P. Robinson and E. dePerters. (2008). Forage quality and testing. Division of Agriculture and Natural Resource. University of California. Publication 8203. 25 p. http://alfalfa.ucdavis.edu/IrrigatedAlfalfa/pdfs/UCAlfalfa8302ForageQuality_free.pdf

Pinos-Rodriguez, J.M., M. Zamudio and S.S. Gonzalez. 2008. The effect of plant age on the chemical composition of fresh and ensiled *Agave salmiana* leaves. S Afr J Anim Sci. 38(1): 43-50.

Van Soest, P.J., J.B. Robertson and B.A. Lewis.1991. Methods for dietary fiber, neutral detergent fiber, and Non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

PO-03-19 Effect of fermented juice of epiphytic lactic acid bacteria (FJLB) levels on quality of oil palm fronds silage

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INTRODUCTION

The main problem of feeding ruminants is roughage not enough especially in tropical regions. Oil palm frond is by-product in oil palm production that to be cut-off during harvest and its high protein (5-6%CP). The utilization of oil palm fronds as alternative offer and easily to apply as resource feed for ruminant in lack regions. Oil palm frond is containing rich amounts of tannins and phenolic compounds (Jaffri et al., 2011), which fermentation can decrease tannin. The problem of ensiling in topical area are difficult because of silage requires epiphytic lactic acid bacteria (LAB) to produce adequate lactic acid for rapid pH reduction (McDonald et al, 1991). Forage that is ensiled properly exhibits rapid pH drop where homo-fermentative bacteria predominate. Lactic acid should be a significant end-product of these fermentations (Ward, 2001). Fermentations that yield more lactic acid typically result in the lowest dry matter losses. The importance of level of moisture in providing suitable conditions for epiphytic organisms that are active during ensiling (Mahanna and Chase, 2003). The fermented juice of epiphytic lactic acid bacteria (FJLB) has been suggested as preservative silage for tropical grass silage (Bureenok et al, 2005). However, the oil palm fronds silage additives with difference FJLB levels were not yet clearly reported. Thus the purpose of this study was to investigate the effect of FJLB levels on quality of oil palm fronds silage.

MATERIALS AND METHODS

The fermented juice of epiphytic lactic acid bacteria (FJLB) was prepared from 200 g fresh oil palm fronds, which was immediately soaked in 1,000 ml of distilled water. The 2% glucose was added and incubated under anaerobic condition for 2 days and was filtered through a double layer of cheesecloth before used as a silage additive.

The samples for making silage were collected from oil palm fronds about 6 year olds oil palm tree in the Pathiu, Chumphon province area to ferment with FJLB. The experiments were divided into five treatments with different FJLB levels namely 0%, 1%, 2%, 3% and 4% v/w of oil palm fronds silage. Thereafter, the experiment silage was packed in plastic bags and air was withdrawn from the plastic bags. The silages were opened on 21 d of ensiling for dry matter, crude protein (nitrogen content x 6.25), ash, ether extract was carried out according to AOAC (1990) and detergent method was following method of Van Soest et al. (1991).

The physical characteristics of silage were evaluated after storage for 21 days. The standards of quality silage by the Thailand department of livestock ministry of agriculture and cooperatives (2004) that explained the sum score of silage (odor, texture and color) were 20-25 = very good, 15-19 = good, 6-14 = medium, 0-5 = low. Moreover, the samples were collected for lactic acid bacteria content (LAB) following method of Kozaki et al. (1992). The data were analyzed using the procedures of SPSS 16 for a completely randomized design (CRD) and compared mean by Duncan's Multiple Rang Test (DMRT).

RESULTS AND DISCUSSION

Chemical compositions of oil palm fronds silage used in this study were not significantly difference ($P>0.05$) between each treatments. The result showed that ash was not significantly different among groups ($P>0.05$). Ash level in sample indicates total mineral level that data shown the range of each groups as 5.87-6.08%DM. In the order of crude protein and acid detergent fiber (ADF) of all treatments were not significantly different ($P>0.05$). Although, in the part of ADF shown that high ADF when increasing FJLB level but not significantly different ($P>0.05$). The higher the ADF value relates to low the digestibility of fiber (Patnum et al., 2008). In addition, the ether extract (EE) of 4% v/w FJLB was higher than that of other treatments ($P<0.05$) and neutral detergent fiber (NDF) of 0% FJLB was lower than that of other groups ($P<0.05$) shown in Table1. NDF gives bulk to the diet and used to predict intake, low NDF is generally preferred. As maturity of the plant at harvest increases, cell wall content of the plant increases cause to NDF increases (Ball et al., 2001).

The lactic acid bacteria content of ensiling oil palm fronds with different FJLB levels at 0%, 1%, 2%, 3% and 4% v/w are show in Figure1. The data showed that 2% v/w of FJLB had lactic acid bacteria content higher than other

groups (P<0.05) with the mean values of 9.44, 9.02, 9.74, 9.35 and 9.24 log cfu/g, respectively, while pH value of oil palm fronds silage were not significant difference among groups (P>0.05). In addition, the evaluation of physical characteristics of silage (color and odor) for all treatments were distinguished by to brownish-yellow in color and fruity odor (P>0.05). Sum score of physical characteristics were not significant different (P>0.05), however the score is in rang every good score (Table2).

CONCLUSION

The chemical composition of oil palm fronds silage (ash, crude protein and ADF) of all treatments were not significantly different (P>0.05), while ether extract of 4% v/w FJLB was higher and NDF of 0% FJLB was lower than that of other groups (P<0.05). Ensiling oil palm fronds 2% v/w of FJLB had lactic acid bacteria content higher than other groups (P<0.05) The evaluation of physical characteristics of silage for all treatments were distinguished by to brownish-yellow in color and fruity odor (P>0.05). The quality of oil palm fronds silage with FJLB was quite suitable and could be used as an alternative way to use as roughage for ruminants.

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The authors acknowledge King Mongkut's Institute of Technology Ladkrabang Prince of Chumphon Campus, Thailand for the facilities and the financial support for this research.

Table1.The chemical composition of oil palm fronds silage with different FJLB level

Components	0% FJLB	1% FJLB	2% FJLB	3% FJLB	4% FJLB
	% DM				
Crude protein	6.67	7.12	6.73	6.82	6.79
Ether extract	1.76^{ab}	1.69^{b}	1.76^{ab}	1.74^{ab}	1.89^{a}
Ash	5.90	6.08	5.96	6.05	5.87
Neutral detergent fiber	73.91^{b}	76.57^{a}	76.62^{a}	74.89^{ab}	75.84^{a}
ADF	59.52	54.52	60.58	66.78	65.65

Notes: FJLB = fermented juice of epiphytic lactic acid bacteria

Table 2 The physical characteristics score of oil palm fronds silage with different FJLB level

Components	0% FJLB	1% FJLB	2% FJLB	3% FJLB	4% FJLB
Color of silage	2.71	2.57	2.43	2.86	2.86
Odor of silage	10.29	10.29	9.71	10.29	10.29
Texture of silage	3.29	3.57	3.43	3.57	4.00
pH	4.68	4.57	4.53	4.55	4.50
Sum score*	20.97	21.00	20.10	21.27	21.65

Notes: FJLB = fermented juice of epiphytic lactic acid bacteria, LAB = Lactic acid bacteria
*Sum score 20-25 = very good, 15-19 = good, 6-14 = medium, 0-5 = low

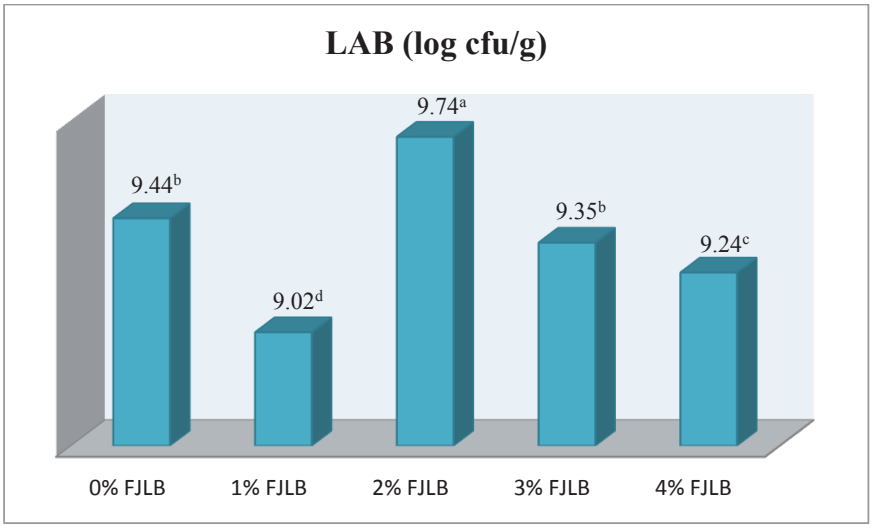

Figure 1 Lactic acid bacteria contain of oil palm fronds silage with different FJLB level

REFERENCES

AOAC. 1990. Official Methods of Analysis, Vol.1, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, USA. 69-90.

Ball, D.M., M. Collins, G.D. Lacefield, N.P. Martin, D.A. Mertens, K.E. Olson, D.H. Putnam, D.J. Undersander and M.W. Wolf. 2001. Understanding forage quality. American Farm Bureau Federation Publication 1-01, Park Ridge, IL

Kozaki, M., T. Uchimura and S. Okada. 1992. Experimental manual of lactic acid bacteria. Asakurashoten, Tokyo, Japan. pp. 6-16.

McDonal, P., A.R. Henderson and S.J.E. Heron. 1991. 2nd ed. Chalcombe Publications, Marlow, UK

Mahanna, W.C. and L.E. Chase. 2003. Practical applications and solutions to silage problems. Pages 855-895 in Silage Science and Technology.

Patnum, D.H., P. Robinson and E. dePerters. (2008). Forage quality and testing. Division of Agriculture and Natural Resource. University of California. Publication 8203. 25 p. http://alfalfa.ucdavis.edu/IrrigatedAlfalfa/pdfs/UCAlfalfa8302ForageQuality_free.pdf

Van Soest, P.J., J.B. Robertson and B.A. Lewis.1991. Methods for dietary fiber, neutral detergent fiber, and Non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Ward, R. 2001. Analysis and Quality Assessments of Corn and Other Silages. Pp. 95-110 in 62nd Minnesota Nutrition Conference & Minnesota Corn Growers Association Technical Symposium. Bloomington, MN.

PO-03-21

Effects of Dietary Supplementation with Andrographis paniculata, Turmeric and Vitamin E on Growth Performance and Meat Quality of Goat

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ABSTRACT

This experiment was conducted to determine the effect of dietary supplementation with *Andrographis paniculata*, Turmeric and vitamin E on growth performance and meat quality of goat. A total of twenty crossbred male goats (Boer x Anglo-Nubian x Native) were randomly allotted in to four dietary groups, consisting of basal diet (control), or basal diet with 0.5% *Andrographis paniculata* (AP) or 0.5% Turmeric (TU) or 400mg/kg vitamin E (VE). All goats were fed the corresponding concentrate diets at 1.5% of body weight and Napier grass *ad libitum* during 120 day period. The meat quality traits were determined using *longissimus dorsi* muscle. The results had shown that the daily weight gain and feed conversion efficiency were significant different between treatments. The ultimate pH value, meat color (CIE *L*,a*, b** values) and drip loss were not affected by the dietary supplements. However, the cooking loss was lower in the VE treatment than the control group ($p<0.05$) and Warner-Bratzler shear force was lower for the TU treatment than the control, AP and VE treatment ($p<0.01$). The present study indicated that vitamin E and Turmeric supplementing have the potential to improve meat quality in term of tenderness and low cooking loss.

INTRODUCTION

Goat meat is leaner than lamb and beef since it incorporates less subcutaneous, intramuscular fat and more internal fat (Webb et al., 2005).The quality of meat depends on the characteristics of the meat such as color, flavor and tenderness. Antioxidant supplementation in diet not only have a preventive effect on the health of animal, but also enhance the final product such as meat in line with now called functional food, due to the increasing consumer's awareness of health (Castillo et al., 2013). Vitamin E (α-tocopherol) is the most frequently used lipid soluble free radical scavenger administered as nutritional supplement (Karami et al., 2010a,b; Sretenovic et al., 2007). High levels of supplemental vitamin E may improve carcass quality by reducing the oxidation of meat (Webb et al., 2005). In recent years, many herb, species, and their extract have been successfully used to reduced lipid oxidation in meat and to improve the sensory characteristics and extend shelf-life (Karami et al., 2011). Dietary antioxidants from *Andrographis paniculata* have potential for improved feed efficiency and addition of turmeric increased average daily gain of male goats (Karami et al., 2010a). Inclusion of AP in diet of goats has significantly improved growth performance (Yusuf et al., 2014). The turmeric antioxidants are active one step above that of vitamin E and are stronger compared to vitamin C and E (Miquel et al., 2002). The objective of this study was to evaluate the effects of antioxidants supplementation with *Andrographis paniculata*, turmeric and vitamin E on growth performance and meat quality traits of the crossbred goat.

MATERIALS AND METHODS

Animals and Samples collection

Twenty four male crossbred (Boer x Anglo-Nubian x Native) goats with a similar weight and age were randomly divided in to four dietary groups, consisting of basal diet (control, CN) and basal diet supplemented with 0.5% *Andrographis paniculata* or 0.5% Turmeric or 400mg/kg vitamin E (α-tocopheryl acetate). Goats were fed the corresponding concentrate diets at 1.5% of body weight and Napier grass *ad libitum*. After 120 days of feeding period, goats were fasted for 12 h with free access to water and slaughtered. Meat quality traits were determined using *longissimus dorsi* (LD) muscle. The pH values were obtained 45 minute (pH_{45min}) and 24 hour (pH_{24h}) after slaughter. Meat color were obtained as lightness (L*), redness (a*) and yellowness (b*) values (CIE color model). Drip loss were measure at 24 hour after slaughter. Meat cuts were cooked for analyzed cooking loss and Warner-Bratzler (WB) shear force.

Data were analyzed by the analysis of variance (ANOVA) to test for the effects of dietary treatments effect. A Duncan's new multiple range test procedure was used to determine significant differences among means at a 5% level of significance.

RESULTS AND DISCUSSION

The result of growth performance are present in Table 1. There were no significant effect of groups on the total feed intake of goats ($p>0.05$). Goats fed diets of AP, TU and VE groups had higher average daily gain and had lower feed conversion ratio than the control diets ($p<0.05$). The present results were also agreement with those of Karami et al. (2010a) who reported dietary antioxidants from AP have potential for improved feed efficiency and addition of turmeric increased average daily gain of male goats and Yusuf et al. (2014) who reported that inclusion of AP in diet of goats has significantly improved growth performance.

Table 1. Growth performance of crossbred goats

Traits	CN	AP	TU	VE	P-value
Body weight gain (kg)	5.78	6.98	6.50	6.78	0.3934
Average daily gain (g/d)[1]	50.97[b]	60.97[a]	58.33[a]	57.92[a]	0.0236
Total feed intake (g/d)	686.07	700.46	701.06	696.72	0.5427
FCR	13.56[a]	11.52[b]	12.07[b]	12.17[b]	0.0363

[1]a-b Means within a row with different superscripts differ significantly differ at $P<0.05$

Table 2. pH value, color, drip loss, cooking loss and shear force of LM muscle in crossbred goats

Traits	CN	AP	TU	VE	P-value
pH_{45min}	6.15	5.83	5.97	6.08	0.030
pH_{24h}	5.74	5.65	5.56	5.66	0.039
Color					
L*	33.48	33.02	33.55	33.94	0.814
a*	11.34	11.22	11.07	12.07	0.428
b*	12.63	12.20	12.51	13.78	0.157
Drip loss (%)	2.67	3.96	2.54	4.36	0.436
Cooking loss (%)[1]	37.09[a]	33.83[ab]	33.78[ab]	31.34[b]	0.045
WB Shear force (kg/cm^2)[2]	5.61[a]	5.06[a]	4.13[b]	5.59[a]	0.004

[1]a-b Means within a row with different superscripts differ significantly differ at $P<0.05$
[2]a-b Mean within a row with different superscripts differ significantly at $P<0.01$

CONCLUSION

The results of this study indicate that supplementation with vitamin E and natural herbs such as *Andrographis paniculata* and Turmeric in diets enhance growth performance of goats and have potential for improved meat quality in term of water holding capacity and tenderness.

Keywords: *Andrographis paniculata*, Turmeric, Vitamin E, Growth performance, Meat quality, Goat

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REFERENCES

Castillo, C.C., Pereira,V., Abuelo, A. & Hernandez, J. 2013. Effect of supplementation with antioxidants on the quality of bovine milk and meat production. The Sci. World J. 2013:1-8.

Karami, M., Alimon, A.R., Sazili A.Q. & Goh, Y.M. 2010a. Effects of dietary herbal antioxidants supplementation on feedlot growth performance and carcass composition of male goats. American J. Anim. & Vet. Sci. 9(24):3039-3047.

Karami, M., Alimon, A. R., Sazili A.Q. & Goh, Y.M. 2010b. Meat quality and lipid oxidation of infraspinatus muscle and blood plasma of goats under dietary supplementation of herbal antioxidants. J. of Anim. & Vet. Adv. 9(24):3039-3047.

Karami, M., Alimon, A.R., Sazili, A.Q., Goh Y.M., & Ivan, M. 2011. Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of *longissimus* muscle in Kacang goat with aging time. Meat Sci. 88(1):102-108.

Miquel, J., Bernd, A., Sempere, J.M., Diaz-Alperi, J. and Ramirez, A. 2002. The curcuma antioxidants: Pharmacological effects and prospects for future clinical use. A review. Arch. Gerontol. Geriatrics. 34:37-46.

Sretenovic, L.J., Aleksic, S., Petrovic, M.P. & Miscevic, B. 2007. Nutritional factors influencing improvement of milk and meat quality as well as productive and reproductive parameters of cattle. Biotechnology in Animal Husbandry 23:217-226.

Webb, E. C., Laure, B., Touil, C. & Scott, S.L. 2005. Goat meat quality. Small Rumin. Res. 60:153-166.

Yusuf, A.L., Goh, Y.M., Samsudin, A.A., Alimon A.R. & Sazili, A.Q. 2014. Growth performance, carcass characteristics and meat yield of Boer goats fed diets containing leaves or whole parts of *Andrographis paniculata*. Asian Australas. J. Anim. Sci. 27(4):503-510.

PO-03-22

INFLUENCE OF ARTOCARPUS LACUCHA MEAL SUPPLEMENTATION ON STRAW BASED DIETS USING IN VITRO GAS FERMENTATION TECHNIQUE

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ABSTRACT

The objective of this study was to determine the influence of *Artocarpus lacucha* (Ma-had(meal supplementation on straw based diets using *in vitro* gas fermentation technique. Two rumen-fistulated cattle were used in a 2 ´ 5 factorial arrangement in CRD. The main factors were two roughage type (rice straw; RS and riceberry straw, RBS) and five supplementation levels of Ma-had meal, 0, 5, 10, 15 and 20 mg. The gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 36, 48, 72 and 96 h of incubation and was used for calculated of gas kinetics and cumulative gas production. It was found that gas production from the soluble fraction (a), insoluble fraction (b), potential extent of gas production (a + b) and cumulative gas production were significantly different by interaction of roughage type and Ma-had level. Moreover, interaction of roughage type and Ma-had level significantly increased (P<0.01) *in vitro* dry matter and organic matter degradability in RBS with 10 mg of Ma-had (55.6% and 67.6%), respectively. In addition, true digestibility was significantly increased (P<0.05) in RBS with 5 and 10 mg of Ma-had supplementation (65.0% and 65.3%), respectively. Microbial biomass was higher in RBS group and supplementation of Ma-had at 10 mg than other group. Based on this study, it is suggested that the RSB group with 10 mg of Ma-had supplementation could improve gas kinetics, gas production, degradability and microbial mass.

INTRODUCTION

Plant secondary compounds such as tannins and saponins have been use as feed additive for improve feed utilization with enhance fibrous feed digestibility, reduce energy and nitrogen losses. The beneficial or detrimental effect of tannins to ruminants is depended upon the amount of consumption, the compound's structure and molecular weight and on the physiology of the consuming species (Hagerman and Butler, 1991).

Artocarpus Lacucha belongs to the family "Moraceae", commonly called as Monkey jack and in Thailand called Ma-had. Ma-had is a tropical fruit and originated from India. This fruit is available in some Asian countries like Bangladesh, Bhutan, Nepal, Myanmar, Sri Lanka, Thailand, Malaysia, Singapore, Vietnam, Cambodia and Laos. Ma-had is a plant species that is rich in bioactive compounds. It was observed that Ma-had hartwood contained flavanoid and tannin (Singhatong et al., 2010). Ma-Had heartwood has been traditionally used as an anti-helmintic (Maneechai et al., 2009). In the previous study found that crude extract of Ma-had have a potential to be used for reduced the parasite (Gautam and Patel, 2006). However, lack information is available on the use of Ma-Had as animal feed. Therefore, the aim of this work was to evaluate the influence of *Artocarpus Lacucha* meal supplementation on straw based diets using *in vitro* gas fermentation technique

MATERIALS AND METHODS

Experimental design and dietary treatments

The experimental design was a 2 ´ 5 factorial arrangement in completely randomized design (CRD). The main factors were two roughage type (rice straw; RS and riceberry straw, RBS) and five supplementation levels of Ma-had meal, 0, 5, 10, 15 and 20 mg with 0.5 g of roughage and concentrate ratio at 60:40. Concentrates were formulated to contain 16% and 76% of crude protein (CP) and total digestible nutrient (TDN), respectively. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1995),

neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991).

Animals and preparation of rumen inoculums

Two male, rumen-fistulated beef cattle with body weight of 500 ± 30 kg were used as rumen fluid donors. Beef cattle rumen fluid was collected from animals fed with 16% CP concentrate at 0.7% body weight and rice straw ad libitum. The animals received the diets for 14 d before the rumen fluid was collected. On day 15, 1000 ml rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

In vitro fermentation of substrates

Samples of 0.5 g of roughage and concentrate at ratio 60:40 with Ma-had level supplementation were weighed into 50 ml serum bottles. For each treatment, three replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39 °C under continuous flushing with CO_2 and 40 ml of rumen inocula mixture were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39°C (72 h) for *in vitro* gas test.

Sample and analysis

During the incubation, data of gas production was measured immediately after incubation at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 36, 48, 72 and 96 h by using a glass syringe. Cumulative gas production data were fitted to the model of ørskov and McDonald (1979) as follows: $y = a + b\ [1\text{-}e^{(-\ ct)}]$ where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a + b) = the potential extent of gas production. y = gas produced at time ''t''. The *in vitro* degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left above was ashed at 550 °C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963). At 48 h post inoculation a set of treatment was determined *in vitro* true digestibility according to Van Soest et al. (1991). The true digestibility was used to calculate microbial mass according to the method of Blümmel et al. (1997).

STATISTICAL ANALYSIS

All data from the experiment were analyzed as a completely randomized design using the GLM procedure of SAS (1998). Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Trend of Ma-had levels responded was performed by orthogonal polynomials.

RESULTS AND DISCUSSIONS

It was found that gas kinetics and cumulative gas production were significantly different by interaction of roughage type and Ma-had level. The gas production from slowly fermentable fraction (b), the potential gas production (a + b) and cumulative gas production of RBS with 10 mg Ma-had was significantly higher than other treatment. Moreover, interaction of roughage type and Ma-had level significantly increased ($P<0.01$) *in vitro* dry matter (IVDMD) and organic matter degradability (IVOMD) in RBS with 10 mg of Ma-had (55.6% and 67.6%), respectively. The higher gas production and degradability observed may be due to the low level of tannins in the Ma-had. However, high level of Ma-had (15 and 20 mg) reduced gas production and *in vitro* degradability. High level of tannins are present in the NDF and ADF fractions and are tightly bound to the cell wall and cell protein and seem to be involved in decreasing digestibility (Reed et al., 1990). Moreover, gas production was negatively correlated with cell wall content (NDF and ADF) in roughage. These may tend to reduce the microbial activity through increasing the adverse environmental conditions as incubation time progress. This is consistent with Njidda and Nasiru (2010), who reported that gas production was negatively related with NDF content and positively with starch. In addition, true digestibility was significantly increased ($P<0.05$) in RBS with 5 and 10 mg of Ma-had supplementation (65.0% and 65.3%), respectively. The two factors, type of roughage and Ma-had level influenced the microbial mass. Microbial biomass was higher in RBS group and supplementation of Ma-had at 10 mg than other group. The beneficial effect of tannins when Ma-had containing low levels of tannins was fed could be due to the protection of protein from microbial degradation by tannins, thus increasing the amount of undegraded protein entering the small intestine. In addition, a higher flow of microbial protein to the intestine as

a result of higher efficiency of microbial protein synthesis (Getachew et al., 2000; Njidda and Nasiru, 2010) has been observed.

CONCLUSION AND RECOMMENDATIONS

The results revealed that the RSB group with 10 mg of Ma-had supplementation could improve gas kinetics, gas production, degradability and microbial mass. However, these findings should be further investigated in *in vivo* study.

KEYWORDS: *Artocarpus lacucha*, *in vitro* gas production, degradability, microbial mass

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Table 1 Ingredients and chemical composition of diets used in the experiment

Item	Concentrate	RS	RBS	*Artocarpus lacucha*
Ingredient, % DM				
Cassava chip	60.2			
Rice bran	10.0			
Soybean meal	13.0			
Whole cottonseed	10.0			
Urea	2.3			
Molasses	2.5			
Salt	0.5			
Sulfur	0.5			
Mineral and vitamin mixture	1.0			
Chemical composition				
Dry matter, %	87.6	95.9	96.6	93.3
	----------% of dry matter----------			
Organic matter	93.8	87.5	85.1	96.8
Crude protein	16.0	3.5	3.9	1.0
Neutral detergent fiber	20.2	74.9	74.0	69.6
Acid detergent fiber	15.0	57.1	50.2	40.7

RS = rice straw, RBS = riceberry straw

Table 2 Effect of *Artocarpus lacucha* meal supplementation on gas kinetics and cumulative gas production

Trt	Roughage source	Ma-had (mg)	Gas kenetics[1]				Gas (96 h) mL/0.5 g DM substrate
			a	b	c	a+b	
1	RS	0	2.1	100.9	0.03	103.0	93.1
2		5	3.4	93.2	0.04	106.9	92.8
3		10	3.2	123.2	0.03	126.4	114.7
4		15	1.7	111.5	0.03	113.1	108.3
5		20	1.5	116.9	0.03	118.4	109.6
6	RBS	0	1.5	126.5	0.04	126.0	121.5
7		5	2.6	126.7	0.03	129.3	122.8
8		10	1.7	134.9	0.04	136.2	130.4
9		15	3.3	120.6	0.03	123.9	115.5
10		20	2.9	116.0	0.03	118.9	111.3
SEM			0.15	0.89	0.01	1.93	1.65
Comparison							
Roughage			*	**	*	**	**
Level of Ma-had			**	**	ns	**	**
Interaction			**	**	*	*	**
Orthogonal polynomial							
Level of Ma-had (lin)			ns	ns	ns	ns	ns
Level of Ma-had (quad)			ns	ns	ns	*	*
Level of Ma-had (cubic)			ns	**	ns	ns	ns
Level of Ma-had (quart)			*	ns	ns	ns	ns

[1]a=the gas production from the immediately soluble fraction; b=the gas production from the insoluble fraction; c=the gas production rate constant for the insoluble fraction (b); a+b=the potential extent (omit gas); SEM=standard error of the mean. ns=non-significant. * P<0.05, ** P<0.01.

Table 3 Effect of *Artocarpus lacucha* meal supplementation on *in vitro* degradability, true digestibility and microbial mass

Trt	Roughage source	Ma-had (mg)	*In vitro* degradability, %		True digestibility	Microbial mass
			IVDMD	IVOMD		
1	RS	0	41.4	54.1	60.1	12.5
2		5	36.6	52.4	57.1	12.6
3		10	45.5	58.3	56.7	13.6
4		15	47.9	59.5	60.4	12.9
5		20	45.7	57.8	57.8	10.4
6	RBS	0	50.8	67.6	60.2	16.5
7		5	53.4	65.7	65.0	18.8
8		10	55.5	68.0	65.3	21.6
9		15	47.7	60.6	62.0	13.8
10		20	44.0	56.8	59.5	17.8
SEM			0.37	0.60	1.02	0.92
Comparison						
Roughage			**	**	**	**
Level of Ma-had			**	**	ns	*
Interaction			**	**	*	ns
Orthogonal polynomial						
Level of Ma-had (lin)			ns	ns	ns	ns
Level of Ma-had (quad)			ns	ns	ns	ns
Level of Ma-had (cubic)			**	**	ns	*
Level of Ma-had (quart)			ns	ns	ns	ns

SEM= standard error of the mean. ns=non-significant. * P<0.05, ** P<0.01.

REFERENCES

AOAC. 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Blümmel, M., H.P.S. Makkar and K. Becker. 1997. *In vitro* gas production: a technique revisited. Journal of Animal Physiology and Animal Nutrition. 77: 24-34.

Gautam, P. and R. Patel. 2014. *Artocarpus Lakoocha* Roxb: An overview. European Journal of Complementary and Alternative Medicine. 1(1):10-14.

Getachew, G., H.P.S. Makkar and K. Becker, 2000. Effect of polyethylene glycol on in vitro degradability of Nitrogen and microbial protein synthesis from tannin rich browse and herbaceous legumes. *British Journal* of *Nutrition*. 84: 73-83.

Hagerman, A.E. and L.G. Butler. 1991. Tannins and lignins. In: Herbivores: their interactions with secondary plant metabolites, Vol I: The chemical participants (Ed. G. A. Rosenthal and M. R. Berenbaum), Academic Press, NY, USA. pp. 355-388.

Maneechai, S., K. Likhitwitayawuid, B. Sritularak, C. Palanuvej, N. Ruangrungsi and P. Sirisa-ard. 2009. Quantitative analysis of oxyresveratrol content in *Artocarpus lakoocha* and 'Puag-Haad'. *Medical Principles* and *Practice.*18: 223-227.

Menke, K.H. and H., Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research Development. 28: 7-55.

Njidda, A.A. and A. Nasiru. 2010. In vitro gas production and dry matter digestibility of tannin-containing forges of semi-arid region of North-Eastern Nigeria. Pakistan Journal of Nutrition. 9(1): 60-66.

Ørskov, E.R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, Cambridge. 92: 499-503.

Reed, J.D., H. Soller and A. Wood. 1990. Fodder tree and straw diets for sheep intake, growth, digestibility and the effect of phenolics on nitrogen utilization. Anim. Feed Sci. Technol., 30: 39-50.

SAS Institute Inc. 1998. User's Guide: Statistic, Version 6, 12th edition. SAS Institute Inc., Cary, NC.

Singhatong, S., D. Leelarungrayub and C. Chaiyasut. 2010. Antioxidant and toxicity activities of *Artocarpus lakoocha* Roxb. heartwood extract. Journal of Medicinal Plants Research. 4(10): 947-953.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd edn. McGraw-Hill Book Company, New York, New York.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for the digestion of forage crops. Journal of the British Grassland Society. 18: 104-111.

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

PO-03-23

The improving nutritional value of banana tree using microorganism on digestibility of beef cattle using In vitro gas production technique

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INTRODUCTION

Crop residues are the main source for ruminant diets in developing countries. These feeds are imbalanced particularly deficient in protein, mineral, vitamins. The banana tree is widely planted in the tropics and is easy to plant and grows well in large areas. The main product is fruit as food for human but also there are by-products especially the stems, although poor quality, protein content ranges from 2.0-4 % and crude fiber about 21.0 % respectively (Moran, 2005). But, no increased protein content when silage without additive feeds (Kandee et al, 2015), however, produce good quality silage when ensiled with high carbohydrate feeds such as molasses or root vegetable (Moran, 2005). Supplementation with protein sources and used of microorganism increased the utilization of low quality roughage in ruminants (Wanapat et al., 2011, Polyorch et al., 2013). The use of microorganism to convert carbohydrates, lignocelluloses and other industrial wastes into foodstuffs rich in protein is possible due to the following characteristics of microorganisms. Gao et al. (2008) showed that as microbial inoculation has proven efficacious in improving ensiling characteristics of straws. Polyorach et al. (2013) reported that yeast fermented cassava chip product (YEFECAP) could enrich of cassava protein content from 2% CP up to 47.5% CP with high in lysine.

Incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition. Because yeast are facultative anaerobic, they may have a beneficial effect on the growth of LAB by utilizing lactic and organic acids (Yang et al., 2006). Therefore, there no only yeast as microorganism for silage, the effective microorganisms (EM) is a best source of microbial for silage too, from a commercial stand point for silage production, EM is very suitable because it is eco-friendly, organic, effective in all conditions, easy to handle and safe for human health. The main functions of EM are to produce substances that can act as an antioxidant, to inhibit harmful microbial species, and to detoxify harmful substances simultaneously. Therefore, the objective of this study was to determine the improving nutritional value of banana tree using microorganism on digestibility of beef cattle using *in vitro* gas production technique.

MATERIAL AND METHODS

Preparation of banana tree silage

Banana tree were chopped into small pieces (2-3 cm length) then mixed with rice bran and microorganism at proportion 10:2:1, packing into plastic bag, then, banana tree silage with microorganism were sampled at 0, 7 and 14 days, respectively.

Experimental design and dietary treatments

This study was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a 2x3 factorial arrangement in a Completely randomized design (CRD). The treatments contained 2 facors, factor A were 2 levels of microorganism sources (yeast and EM) and factor B were 3 levels of silage time (0, 7 and 14 days). Rice straw was used as a roughage source. Samples of banana tree silage with microorganism were dried at 60 °C, then ground to passa1-mm sieve (Cyclotech Mill,Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1995), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991). The ingredients and chemical compositions of concentrate and rice straw

used in the *in vitro* experiment are shown in Table 1.

Animals and preparation of rumen inoculums

Two, male 2-year-old, rumen fistulated beef cattles with an initial BW of 280 ± 15 kg were used as rumen fluid donors. Beef cattles rumen fluid was collected from animals fed with concentrate (14.0 % CP and 80.6 % TDN) at 0.5% of BW in to equal portions, at 07.00 h and 16.00 h and rice straw was fed on ad libitum basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 20 d before the rumen fluid was collected. On day 20, 1000 ml rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

In vitro fermentation of substrates

Sample of each total mixed substrate (200 mg), following respective treatments were weighed into 50 ml serum bottles. For each treatment, three replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39 °C under continuous flushing with CO_2. Thirty milliliters of rumen inoculum mixture were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39 °C (96 h) for *in vitro* gas test. Thirty minutes after starting the incubation, the bottles were gently mixed and then mixed three times every 3 h. For each sampling time, three bottles containing only the rumen inocula were included within each run and the mean gas production values of these bottles were used as blank. The blank values were subtracted from each measured value to give the net gas production.

Sample and analysis

During the incubation, data of gas production was measured immediately after incubation at 0, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

$$y = a + b(1-e^{(-ct)})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a + b) = the potential extent of gas production. y = gas produced at time "t".

In vitro degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left from above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963).

Statistical analysis

All data were analyzed as a 2x3 factorial arrangement in a Completely randomized design (CRD) using the general linear procedures in PROC GLM of SAS (1998). Data were analyzed using the model:

$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ij}$

Where Y is observations, μ is overall mean, A_i is effect of factor A (Yeast sources, i = 1 to 2), B_j is effect of factor B (level of urea supplementation, j = 1 to 3), AB_{ij} is interaction between factor A and B and C, and ε_{ij} is the residual effect.

Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980), and differences among means with P.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of banana tree silage as difference sources of microorganisms and during difference times of silage were showed in Table 1. Interaction between microorganism source and time of silage did not affect ($p>0.05$) on DM, OM, NDF and ADF while CP was highest ($p<0.05$) when using EM as microorganism source and fermented for 14 days (3.73%CP).

The DM, OM, CP and NDF of silage were significant difference ($p<0.05$) by DM and NDF were decreased while OM and CP were increased when increasing silage time. However, DM, NDF and ADF were non-significant difference ($p<0.05$) when compared between microorganism sources while OM and CP in EM group were significant higher ($p<0.05$) than yeast group.

Cumulative gas production for each of the substrate treatments is presented as gas production and values for estimated parameters obtained from the kinetics of gas production model for substrates studied are given in Table

2. Microorganism source, silage time and interaction between microorganism sources and silage time did not affected on the intercept value (a), gas production from the insoluble fraction (b), gas production rate constants for the insoluble fraction (c), potential extent of gas production (a + b) and cumulative gas production at 96 h., and *In vitro* degradability. However, *In vitro* degradability tended to increase IVDMD and IVOMD of banana tree silage when increasing time of fermented of silage.

The process of protein enrichment of animal feed using the microorganism to improve the nutritional value of local feed resources has been evaluated (Oboh, 2006, Wanapat et al., 2011, Polyorach et al., 2013, 2014). This method of upgrading the protein content of local feed resources has been developed. Recently, Polyorch et al. (2012) demonstrated that using microorganism (*Saccharomyces cerevisiae*) could improve nutritional value of cassava chip especially crude protein from 2% CP up to 47.5% CP and when using yeast fermented cassava product (YEFECAP) with 300 g/h/d mangosteen peel powder supplementation could improve rumen fermentation efficiency, digestibility, milk production and protein content, and economical return of lactating dairy cows fed on rice straw (Polyorach et al., 2015).

CONCLUSION

Base on this study could be concluded that microorganism could improve nutritional value of banana tree by when increasing silage time, DM and NDF were decreased while OM and CP were increased, in additional, OM and CP in EM group were higher than in yeast group. For *In vitro* degradability was tended to increase when increasing silage time. However, the used of microorganism fermented with banana tree in *in vivo* trial should be conducted.

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Table 1. Effects of microorganism on chemical composition of banana tree silage at different silage time.

Microorganism source	Time silage (day)	Chemical composition (% of DM)				
		DM	OM	CP	NDF	ADF
Yeast	0	21.65[a]	77.16[c]	3.48[b]	63.67[a]	47.27
	7	11.08[b]	86.00[b]	3.66[b]	62.64[a]	43.96
	14	9.77[b]	88.70[a]	4.01[b]	51.65[b]	41.82
EM	0	22.85[a]	78.35[c]	3.46[b]	63.07[a]	47.86
	7	11.31[b]	88.92[a]	5.04[b]	62.35[a]	43.28
	14	10.53[b]	90.24[a]	7.46[a]	52.16[b]	42.88
SEM		0.86	0.89	0.69	2.99	2.64
Comparison						
Microorganism source		ns	*	*	ns	ns
Time silage		**	**	*	**	ns
Interaction		ns	ns	*	ns	ns

ns = Non-significant, SEM = Standard error of the mean, EM = Effective microorganism.

Table 2. Effects of microorganism on the gas production value and In vitro degradability and cumulative of gas production of banana tree silage at different silage time.

Microorganism source	Time silage (day)	Gas kinetics[3]				Gas (96h) mL /0.2 g DM substrate	*In vitro* degradability	
		a	b	c	a+b		IVDMD	IVOMD
Yeast	0	1.44	33.75	0.051	35.19	35.05	55.57	73.78
	7	1.89	40.41	0.071	42.31	42.047	56.78	75.91
	14	2.11	43.16	0.049	45.28	46.13	58.56	77.38
EM	0	1.39	34.94	0.107	36.27	37.013	51.96	70.78
	7	2.68	39.62	0.044	42.31	42.03	61.34	75.88
	14	2.28	43.44	0.056	45.73	46.39	60.54	78.63
SEM		0.214	0.163	0.19	0.176	0.195	0.566	0.511
Comparison								
Microorganism source		ns	ns	ns	ns	ns	ns	ns
Time of fermented		ns	ns	ns	ns	ns	ns	ns
Interaction		ns	ns	ns	ns	ns	ns	ns

ns = Non-significant, SEM = Standard error of the mean, EM = Effective microorganism, [3]a = The gas production from the immediately soluble fraction, b = The gas production from the insoluble fraction, c = The gas production rate constant for the insoluble fraction (b), a+b = The gas potential extent of gas production.

REFERENCES

AOAC., 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Gao, L., Yang, H., Wang, X., Huang, Z., Ishii, M., Igarashi, Y., Cui, Z., 2008. Rice straw fermentation using lactic acid bacteria. Bioresour. Technol. 99, 2742–2748.

Kandee, T., Chanthakoun, V., and Keohavong, B. 2015. The effect of taro and banana pseudo-stem (*Musa Aduminata*) silage with rice bran as basal diet on the growth performance of Lao native pigs. Souphanouvong Journal 1:16-20.

Moran, J. 2005. Tropical dairy farming: Feeding management for small holder dairy farmers in the humid tropics. Department of Primary Industries. 312 pp.

Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of *Sacchromyces cerevisiae* and *Lactobacillus* spp. solid media fermentation technique. Electron. J. Biotechnol. 9:46-49.

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Polyorach, S., Wanapat, M., and Wanapat, S. 2013. Enrichment of protein content in cassava (*Manihot esculenta* Crantz) by supplementing with yeast for use as animal feed. Emirat. J. Food Agric. 25:142-149.

Polyorach, S., Wanapat, M., and Cherdthong, A. 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on fermentation parameters using *in vitro* gas production technique. Asian-Aust. J. Anim. Sci. 27(1): 36-45.

SAS., 1998. User's Guide: Statistic, Version 6, 12th Edition. SAS Inst. Inc., Cary, NC.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics, McGraw Hill Book Co, New York.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops Current Contents. Journal of the British Grassland Society. 18:104-111.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583–3597.

Wanapat, M., Polyorach, S., Chanthakhoun, V. and Sornsongnern, N. 2011. Yeast-fermented cassava chip protein (YEFECAP) concentrate for lactating dairy cows fed on urea–lime treated rice straw. Livest. Sci., 139: 258–263.

Yang, S.Y., Ji, K.S., Baik, Y.H., Kwak, W.S., McCaskey, T.A., 2006. Lactic acid fermentation of food waste for swine feed. Bioresour. Technol. 97: 1858–1864.

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In vitro Gas Production Kinetics and Digestibility as Influenced by Spent Coffee Grounds and Napier Grass Varieties

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ABSTRACT

This study aimed to investigate the effect of spent coffee grounds levels and two Napier grass (*Pennisetum purpureum)* varieties as a forage source on gas production kinetics and digestibility by using *in vitro* techniques. The experimental design was a 2×5 factorial arrangement in a completely randomized design (CRD). Factor A was napier grass varieties (NGV): green (GNG) and purple (PNG) napier grass and factor B was five levels of spent coffee grounds (SCG) supplementation at 0, 5, 10, 15 and 20 mg with 0.5 g of forage and concentrate ratio at 60:40. The gas production from the insoluble fraction (b), gas production rate (c), the potential extent of gas production (a + b) and cumulative gas production (96 h of incubation) were affected by NG and NG*SCG ($p<0.05$). *In vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) were increased by PNG, while there were decreased linearly when increasing levels of SCG ($p<0.01$). In addition, *in vitro* true digestibility was higher for PNG than for GNG ($p<0.01$). Microbial mass was decreased linearly ($p<0.05$) when supplementation of SCG at 20 mg. Based on this study, it could be concluded that PNG could improve the gas production kinetics and digestibility, whereas supplementation of SCG at 5-10 mg maintaining gas production kinetics, digestibility and microbial mass.

INTRODUCTION

Napier grass (*Pennisetum purpureum* schum.) is a perennial forage crop with high growth rate, high productivity and good nutritive value and mostly used for cut and carry system over the tropical and sub-tropical area in the world (Wijitphan et al., 2009). It is the most popular perennial forage recommended for the intensively managed smallholder crop-livestock farming systems in Thailand. However, limit information is available on the use of purple napier grass in animal diets.

Agricultural by-products from processing crops and food products have received much attention as feed alternatives because of their consistent and mass production. Food by-products would also likely be inexpensive because of their classification as a waste product (Seo et al., 2015). Coffee is one of the world's most widely consumed beverages, and spent coffee grounds (SCG), the solid residues obtained from the treatment of coffee powder with hot water to prepare instant coffee, are the main coffee industry residues with a worldwide annual generation of 6 million tons (Tokimoto et al., 2005). The SCG has been studied by nutritional researchers as a source of dietary fiber and grains in animal feeds (Campbell et al., 1976; Xu et al., 2007). However, information on the use of SCG supplementation and comparison of purple and green Napier grass in ruminants has been limited. Therefore, the aim of this study was to determine the effect of levels of SCG and two varieties of napier grass on ruminal digestibility and kinetics of *in vitro* gas production.

MATERIALS AND METHODS

Treatments and experimental design

The experimental design was a 2x5 factorial arrangement in a completely randomized design (CRD) and the dietary treatments were two Napier grass: cv. Pakchong1 (green) and (purple) and five levels of SCG supplement (0, 5, 10, 15 and 20 mg) with 0.5 g of forage and concentrate ratio at 60:40. Napier grass was harvested at the maturing stage after 45 d of re-growth. SCG was collected from Amazon coffee shop, Phangkhon, Sakon Nakhon, Thailand; and sundried for 2 days. Samples of SCG, forage and concentrates were dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analy4sis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1995), neutral detergent ?ber (NDF) and acid detergent ?ber (ADF) according to Van Soest *et al.* (1991). The ingredients

and chemical compositions of concentrate, NG and SCG used in the *in vitro* experiment are shown in Table 1.

Animals and preparation of rumen inocula

Two male, rumen-fistulated crossbred (Brahman×native) beef cattle with body weight (BW) of 400 ± 40.2 kg were used as rumen fluid donors. Rumen fluid was collected from animals fed with concentrate (16% CP) at 0.5% of BW in two equal portions, at 07:00 h and 16:00 h and rice straw was fed on ad libitum basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 14 days before the rumen fluid was collected. On day 15, 1000 mL rumen liquor was obtained from each of the cattle before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks and then transported to the laboratory. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39° C under continuous flushing with carbon dioxide and 40 ml of rumen inocula mixture were added into each bottle under carbon dioxide flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39° C for in vitro gas test. During the incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 36, 48, 72 and 96 h. Cumulative gas production data were fitted to the model of Ørskov & McDonald (1979).

Two male, rumen-fistulated crossbred (Brahman×native) beef cattle with body weight (BW) of 400 ± 40.2 kg were used as rumen fluid donors. Rumen fluid was collected from animals fed with concentrate (16% CP) at 0.5% of BW in two equal portions, at 07:00 h and 16:00 h and rice straw was fed on *ad libitum* basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 14 days before the rumen fluid was collected. On day 15, 1000 mL rumen liquor was obtained from each of the cattle before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks and then transported to the laboratory. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39°C under continuous flushing with carbon dioxide and 40 ml of rumen inocula mixture were added into each bottle under carbon dioxide flushing. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39°C for *in vitro* gas test. During the incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 36, 48, 72 and 96 h. Cumulative gas production data were fitted to the model of Ørskov & McDonald (1979).

Determination of fermentation parameters

In vitro degradability was determined after termination of incubation at 96 h, when the contents were ?ltered through pre-weighed Gooch crucibles (40 mm of porosity) and residual DM was estimated. The percent loss in weight was determined and presented as *in vitro* DM degradability (IVDMD). The dried feed sample and residue left above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry 1963). At 48 h post inoculation a set of sample was determined *in vitro* true digestibility according to Van Soest *et al.* (1991). The amount of microbial mass was calculated according to the following equation: microbial mass (mg) = mg substrate truly degraded - (ml gas volume x 2.2) (Blümmel *et al.* 1997).

Statistical analysis

All data were analyzed as a 2x5 factorial arrangement in a completely randomized design (CRD) using the general linear procedure in PROC GLM of SAS (1996). The statistical model included terms for NG, SCG level, and the NGxSCG level interactions. Trend of SCG level responded was performed by orthogonal polynomials.

RESULTS AND DISCUSSION

Cumulative gas production and parameters of kinetics gas

Gas production kinetics including the gas production from the insoluble fraction (b), the potential extent of gas production (a + b) and accumulated gas production (at 96 h of incubation) were affected by NG ($P < 0.05$), while were not affected by SCG supplementation ($P > 0.05$) (Table 2). In contrast, Seo et al. (2015) reported that including SCG as a roughage source in the diet decreased total gas production. Furthermore, the gas production from the immediately soluble fraction (a) and the gas production rate constant for the insoluble fraction (c) were not affected by dietary treatments ($P > 0.05$).

In vitro digestibility and microbial mass

The IVDMD and IVOMD were linearly decreased with increasing level of SCG ((P < 0.05) (P < 0.05) and were influenced by NG (P<0.01) (Table 3). This result was probably due to the increased fiber contents, since the SCG contained 73.7% and 39.1% of NDF and ADF, respectively. Similarly, Seo et al. (2015) reported *in vitro* NDF digestibility of the diets containing SCG were lower than those of the control group. Moreover, *in vitro* true digestibility was affected by NG (P < 0.05) (P < 0.01). The MB produced in the rumen by micro-organisms is the major source of protein for the ruminants and the prediction of efficiency of MB production is very important in ruminant nutrition (Weisbjerg et al., 1996). Microbial mass was linearly decreased when supplemented with SCG at 20 mg.

CONCLUSIONS

Based on this experiment, it could be concluded PNG improved kinetics gas, cumulative gas production (96 h), *in vitro* degradability and *in vitro* true digestibility, while supplementation of SCG at 5-10 mg of substrate maintaining *in vitro* kinetics gas, digestibility and microbial mass.

Keywords: *Pennisetum purpureum*, spent coffee grounds, gas production kinetics, digestibility

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Table 1. Ingredients and chemical composition of concentrate, napier grass (NG) and spent coffee grounds (SCG).

Item	Concentrate	Green Napier grass	Purple Napier grass	SCG
Ingredient	----------% of DM ----------			
Cassava chip	66.8	-	-	-
Whole cottonseed	1.0	-	-	-
Soybean meal	9.0	-	-	-
Rice bran	7.0	-	-	-
Urea	2.2	-	-	-
Molasses	3.0	-	-	-
Salt	0.5	-	-	-
Sulfur	0.5	-	-	-
Mineral and vitamin mixture	1.0	-	-	-
Chemical composition				
Dry matter, g/kg	87.2			
	---------- % of DM ----------			
OM	92.9	89.9	90.9	98.3
CP	16.1	10.6	10.1	16.2
NDF	21.4	72.2	70.3	73.7
ADF	13.0	47.2	46.7	39.1
Ash	7.1	10.1	9.1	1.7

Table 2. The effect of spent coffee grounds (SCG) supplementation and napier grass (NG) varieties on gas kinetics and cumulative gas production (96 h).

Trt	NG	SCG (mg)	Gas kinetics[1]				Gas[2]
			a	*b*	*c*	*a*+*b*	
T1	GNG	0	2.7	117.6	0.04	120.2	115.5
T2		5	2.3	117.6	0.05	119.9	119.9
T3		10	3.5	108.9	0.05	112.4	113.4
T4		15	2.3	122.9	0.04	125.1	124.9
T5		20	1.7	125.1	0.05	126.8	127.3
T6	PNG	0	3.7	114.8	0.06	118.5	120.4
T7		5	3.0	128.9	0.04	131.9	129.3
T8		10	3.6	139.9	0.04	143.5	140.3
T9		15	4.8	132.5	0.04	137.2	132.0
T10		20	3.7	136.0	0.03	139.6	130.7
SEM			0.55	4.40	0.003	4.10	4.20
Comparison							
NG			ns	*	ns	*	*
SCG			ns	ns	ns	ns	ns
NG*SCG			ns	ns	ns	ns	ns
Orthogonal polynomial							
L			ns	ns	ns	ns	ns
Q			ns	ns	ns	ns	ns
C			ns	ns	ns	ns	ns

[1]a, The gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); a+b, the potential extent of gas production; [2]Gas, Gas (96 h), mL/0.5 g dry matter substrate.

Table 3. The effect of spent coffee grounds (SCG) supplementation and napier grass (NG) varieties on digestibility and microbial mass.

Trt	NG[1]	SCG[2] (mg)	Degradability (%)		True digestibility (%)	Microbial mass, mg
			IVDMD	IVOMD		
T1	GNG	0	55.3	66.5	62.9	24.3
T2		5	53.8	62.9	68.0	26.6
T3		10	51.5	59.7	64.5	20.4
T4		15	52.0	60.2	64.7	23.1
T5		20	53.6	62.4	60.8	18.3
T6	PNG	0	60.6	69.1	75.9	21.9
T7		5	60.1	69.6	71.9	21.6
T8		10	59.2	67.3	70.7	22.7
T9		15	54.8	62.1	67.7	23.2
T10		20	51.5	60.2	70.0	14.7
SEM			0.93	0.96	0.75	1.16
Comparison						
NG			**	**	**	ns
SCG			*	**	ns	*
NG*SCG			ns	ns	ns	ns
Orthogonal polynomial						
L			**	**	ns	*
Q			ns	ns	ns	ns
C			ns	ns	ns	ns

REFERENCES

AOAC. 1995. Official Method of Analysis, 16th ed. Animal Feeds: Association of Official Analytical Chemists, VA, USA.

Blümmel, M., H. P. S. Makkar, and K. Becker. 1997. *In vitro* gas production: a technique revisited. J. Anim. Physiol. Anim. Nutr. 77:24–34.

Campbell, T. W., E. E. Bartley, R. M. Bechtle, and A. D. Dayton. 1976. Coffee grounds. I. Effects of coffee grounds on ration digestibility and diuresis in cattle, on *in vitro* rumen fermentation, and on rat growth. J. Dairy Sci. 59:1452–1460.

Menke, K. H., and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev. 28:7–55.

ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., Camb. 92:499–503.

SAS. 1996. SAS/STAT user's guide: statistics. 5th edn. SAS Inst., Cary, NC, USA.

Seo, J., J. K. Jung, and S. Seo. 2015. Evaluation of nutritional and economic feed values of spent coffee grounds and *Artemisia princeps* residues as a ruminant feed using *in vitro* ruminal fermentation. PeerJ 3:e1343. doi 10.7717/peerj.1343

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the digestion of forage crops. J. Br. Grassl. Soc. 18:104–111.

Tokimoto, T., N. Kawasaki, T. Nakamura, J. Akutagawa, and S. Tanada. 2005. Removal of lead ions in drinking water by coffee grounds as vegetable biomass. *J. Colloid Interface Sci*. 281:56–61.

Xu, C. C, Y. Cai, J. G. Zhang, and M. Ogawa. 2007. Fermentation quality and nutritive value of a total mixed ration silage containing coffee grounds at ten or twenty percent of dry matter. J. Anim. Sci. 85:1024–1029.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Weisbjerg, M. R., T. Hvelplund, S. Hellberg, S. Olsson, and S. Same. 1996. Effective rumen degradability and intestinal digestibility of individual amino acids in different concentrates determined *in situ*. Anim. Feed Sci.Technol. 62:179–188.

Wijitphan, S., P. Lorwilai, and C. Arkaseang. 2009. Effect of cutting heights on productivity and quality of king napier grass (*Pennisetum purpureum* cv. King grass) under irrigation. Pak. J. Nutr. 8:1244–1250.

PO-03-25 Effects of Lactobacillus plantarum on the nutritive value of TMR prepared mainly Italian Ryegrass silage for lactational dairy cows

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Introduction

The feed costs in the dairy industry are a big burden for farmers because of vicious circle by lowing the feed self-sufficiency rate and increasing the price of corn. In fact, silages are the process used for preserving forage crops long term in some countries including Korea, Japan, and China.

Although it is known that Italian Ryegrass (IRG) has a high nutritive value, there is some difficulty to ensile with good quality because of many negative factors during the manufacturing and storage process of forage. Microbial inoculants often used as an additives to improve the fermentative characteristics and storability of silages. *Lactobacillus* strains have been used as silage inoculants leading to increase lactate concentration and decrease pH, gas, and protein decomposition. Also, it is fed to farm animals to improve intestinal microbial balance due to elimination or reduction of undesirable microorganisms. In recent years, it is evident that *L. plantarum* could be improve silage fermentative characteristics, used as a starter or adjacent culture for fermented foods, and enhance the antifungal and probiotic effects (Ely et al., 1981; Filya, 2003; Tyrolova and Vyborna, 2008; Valan Arasu et al., 2014).

Objective

This study were conducted to evaluate effects of *Lactobacillus plantarum* on the nutritive value of TMR, which made with Italian Ryegrass silage (IRGS) mainly, milk production and blood metabolites in lactational dairy cows.

Materials and Methods

Twenty four multiparous Holstein lactating cows were randomly assigned to four treatments (IRGS 25.01% (T1), IRGS 25.01% + *L. plantarum* culture (T2), IRGS 17.32% + Alfalfa 9.24% (T3), IRGS 17.32% + Alfalfa 9.24% + *L. plantarum* culture (T4)) and blocked according to parity, expected calving date, and level of milk production during their previous lactation.

The diets were formulated to supply sufficient metabolizable energy for cows producing 32 kg/d of milk with a predicted DMI of 22.0 kg/d and NEL 32.5 M?/d. Feed samples were collected once twice weekly, determined according to the method of the AOAC (1995) for chemical composition, and determined according to the methods of Van Soeat et al. (1991) for NDF and ADF (Table 1).

The culture of *L. plantarum* (2x107cfu/ml * 5ml/head) (T2 and T4) was dissolved by distilled water (5ml/1000ml) and was sprayed just prior to feeding experimental TMR one times daily at 09:00 h. It was sprayed only water (1000ml) for T1 and T2. Cows weighed biweekly, and milked at 06:00 and 16:00 h and analyzed using the LactoScope (MK2, Delta Instruments, Netherlands) for milk composition.

Blood metabolites were determined using a Blood Autoanalyzer (Hitachi, 7180, Japan).

Data were analyzed by ANOVA using the general linear models procedure of SPSS version 17.0 software. Differences among means were tested using the Duncan's multiple range-test and declared significant at $p < 0.05$ unless otherwise noted.

Results and Discussion

Middle size (8-19㎜) of particle distribution was increased at TMR (T3 and T4, 27.30%) added Alfalfa hay than TMR (T1 and T2, 23.34%) with IRGS in the main. Also, peNDF8.0 was higher in TMR fed T3 and T4 treatments (17.90%) than TMR fed T1 and T2 treatments (16.78%)

Although there were no significant differences among treatments in milk production, cows fed the TMR supplied with *L. plantarum* culture tended to have a higher milk yield than those fed TMR no addition.

In milk composition, total solid content was lower for those having high milk yield than for cows having low milk yield, and was increased for cows fed TMR blending of alfalfa when adding with *L. plantarum* culture ($p<0.05$).

Nocek et al. (2002) showed an increase in mean daily low ruminal pH, mean nadir pH, and DM digestibility

of specific forages and high-moisture corn with no effect on DMI when cows were supplemented with similar microbial products. In the present study, DMI was decreased among groups of cows that had fed TMR supplied with *L. plantarum* culture. However, feed efficiency tend to be higher for was cows that had fed TMR supplied with *L. plantarum* culture than for those fed TMR without *L. plantarum* culture.

Blood metabolites ranged within normal limits in all treatments. Supplementing with *L. plantarum* culture had no influence on blood glucose, AST, ALT, and cholesterol compared with not receiving *L. plantarum* culture in dairy cows fed experimental TMRs. In particular, blood urea nitrogen (BUN) was significantly decreased in treatments (T2 and T4) with *L. plantarum* ($p<0.05$). According to these results, it suggest that *L. plantarum*-treated TMR was increased milk production and milk solid content, and improved feed efficiency on the lactational dairy cows fed IRG silage-based TMR to 17.32% ratio.

Table 1. Ingredient and chemical composition of TMRs used during experiment

	T1	T2	T3	T4
Ingredient composition	……*(% of DM)*……			
Concentrates	39.39		45.45	
Corn	7.91		9.12	
Full-fat soybeans	2.06		-	
DDGS	3.69		2.13	
Corn silage	12.10		13.97	
IRG silage	**25.01**		**17.32**	
RS silage	**7.43**		-	
Alfalfa	-		**9.24**	
Mixture[1)]	2.41		2.77	
Total	100.0		100.0	
L. plantarum, $2x10^7$cfu/ml	-	5ml/head	-	5ml/head
Chemical composition	……*(% of DM)*……			
Dry Matter	61.47		60.24	
Crude Protein	15.85		15.46	
Ether extract	6.75		6.18	
Crude Fiber	19.57		19.54	
Crude Ash	10.74		10.16	
Neutral detergent Fiber	37.06		36.33	
Acid detergent fiber	25.15		26.83	
Ca	1.40		1.30	
P	0.42		0.41	

[1)] limestone, Sodium bicarbonate, Salts, vitamin, and mineral

Table 2. Effects of *L. plantarum* culture addition on milk production in dairy cows

	IRG TMR				
	T1	T2	T3	T4	*p*-value
Milk yield(ℓ/day/head)	33.75	33.89	37.30	37.85	0.696
Milk Fat(%)	3.83	3.60	4.25	4.34	0.177
Milk Protein(%)	3.00	3.10	3.18	3.24	0.826
Lactose(%)	4.79	4.79	4.76	4.81	0.867
Total solids(%)	13.83^{a}	13.69^{a}	12.10^{c}	12.57^{b}	<0.05
Milk Urea Nitrogen(mg/dL)	19.07^{a}	18.36^{a}	8.37^{b}	8.82^{b}	<0.05
Citrate, mg/L	3.10	3.00	2.86	2.87	0.335
Free fatty acid, mEq/dL	1.40	1.53	1.04	1.09	0.155

T1: IRG 25.01%, T2: IRG 25.01%+*L. plantarum*, T3: IRG 17.32%+Alfalfa 9.24%, T4: IRG 17.32%+Alfalfa 9.24%+*L. plantarum*

Table 3. Effects of *L. plantarum* culture addition on feed intake, feed efficiency, and body weight in dairy cows

Items	T1	T2	T3	T4	*p*-value
Feed Intake(kg/day/head)	34.87^{ab}	32.69^{b}	35.89^{a}	34.13^{ab}	<0.05
DMI Intake(kg/day/head)	21.01	19.70	21.62	20.56	-
Particle size distribution					
>19㎜	19.65		18.40		-
8-19㎜	23.34		27.30		-
<8㎜	57.01		54.30		-
peNDF$_{8.0}$	16.78		17.90		-
peNDF$_{19.0}$	9.11		10.20		-
Feed efficiency	0.968	1.037	1.039	1.109	-
BW(kg)	666.6	640.0	674.0	701.6	-
Change in BW(kg/day)	-0.1	-0.6	0.5	0.4	-

T1: IRG 25.01%, T2: IRG 25.01%+*L. plantarum*, T3: IRG 17.32%+Alfalfa 9.24%, T4: IRG 17.32%+Alfalfa 9.24%+*L. plantarum*

Table 4. Effects of *L. plantarum* culture addition on blood metabolites in dairy cows

Items	T1	T2	T3	T4	*p*-value
Glucose(mg/dl)	54.00	51.83	54.17	51.00	0.855
AST(IU/L)	82.50	88.67	83.67	83.50	0.946
ALT(IU/L)	23.67	24.00	29.00	24.50	0.356
Cholesterol(mg/dl)	211.33	220.83	215.50	206.33	0.922
BUN(mg/dl)	19.97[a]	17.08[a]	17.70[a]	13.10[b]	$p<0.05$

T1: IRG 25.01%, T2: IRG 25.01%+*L. plantarum*, T3: IRG 17.32%+Alfalfa 9.24%, T4: IRG 17.32%+Alfalfa 9.24%+*L. plantarum*

References

Ely, L. O., Sudweeks, E. M., and Moon, N. J. 1981. Inoculation with *Lactobacillus plantarum* of alfalfa, corn, sorghum, and wheat silages. Journal of Dairy Science. 64:2378-2387.

Filya, I. 2003. The effect of *Lactobacillus buchneri* and *L. plantarum* on the fermentation, aerobi c stability, and ruminal degradability of low dry matter corn and sorghum silages. Journal of Dai ry Science. 86:3575-3581.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle, and J. G. Allman. 2002. Ruminal supplementation of dir ect-fed microbials on diurnal pH variation and situ digestion in dairy cattle. J. Dairy Sci. 85:429-43 3.

Tyrolova, Y. and V´yborna, A. 2008. Effect of the stage of maturity on the leaf percentage of lu cerne and the effect of additives on silage characteristics. Czech Journal of Animal Science. 53 (8): 330-335.

Valan Arasu, M., Jung, M.W., Kim, D.H., Ilavenil, S., Lee, K.D., Choi, G.J., Al-Dhabi, NA and Choi, K.C. 2014. Isolation and characterization of Lactobacillus plantarum KCC-19 from crimson silage. Journal of Pure and Applied Microbiology. 8(5):3575- 3587.

PO-03-27

Effects of dietary protein levels on digestibility, nitrogen-balance and growth performance of fattening black goat (Capara hircus coreanae)

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Abstract

This experiment was to determine the nutrient digestibility and growth performance of fattening goats, which were fed diets with different crude protein (CP) levels. Six fattening weathers of black goats [32.43 ± 2.67 kg body weight (BW)] were used in each trial in a replicated 3x3 Latin square design. Weathers individually housed in metabolic cages with 10 days of adaption to diets and 5 days of experimental measurements. Each of fattening Korea native goat was fed three diets that were formulated to contain low (14%, trial 1), medium (16%, trial 2), and high (19%, trial 3) levels of crude protein (CP). In chemical composition, CP contents analyzed were 13.92%, 15.99%, and 19.36% for low, medium, and high CP diets, respectively. Intakes of dry matter were higher in trial 2 than others, no significantly($P>0.05$). Dry matter intake (DMI) were 778.72, 822.36 and 772.53 g/day. Average daily gains (ADG) were 154.17, 158.33 and 155.09 g/day for each trial.In study increase in protein intake did not affects ADG and intake of DM. Nitrogen intake,digestible nitrogenincreased as the concentration of dietary protein increased ($p<0.05$). Apparent digestibility of crude protein was higher in trial 3 than others, while digestibility of neutral detergent fiber digestibility higher in trial 1 than others, significantly ($p<0.05$). The present findings indicate that the estimate of protein level for fattening goats was suitable range of 14 to 16 percent.

Objective

The economic importance of goats in the provision of animal proteins in developing countries has been extensively reviewed (Devendra, 1981). In Korea, consumption of goat meat increases recently because goat meat is a good source of desirable fatty acids, since goats deposit higher amounts of polyunsaturated fatty acids (PUFA) than other ruminants (Banskalieva et al., 2000; Mahgoup et al., 2002) and the main feed resources are natural pastures or poor quality grasses (S. H. Choi et al., 2005). Goats are well adapted to a harsh environment and limited feed and utilize marginal land to produce high protein products. However, investigation of effects of diet composition on goat carcass characteristics has been limited and there are no references on protein requirement of fattening Korea native goats.

Research has not been conducted on the effect of dietary crude protein level on intake, fattening of Korea native goats in different geographic environments. Accordingly, This study was conducted to evaluate the protein requirement for fattening Korea native goat.

Methodology

Animals and diets

The experiment was carried out using six 8-months-old male goats whose mean body weight was 32.43 ± 2.67 kg. During the experiment, the animals were kept in individual metabolism pens (1m ± 1.6 m ± 1.5 m) that permitted separate collection of feces and urine.

The experiment feed ingredients are presented in Table 1. In chemical composition, CP contents analyzed were 13.92%, 15.99%, and 19.36% for low, medium, and high CP diets, respectively.

The experiment had a replicated duplicated 3×3 Latin square design for balancing carryover effects. Diet trial order was randomly selected for each animal. Weathers individually housed in metabolic cages with 10 days of adaption to diets and 5 days of experimental measurements.

Feed was restricted and offered twice per day (at 0800 and 1700h). Animals had free access to water.

Sampling procedures

Urine was collected in a collection jar containing 15ml 4N H2SO4. After the urine was weighed, 2% of the urine volume was sampled, refrigerated and bulked for the collection period. Fecal and urine samples, each about 100g (or mL), were separately pooled and thoroughly mixed. Feed samples were collected once weekly, and orts were collected daily and DM determination on a weekly basis. Feed offered and orts were measured and recorded daily during the last 7 d of the period to calculate feed intake. These samples were dried in a forced-air oven at 60℃

and stored for further analysis.

Analytical techniques

Daily feed and orts samples were frozen and analyzed for dry matter (DM), organic matter (OM), CP, and acid detergent fiber (ADF) (AOAC, 1990). Daily fecal samples were dried in a 60℃ dry oven and analyzed for DM, OM, CP, and ADF as above. The nitrogen content of urine was determined (AOAC, 1990).

Preliminary analyses with replication as a factor in the model indicated no differences between first and second replication; therefore, the data were pooled across replicates.

Statistical analysis

A randomized complete block ANOVA was used to compare nitrogen balance data (nitrogen intake, fecal nitrogen, digestible nitrogen, urinary nitrogen and retained nitrogen) with apparent digestibility characteristics among treatments for each trial (SAS, 2009). Treatment means were compared using Tukey's test, when necessary. A statistical program (SAS, 2009) was used to perform regression analysis and create equations and graphs.

Result

Intake, daily gain, feed conversion ratio and apparent digestibility of nutrients

Average daily gain (ADG), DM intake (DMI) and feed conversion ratio of Korea native goat are presented in Table 2. ADG were 154.17, 158.33, and 155.09 g/d in the low, medium, and high protein, respectively. In the other hand, DMI was 822.36 g/d in the medium protein group and this was higher than those of low protein and high protein which were 778.72(low) and 772.53(high), no significantly($P>0.05$). Feed conversation ratio was 5.16, 5.68, and 5.79 for 13.9%, 15.9%, and 19.3% treatments of dietary CP respectively.

The apparent digestibility of nutrients is presented in Table 3. Dietary CP levels did not affect the apparent digestibility of DM, EE, CF, Ash, or ADF in treated diets. However, the apparent digestibility of CP increased linearly as dietary CP increased ($P<0.05$).

nitrogen balance and protein requirement for fattening black goat

The nitrogen intake(NI) linearly increased ($P<0.05$) as dietary CP increased. Fecal nitrogen and urinary nitrogen were 9.24%, 9.70% in the medium protein group and this was higher than those of low protein and high protein which were 8.68%, 7.70%(low) and 8.00%, 8.88%(high), no significantly($P>0.05$). Digestible nitrogen was higher in trial 3 than others, significantly($P<0.05$). Retained nitrogen(RN) was higher in trial 3 than others, no significantly($P>0.05$)

conclusion

Protein is an essential nutrient for animal growth and plays an important role in muscle growth and animal development (Mtenga and Kitaly, 1990), and earlier studies reported that ADG increased as CP level in feed increased (Hart et al., 1993; Jia et al., 1995; Choi et al., 2005). However, in the present study, dietary CP levels did not affect intake and daily gain.

The results from this study suggested that an adequate CP level in diet for achieving optimal growth performance of fattening Korean native goats might be 14% of dietary CP level above 14% seemed not to further increase fattening performance

Keywords : black goat, digestibility ,dietary crude protein, nitrogen balance

Table 1. Chemical composition of the experimental TMR feed at different crude protein contents.

Item	Treatment, CP (%)*		
	T1	T2	T3
Chemical composition**			
DM (%)	90.35±0.22^{b}	90.84±0.25^{a}	90.70±0.18^{ab}
	% DM		
CP	13.92±0.06^{c}	15.99±0.20^{b}	19.36±0.15^{a}
EE	4.59±0.20^{b}	5.86±0.20^{a}	4.77±0.28^{b}
CF	17.45±0.81^{a}	13.65±0.24^{b}	13.60±0.77^{b}
Ash	7.23±0.10^{a}	6.04±0.12^{b}	5.58±0.06^{c}
NDF	49.41±0.66^{a}	43.86±0.51^{b}	44.23±0.23^{b}
ADF	23.57±0.88^{a}	20.06±0.20^{b}	18.13±0.47^{c}

* T1: Low protein, T2: Medium protein, T3: High protein

** DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, Ash: crude ash NDF: neutral detergent fiber, ADF: acid detergent fiber

$^{a,b,and\ c}$Means with different superscript in the same row are different

Table 2. Effects of dietary crude protein level on body weight and feed intake of black goat

Item	Treatment, CP(%)*		
	T1	T2	T3
Initial body weight (kg)	33.98±1.72	33.87±2.87	33.81±3.83
Average daily gain (ADG, g/d)	154.17±33.23	158.33±80.81	155.09±53.65
Dry matter intake (DMI, g/d)	778.72±277.09	822.36±284.87	772.53±97.66
Feed conversion ratio			
DMI/ADG (g/g)	5.16±1.92	5.68±2.00	5.79±3.29

* T1: Low protein, T2: Medium protein, T3: High protein

Table 3. Apparent nutrient digestibility in black goat fed TMR feed at different crude protein contents

Item	Treatment, CP(%)*		
	T1	T2	T3
	% DM		
DM**	78.33±1.77	77.26±5.42	76.90±5.04
CP	77.19±2.54^{b}	79.61±3.52^{ab}	82.29±3.88^{a}
EE	89.70±2.32	90.11±4.37	89.72±2.34
CF	65.37±1.97^{a}	48.24±5.81^{c}	56.99±3.37^{b}
Ash	62.06±5.02^{a}	43.81±6.68^{b}	53.97±4.34^{a}
NDF	74.20±2.43^{a}	65.72±4.24^{b}	70.21±3.65^{ab}
ADF	69.72±2.01^{a}	58.48±4.38^{b}	59.39±5.68^{b}

* T1: Low protein, T2: Medium protein, T3: High protein

** DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, Ash: crude ash NDF: neutral detergent fiber, ADF: acid detergent fiber

$^{a,b \text{ and } c}$Means with different superscript in the same row are different

Table 4. Nitrogen balance in black goat fed TMR feed at different crude protein contents

Item	Treatment, CP(%)*		
	T1	T2	T3
	g/d		
Nitrogen intake(NI)	19.56±3.27^{b}	20.81±1.81^{ab}	23.93±3.03^{a}
Fecal nitrogen	8.68±1.40	9.24±1.02	8.00±0.77
Digestible nitrogen	11.03±1.92^{b}	11.92±1.62^{b}	15.93±3.37^{a}
Urinary nitrogen	7.70±1.18	9.70±3.70	8.88±3.40
Retained nitrogen(RN)	3.42±1.11	3.60±3.67	6.38±4.72
RN/NI(%)	17.20±4.29	27.43±22.88	27.35±22.28

* T1: Low protein, T2: Medium protein, T3: High protein

$^{a,b \text{ and } c}$Means with different superscript in the same row are different

Reference

Banskalieva,V., Sahlu, T., Goetsch, A.L., 2000. Fatty acid composition of goat muscles and fat depots: a review. Small Rumin. Res. 37, 255–268.

Cheva-Isarakul, B., B. Rengsirikul, and W. Liamkaeo. 1991. The minimum protein requirement for goats. In: Goat Production in the Asian Humid Tropics (Ed. S. Saithanoo and B. W. Norton), Proc. Int. Seminar, Hat Yai, Thailand, pp. 136-143.

Choi, S. H., S. W. Kim, B. Y. Park, B. D. Sang, Y. K. Kim, J. H. Myung and S. N. Hur. 2005. Effects of dietary crude protein level on growth and meat quality of Korean native goats. J. Anim. Sci. Technol. (Kor.) 45:783-788.

Cowan, R. L., E. W. Hartsook, J. B. Whelan, T. A. Long, and R. S. Wetzel. 1969. A cage for metabolism and radio-isotope studies with deer. J. Wildl. Manage. 33:204-208.

Silva, J. F. C. 1996. Methodologies of nutritional requirement determination in sheep. In: Sheep Nutrition (Ed. A. G. Silva Sobrinho, A. M. V. Batista, E. R. Siqueira, and J. F. C. Silva). FUNEP, Jaboticabal, pp. 1-68.

Smith, S. H., J. B. Holter, H. H. Hayes, and H. Silver. 1975. Protein requirement of white-tailed deer fawns. J. Wildl. Manage. 39:582-589.

PO-03-28

Effect of Thyme Essential oil and Di-sodium Fumarate on In Vitro Ruminal Nutrient Disappearance and Partitioning Factor

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INTRODUCTION

Rumen fermentation has some disadvantages because loss of energy and protein from methane emission and ammonia nitrogen ($N\text{-}NH_3$) production. Therefore, researchers have become interested in studying other alternatives for improving rumen fermentation, such as yeast extracts, organic acids (fumarate, malate), plant extracts and essential oils (EO), and probiotics (Calsamiglia et al., 2007). It is recognized that thymol and carvacrol are the main secondary compounds of thyme EO. Universally, phenolic compounds such as thymol, as regards owning a hydroxyl group have more antibacterial activity compared to other non-phenolic plant secondary metabolites (Ultee et al., 2002). Previous studies showed that the use of thyme EO and thymol resulted in a decrease in $N\text{-}NH_3$ concentration and crude protein (ICPD) disappearance (Jahani-Azizabadi et al., 2011)

Fumarate is a hydrogen sink and acting as propionate precursor in the rumen. Several studies used of fumarate (form of salts and free acid) and malate in *in vitro* and *in vivo* experiment for rumen manipulation and enhance the revenue of rumen fermentation process (García-Martínez et al., 2005; Lin et al., 2013b). Previous studies have been reported reduce in rumen fluid $N\text{-}NH_3$ concentration and increase in OMD (García-Martínez et al., 2005) with fumarate supplementation. It appears that data on the optimum doses of DSF and TEO on the *in vitro* experiments is still missing. The aim of this study was to investigate the effect of different levels of DSF and TEO on the *in vitro* ruminal nutrient disappearance and partitioning factor of a 50:50 forage: concentrate diet.

MATERIALS AND METHODS

The experimental diet used for batch cultures was a 50:50 alfalfa hay: concentrate diet which was ground to pass through 1.5-mm screen. Rumen contents were collected from 2 adults ruminally fistulated sheep (35 ± 2.5 kg, body weight) before the morning feeding and immediately strained through the four layers of cheesecloth. Animals were fed with the same diet. In an anaerobic condition,, 50 ml of buffered rumen fluid [ratio buffer to rumen fluid was 2:1 by (McDougall, 1948)] were dispensed int 125 ml bottles (N=9 per each treatment) containing 500 mg of dried experimental diet, and incubated for 24 h at 38.6º C. To prevent the over-accumulation of gas produced, head space gas pressure in each bottle was recorded using a pressure transducer at 7, 14 and 24 h of the incubation, and gas released. Gas pressure was converted into volume using an experimentally calibrated curve. Treatments were: control (no additive), 8, 10 or 12 mmol of fumarate (Disodium salt; Sigma Chemical Co., Poole, and Dorset, UK) and 100, 200, 300 or 400 µl/L (9 replicates for each treatment in 2 runs) of TEO (MONIN Company, France). Following 24 h past of the incubation, the bottles were respectively transferred to an ice bath for stoping fermentation. Then, the bottle contents were filtered (48 µm, pore size). The solid residues were oven dried (55°C for 48 hours) and used to estimate the IDMD, IOMD and ICPD disappearances. Incubated or non-incubated samples were analyzed for DM, CP, and OM (AOAC, 1997).

Partitioning factor (PF) at 24h past of the incubation was estimated as the ratio of truly degraded substrate (TDS) to the ml gas produced (Blümmel et al., 1999). Data were analyzed as a completely random design (GLM procedure, SAS 9,1) and Tukey test was used to compare the means ($P<0.05$).

RESULTS AND DISCUSSION

Effects of DSF and TEO on *in vitro* ruminal nutrients disappearance of a 50:50 alfalfa hay: concentrate diet after 24h post of the incubation are presented in **Table 1** and **2**. Relative to the control, DSF and TEO had no significant effect on ICPD and IDMD (except at 400 µl TEO/L). Results of the present study showed an 11.3 % decrease in IDMD at 400 µl/L TEO. Relative to the control, the addition of DSF at 8, 10 and 12 Mm/L resulted in an increase ($P<0.001$) in IOMD (44.3, 42 or 45.2 %, respectively) and PF (3.5, 3.4 and 3.5%, respectively) relative to those of the control. Relative to the control, the addition of 100 µl of TEO/L increased ($P<0.05$) the IOMD (47.6 %) and PF (69%). Previous studies showed that the use of fumarate resulted in an increase in the enumeration of cellulolytic and some fumarate-utilizing bacteria (Asanuma et al., 1999; Newbold et al., 2005). It seems that fumarate-utilizing

bacteria accelerate the metabolism of the intermediate products of fibrolytic bacteria such as hydrogen (Mao et al., 2007). It is recognized that phenolic compounds such as thymol, as regards owning a hydroxyl group, have antibacterial and inhibitory effects on ruminal bacteria fermentation (Ultee et al., 2002).

CONCLUSION

Findings of the present study demonstrated that DSF and TEO can result to increase in feed use efficiency and improve in the ruminal fermentation at the condition of the present study.

Table 1. Effect of di-sodium fumarate on in vitro ruminal dry matter, crude protein, organic matter disappearance and partitioning factor after 24h of incubation

	DSF (mM)				
Item	0	8	10	12	SEM*
In vitro nutrient disappearance (%)					
Dry matter	68.1	66.6	68.0	66.9	0.77
Crude protein	60.6	62.4	64.6	62.0	1.68
Organic matter	54.6^{a}	78.8^{b}	77.5^{b}	79.3^{b}	1.60
Partitioning factor	2.00^{a}	3.53^{b}	3.42^{b}	3.50^{b}	0.122

* Standard errors of the mean

$^{a, b}$ Means within a row with different letters are significantly different ($P < 0.05$).

Table 2. Effect of thyme essential oil on *in vitro* ruminal dry matter, crude protein organic matter disappearance and partitioning factor

	TEO (µl)					
Item**	0	100	200	300	400	SEM*
In vitro nutrient disappearance (%)						
Dry matter	68.1^{a}	67.3^{ab}	64.3^{ab}	62.2^{ab}	60.4^{b}	0.79
Crude protein	60.6	62.9	61.1	57.7	52.3	1.49
Organic matter	54.6^{a}	80.6^{b}	72.5^{b}	71.4^{ab}	68.6^{ab}	1.77
Partitioning factor	2.00^{a}	3.38^{b}	3.19^{ab}	2.90^{ab}	2.88^{ab}	0.123

* Standard errors of the mean

$^{a, b, c}$ Means within a row with different letters are significantly different ($P < 0.05$).

REFERENCES

AOAC, International. 1997. Official Methods of Analysis. AOAC International, Gaithersburg, MD.

Asanuma, N., Iwamoto, M. and Hino, T. 1999. Effect of the addition of fumarate on methane production by ruminal microorganisms in vitro. J. Dairy sci., 82: 780-787.

Blümmel, M., Mgomezulu, R., Chen, X., Makkar, H., Becker, K. and Ørskov, E. 1999. The modification of an in vitro gas production test to detect roughage related differences in *in vivo* microbial protein synthesis as estimated by the excretion of purine derivatives. J. Agr. Sci., 133: 335-340.

Calsamiglia, S., Busquet, M., Cardozo, P., Castillejos, L. and Ferret, A. 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J. Dairy sci., 90: 2580-2595.

García-Martínez, R., Ranilla, M., Tejido, M. and Carro, M. 2005. Effects of disodium fumarate on in vitro rumen microbial growth, methane production and fermentation of diets differing in their forage: concentrate ratio. Brit. J. Nutr., 94: 71-77.

Jahani-Azizabadi, H., Mesgaran, M. D., Vakili, A. R., Rezayazdi, K. and Hashemi, M. 2011. Effect of various medicinal plant essential oils obtained from semi-arid climate on rumen fermentation characteristics of a high forage diet using in vitro batch culture. Afric. J. Microbiol. Res., 5: 4812-4819.

Lin, B., Wang, J., Lu, Y., Liang, Q. and Liu, J. 2013b. Effects of essential oil combinations on sheep ruminal fermentation and digestibility of a diet with fumarate included. J. Anim. Feed Sci. Technol., 184: 24-32.

Mao, S., Zhang, G. and Zhu, W. 2007. Effect of disodium fumarate on in vitro rumen fermentation of different substrates and rumen bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA. Asian Australas. J. Anim. Sci., 20: 543-549.

McDougall, E. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J., 43: 99-109.

Newbold, C., López, S., Nelson, N., Ouda, J., Wallace, R. and Moss, A. 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation in vitro. Brit. J. Nutr., 94: 27-35.

Ultee, A., Bennik, M. and Moezelaar, R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Appl. Environ. Microbiol., 68: 1561-1568.

PO-03-30

Effect of glucosinolate (Sinigrin) for reducing methane emission rumen parameters and microbial community in vitro

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Introduction

The globally averaged combined land and ocean surface temperature data as calculated by a linear trend, show a warming of 0.85 ºC, over the period 1880 to 2012, when multiple independently produced datasets exist (Stocker, 2014). There have been increasing worries over greenhouse gas emissions because of their effects on climate change. Methane is one of the greenhouse gasses, which is normally generated as a result of microbial fermentation of feed components. One important group of feed additives of reducing methane (CH_4) emission is represented by glucosinolates (GSL) of glucosinolates hydrolysis products with ruminant feed resources. Sinigrin is a GSL found in *Brassicaceae* of feed additive. SIN is converted to allyl isothiocyanate (AITC) at pH 7 (Bones and Rossiter, 1996). Lila et al. (2003) reported that AITC decreased methanogenic bacteria and CH_4 production. For that reason, using SIN of dietary additive of ruminant animal diet can be practices of reducing CH_4 emission by rumen microbial fermentation.

The objective of this *in vitro* experiment was conducted to determine the effect of levels SIN and AITC of glucosinolate hydrolysate concentration of reduced CH_4 emissions by rumen microbial fermentation *in vitro*.

Materials and methods

In vitro test of rumen microbial fermentation was control and 4 treatments, composition of different types SIN (LKT laboratories, Inc. Japan) concentration as feed additives, mixed level of each 20, 40, 60 and 80 μmol of in the experimental diet (rice straw : concentration diet was 7:3). Control and treatments were 3 replications to investigate of the rumen microbial fermentation parameters at each 0, 2, 4, 6, 8, 10, 12 and 24 h for incubation time. Rumen fluid of inoculum (5%, v/v) was anaerobically transferred to serum bottles containing 100 mL rumen buffer with 1.7 g of substrates and incubated at 39°C. During the incubation, determined total gas production, CO_2 and CH_4 production by gas chromatography. At the end of each incubation times, supernatants were collected for pH value, NH_3-N concentration, microbial protein synthesis, DM digestibility and VFAs. All data were analyzed by using the PROC MIXED procedure of SAS. SIN was quantified by high performance liquid chromatography following desulfatation according to the optimized official method ISO 9167-1. Also DGGE was conducted using a D-Code system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with 8% (w/v) polyacrylamide gels containing a 35% to 45% denaturant gradient.

Results and discussion

Control and SIN treatments were increased total gas production, total VFA and DM digestibility, but pH and NH_3-N concentration were shown decrease during incubation time. Total gas production was observed as significantly higher control than in supplemented treatments after 6 h incubation ($P < 0.05$). CH_4 production was increased control compared to SIN treatments. pH value of SIN treatments was higher than control. Microbial protein synthesis of SIN treatments from 58.50 to 68.43 mg/100mL was lower than control at 24h incubation ($P < 0.05$). The SIN was efficient at CH_4 mitigation than control for all incubation times. The HPLC analysis of SIN show a decreased SIN concentration during incubation. SIN hydrolysate of AITC conversion of SIN to occur with the SIN treatments, which was high level SIN concentration. In conclusion, SIN treatment of ruminant feed additive was inhibited methane emission from ruminants with optimal ruminal fermentation. PCR-DGGE was using the samples obtained in vitro fermentation at 8 h, 10 h, 12 h and 24 h of incubation time to identify possible change in the community of methanogens associated with variations in concentration of the SIN additives. We compared different concentration of SIN with respect to their effects on methane production and fermentation. The DGGE profiles showed that variation in percent similarity was observed at latter incubation time. This means that the change was occur in the microbial community.

Conclusion

This experiment was used pure compound of SIN. It was showed that methane production was drastically reduced by SIN additives since early incubation. In addition, DM digestibility, total VFA and microbial protein synthesis were slight affected overall incubation but, it was the normal ruminal microbial fermentation condition. This experiment was confirmed that it is a powerful methane reduction substrate such as AITC. Therefore, further research is needed to determine the effect of the decreased methanogen is this experiment on an *in vivo* rumen microbial population.

Acknowledgments

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Figure 1. Effect of sinigrin additives on rumen microbial community of acrchaeal 16S rRNA determined by denaturing gradient gel electrophoresis at 16h-24h

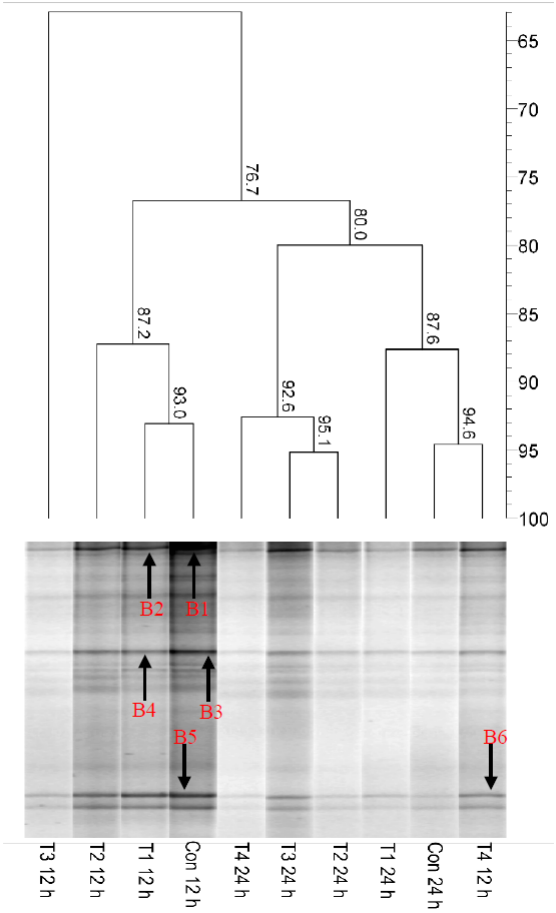

Table 1. In vitro rumen fermentation characteristics using sinigrin additives_page_1

Items	Incubation time (h)							
	0	2	4	6	8	10	12	24
pH								
Control	6.94^{c}	6.92^{c}	6.9^{b}	6.74^{c}	6.68^{c}	6.23^{b}	6.61^{b}	6.57^{b}
Sinigrin 20 μmol	6.95^{bc}	6.94^{bc}	6.91^{b}	6.75^{bc}	6.69^{bc}	6.64^{ab}	6.61^{ab}	6.62^{a}
Sinigrin 40 μmol	6.97^{a}	6.97^{ab}	6.95^{a}	6.77^{a}	6.71^{ab}	6.66^{a}	6.64^{a}	6.63^{a}
Sinigrin 60 μmol	6.96^{ab}	6.96^{abc}	6.93^{a}	6.77^{a}	6.72^{a}	6.66^{a}	6.64^{ab}	6.65^{a}
Sinigrin 80 μmol	6.97^{ab}	6.99^{a}	6.94^{a}	6.75^{b}	6.71^{a}	6.66^{a}	6.63^{ab}	6.63^{a}
S.E.M.	0.008	0.014	0.006	0.004	0.006	0.010	0.010	0.013
p value	0.0347	0.0417	0.0006	0.0011	0.0103	0.0614	0.1007	0.0091
L	0.0075	0.0049	0.0002	0.0038	0.0014	0.0095	0.0355	0.0025
Q	0.1728	0.5406	0.0217	0.0007	0.1539	0.1918	0.1662	0.0182
Total Gas Production (mL)								
Control	-	2.00^{c}	6.03^{b}	55.77^{a}	83.27^{a}	104.97^{a}	106.10^{a}	146.13^{a}
Sinigrin 20 μmol	-	4.03^{b}	2.13^{c}	34.43^{c}	56.23^{c}	71.90^{c}	66.97^{c}	108.67^{c}
Sinigrin 40 μmol	-	8.57^{a}	7.70^{a}	26.53^{d}	50.17^{d}	60.57^{d}	60.47^{d}	99.80^{d}
Sinigrin 60 μmol	-	2.73^{bc}	2.43^{c}	35.83^{c}	58.63^{c}	70.53^{c}	68.00^{c}	109.20^{c}
Sinigrin 80 μmol	-	3.10^{bc}	1.70^{c}	39.13^{b}	65.13^{b}	78.60^{b}	79.13^{b}	125.47^{b}
S.E.M.	-	0.475	0.278	0.987	1.413	0.900	1.347	1.707
p value	-	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
L		0.5621	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Q		<.0001	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001
CH_4 Production (mL)								
Control	-	0.009^{bc}	0.011^{a}	0.107^{a}	0.134^{a}	0.346^{a}	0.346^{a}	0.765^{a}
Sinigrin 20 μmol	-	0.011^{ab}	0.007^{a}	0.030^{b}	0.043^{b}	0.08^{b}	0.050^{b}	0.096^{b}
Sinigrin 40 μmol	-	0.015^{a}	0.010^{a}	0.051^{b}	0.040^{b}	0.026^{b}	0.047^{b}	0.056^{bc}
Sinigrin 60 μmol	-	0.005^{c}	0.001^{b}	0.052^{b}	0.053^{b}	0.045^{b}	0.048^{b}	0.059^{bc}
Sinigrin 80 μmol	-	0.005^{c}	0.002^{b}	0.027^{b}	0.070^{b}	0.050^{b}	0.058^{b}	0.049^{c}
S.E.M.	-	0.002	0.002	0.015	0.013	0.032	0.029	0.014
p value	-	0.0077	0.0021	0.0213	0.0026	0.0002	<.0001	<.0001
L		0.0246	0.0004	0.0144	0.0164	<.0001	<.0001	<.0001
Q		0.0141	0.6406	0.1643	0.0008	0.0004	0.0002	<.0001
Total VFA (mmol)								
Control	3.29	3.21^{ab}	4.16^{ab}	5.50^{a}	6.99^{a}	5.88^{a}	6.68^{a}	14.11^{a}
Sinigrin 20 μmol	2.96	3.09^{b}	3.85^{b}	4.68^{b}	4.55^{b}	5.25^{b}	4.97^{b}	7.97^{d}
Sinigrin 40 μmol	2.73	3.18^{ab}	3.93^{ab}	4.65^{b}	4.00^{b}	4.50^{c}	4.81^{b}	7.92^{d}
Sinigrin 60 μmol	2.84	3.56^{a}	3.97^{ab}	5.26^{a}	3.81^{b}	5.04^{b}	5.12^{b}	10.45^{c}
Sinigrin 80 μmol	2.93	3.47^{ab}	4.18^{a}	5.28^{a}	4.13^{b}	5.06^{b}	4.75^{b}	12.91^{b}
S.E.M.	0.191	0.141	0.105	0.128	0.395	0.146	0.179	0.328
p value	0.3632	0.1699	0.1721	0.0022	0.0011	0.0009	<.0001	<.0001
L	0.1904	0.0524	0.6389	0.7235	0.0004	0.0024	<.0001	0.9449
Q	0.1209	0.5439	0.027	0.0006	0.0026	0.0008	0.0009	<.0001
Acetate (mmol)								
Control	2.45	2.46	2.96	3.85^{a}	4.75^{a}	4.20^{a}	4.70^{a}	6.08^{a}
Sinigrin 20 μmol	2.23	2.38	2.75	3.41^{bc}	3.62^{b}	4.13^{a}	4.14^{b}	5.02^{b}
Sinigrin 40 μmol	2.09	2.30	2.82	3.38^{c}	3.36^{c}	3.71^{b}	4.08^{b}	5.22^{b}
Sinigrin 60 μmol	2.17	2.59	2.83	3.73^{a}	3.21^{c}	4.03^{ab}	4.38^{b}	5.77^{a}
Sinigrin 80 μmol	2.23	2.54	2.87	3.67^{ab}	3.30^{c}	3.98^{ab}	3.93^{ab}	5.98^{a}
S.E.M.	0.133	0.115	0.074	0.084	0.080	0.111	0.162	0.140
p value	0.4408	0.4207	0.4124	0.0111	<.0001	0.0853	0.05	0.001
L	0.2563	0.3349	0.6798	0.8653	<.0001	0.1634	0.028	0.2378
Q	0.1483	0.3355	0.1505	0.0047	<.0001	0.0897	0.3554	0.0003
Propionate (mmol)								
Control	0.44^{a}	0.39^{b}	0.72^{ab}	0.94^{a}	1.40^{a}	0.74^{a}	0.97^{a}	6.68^{a}
Sinigrin 20 μmol	0.29^{ab}	0.36^{b}	0.56^{c}	0.70^{c}	0.53^{b}	0.40^{b}	0.41^{b}	2.58^{d}

Table 1. In vitro rumen fermentation characteristics using sinigrin additives_page_2

Sinigrin 40 µmol	0.23^{b}	0.46^{ab}	0.60^{c}	0.69^{c}	0.36^{b}	0.39^{b}	0.36^{b}	2.14^{d}
Sinigrin 60 µmol	0.36^{ab}	0.56^{a}	0.64^{bc}	0.82^{b}	0.29^{b}	0.42^{b}	0.40^{b}	3.86^{c}
Sinigrin 80 µmol	0.28^{ab}	0.52^{a}	0.76^{a}	0.85^{ab}	0.34^{b}	0.44^{b}	0.39^{b}	5.83^{b}
S.E.M.	0.058	0.042	0.036	0.034	0.232	0.039	0.037	0.246
p value	0.19	0.0333	0.0159	0.0016	0.034	0.0005	<.0001	<.0001
L	0,2029	0.0058	0.1894	0.6146	0.0094	0.001	<.0001	0.5983
Q	0.1673	0.8695	0.0023	0.0003	0.0488	0.0005	<.0001	<.0001
A/P Ratio								
Control	5.89^{b}	6.37^{ab}	4.13^{bc}	4.08^{c}	4.27^{c}	5.74^{b}	4.89^{b}	0.92^{d}
Sinigrin 20 µmol	7.97^{ab}	6.53^{a}	4.99^{a}	4.91^{a}	6.93^{b}	10.32^{a}	10.46^{a}	1.95^{b}
Sinigrin 40 µmol	9.03^{a}	5.05^{bc}	4.68^{ab}	4.93^{a}	9.27^{a}	9.52^{a}	11.52^{a}	2.49^{a}
Sinigrin 60 µmol	6.54^{b}	4.61^{c}	4.52^{abc}	4.58^{ab}	10.98^{a}	9.95^{a}	11.05^{a}	1.50^{c}
Sinigrin 80 µmol	7.98^{ab}	4.92^{c}	3.80^{c}	4.31^{bc}	9.66^{a}	9.15^{a}	10.33^{a}	1.03^{d}
S.E.M.	0.683	0.430	0.270	0.150	0.648	0.768	1.025	0.109
p value	0.0541	0.0287	0.0737	0.0091	0.0002	0.0115	0.0060	<.0001
L	0.2351	0.0053	0.2113	0.7702	<.0001	0.0242	0.0053	0.532
Q	0.089	0.4268	0.0138	0.001	0.0053	0.0077	0.0042	<.0001
Dry matter digestibility (%)								
Control	-	21.72^{ab}	24.18^{a}	24.23^{c}	27.72^{a}	29.38^{ab}	29.89	32.13
Sinigrin 20 µmol	-	22.44^{a}	23.19^{bc}	24.81^{bc}	27.60^{a}	29.71^{a}	30.00	31.67
Sinigrin 40 µmol	-	22.29^{ab}	23.71^{ab}	25.51^{a}	27.72^{a}	29.27^{ab}	29.35	31.02
Sinigrin 60 µmol	-	20.98^{ab}	23.30^{bc}	25.45^{ab}	27.25^{a}	28.28^{bc}	29.31	31.02
Sinigrin 80 µmol	-	20.75^{b}	22.61^{c}	24.94^{ab}	26.57^{b}	27.83^{c}	29.49	31.56
S.E.M.	-	0.515	0.267	0.219	0.165	0.369	0.255	0.433
p value	-	0.1458	0.0194	0.0119	0.0026	0.0218	0.2535	0.3747
L	-	0.0628	0.0049	0.0141	0.0005	0.0031	0.0908	0.2197
Q	-	0.1431	0.7577	0.005	0.0187	0.1571	0.4464	0.1327
Microbial protein synthesis (mg/100 mL)								
Control	69.07	77.52	73.50^{ab}	65.90^{bc}	69.70	69.70	68.01	74.77^{a}
Sinigrin 20 µmol	67.80	76.04	71.81^{ab}	63.79^{c}	66.74	69.28	65.47	60.87^{b}
Sinigrin 40 µmol	68.22	75.62	68.01^{b}	68.22^{bc}	60.76	68.22	66.95	68.43^{ab}
Sinigrin 60 µmol	69.49	75.41	75.20^{ab}	69.49^{ab}	62.47	69.07	63.57	57.02^{b}
Sinigrin 80 µmol	69.70	76.68	79.63^{a}	73.50^{a}	61.37	68.64	65.68	58.50^{b}
S.E.M.	1.489	1.192	3.119	1.577	4.773	1.854	2.541	4.364
p value	0.8691	0.7243	0.1896	0.0133	0.6377	0.9818	0.7812	0.0755
L	0.5427	0.5503	0.1439	0.0018	0.1957	0.6998	0.4335	0.0249
Q	0.5091	0.2298	0.0744	0.1545	0.5373	0.7897	0.6507	0.4869
NH_3-N concentration (mg/100 mL)								
Control	0.93	0.93^{ab}	1.00	0.71	0.64	0.66	0.65	0.63^{ab}
Sinigrin 20 µmol	0.99	1.03^{a}	1.04	0.72	0.64	0.65	0.63	0.66^{ab}
Sinigrin 40 µmol	0.91	0.96^{ab}	1.04	0.76	0.73	0.59	0.64	0.69^{a}
Sinigrin 60 µmol	0.84	0.88^{b}	1.02	0.82	0.66	0.69	0.61	0.62^{ab}
Sinigrin 80 µmol	0.94	0.91^{ab}	0.96	0.61	0.60	0.69	0.65	0.60^{b}
S.E.M.	0.059	0.040	0.066	0.084	0.047	0.051	0.027	0.028
p value	0.4973	0.1505	0.9116	0.5324	0.4916	0.6485	0.8283	0.2338
L	0.5106	0.1434	0.6302	0.7244	0.6804	0.5379	0.9098	0.2296
Q	0.6799	0.3483	0.4281	0.2158	0.1769	0.3960	0.4687	0.0854

[a,b] Mean with different letter differ significantly between treatments ($p < 0.05$).

S.E.M. = standard error of the mean

Table 2. Identified bands from archaeal 16S rRNA by denaturing gradient gel electrophoresis

PCR-DGGE band	Most related taxon (GenBank accession no.)	Similarity (%)
B1	*Methanobrevibacter smithii* strain PS (NR074235)	94
B2	*Methanobrevibacter smithii* strain PS (NR074235)	97
B3	*Methanobrevibacter oralis* strain ZR 16S (NR104878)	91
B4	*Methanobrevibacter oralis* strain ZR 16S (NR104878)	92
B5	*Methanobrevibacter millerae* strain ZA-10 (NR042785)	95
B6	*Methanobrevibacter millerae* strain ZA-10 (NR042785)	92

Reference

Bones, A. M., & Rossiter, J. T. (1996). The myrosinase-glucosinolate system, its organisation and biochemistry. Physiologia Plantarum, 97(1), 194-208.

Lila, Z. A., Mohammed, N., Kanda, S., Kamada, T., & Itabashi, H. (2003). Effect of α-cyclodextrin allyl isothiocyanate on ruminal microbial methane production in vitro. Journal of Animal Science, 74(4), 321-326. doi:10.1046/j.1344-3941.2003.00123.x

Stocker, T. (2014). Climate change 2013 : the physical science basis : Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change. New York: Cambridge University Press.

PO-03-37 Changes in Gut Microbiota in Japanese Black and Holstein Calves during Growing Stage

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[Objective]

Gut microbiota is now considered an additional organ and a component of the host animal's genome, that influences metabolic function in the adult as well as development in the neonate. The intestinal tract is sterile at birth and immediately colonized by a succession of bacteria after birth, and then, gradually accesses the climax pattern with increasing abundance and diversity of bacteria. These initial bacteria contribute to functional development of intestinal tract such as angiogenesis, epithelial tissues, and mucosal system (Callaway, 2011), and also help protect against pathogen invasion (Marques et al., 2010). Bacterial structure stability could be changed throughout life by many variables like diet, physical activity and stress, and the use of antibiotics, probiotics, and prebiotics (Callaway, 2011; Mujico et al., 2013). It is said that, despite the bacterial composition is somehow individually different, the developmental and climax populations could be manipulated to improve animal wellbeing and resistance to stress and diseases (Callaway, 2011).

For years, although several studies have used molecular based culture-independent techniques to investigate the profiles of gut bacteria (McEwan et al., 2005; Guan et al., 2008), or to examine the influence of some elements (diet, feeding level, antibiotics, and probiotics usage) on the gut bacteria (Penders et al., 2006; Rist et al., 2012; Mujico et al., 2013; Scott et al., 2013; Ng et al., 2013), few studies concerned how gut bacterial structure changes during growing of Japanese Black cattle. In this study, seven Holstein heifers and seven Japanese Black calves were examined about the community structures of total bacteria and three different bacterial groups (*Bacteroides*, *Clostridium* and *Lactobacillus*) at different growing stages using feces samples. The aim of this study was to understand how gut bacterial communities changes over time and if impact of weaning differs between dairy and beef breeds.

[Methodology]

Fecal sample collection and DNA extraction – Fecal samples were taken from seven Holstein heifers and seven Japanese Black calves three times during growing: prior and posterior to weaning, and at the termination of growing stage (3 weeks, 4 months, and 8 months after calving). Bacterial DNA was extracted using repeated bead beating plus column method (Yu and Morrison, 2004).

PCR amplification – Extracted DNA was subjected to PCR with universal primers including GC357f and 517r for V3 region amplification. PCR was also performed using the primer pairs of Bac303f and Bac708r for targeted *Bacteroides* community (Bartosch et al., 2004), Clost-f and Clost-r for targeted *Clostridium* community (Hung et al., 2008), Lab159f and Lab617r for targeted *Lactobacillus* community (Heilig et al., 2002) respectively. The PCR products of *Bacteroides*, *Clostridium,* and *Lactobacillus* groups were further amplified by nested PCR procedure with the universal primers described above.

DGGE analysis – PCR products were separated by DGGE by using the DCode Universal Mutation Detection System (Bio-Rad Laboratory Inc.). Polyacrylamide gels consisted of 8% (v/v) polyacrylamide were used for the separation. Electrophoresis was performed for 8 h at 150 V at a constant temperature of 60°C. Gels were stained with SYBR Green, observed by UV transilluminator, and photographed.

Quantitative PCR – A master mix for quantification of total bacteria, *Bacteroides* spp., *Clostridium* spp., and *Lactobacillus* spp. was prepared with KAPA SYBR FAST qPCR Master Mix (Kapa Biosystem Inc.), forward and reverse primers, and standard plasmid. MiniOpticon Real-Time PCR Detection System (Bio-Rad Laboratory, Inc.) was used for analysis. Amplification program described by Rinttilä et al. (2004) was followed.

Statistical analysis – Based on DGGE banding patterns, binary matrix was created to describe the presence or absence of individual band in all lanes. Cluster analysis was performed to demonstrate similarity and differences between sampling times and individual calves. Data for bacterial population were subjected to repeated measures of ANOVA and mean values were separated by Tukey's multiple range test.

[Results]

Changing pattern of bacterial structure during growing stage

DGGE banding patterns showed various changes in composition over sampling times during growing (Figures 1 and 2). Similarity between cattle appeared to increase after weaning period for both Japanese Black calves and Holstein heifers. Holstein heifers revealed significant changes in composition before and after weaning but no change was observed thereafter. In Japanese Black calves, bacterial structure changed at every single sampling time.

Taking an insight into individual groups, the changing pattern was different between groups and breeds. *Bacteroides* spp. and *Clostridium* spp. in Holstein heifers formed two clear separated groups before and after weaning point, indicating that great impact of weaning on these two bacterial genera. The structural synchronization between individual heifers greatly increased with aging (Figures 1). In Japanese Black calves, *Bacteroides* spp. and *Clostridium* spp. communities showed different banding pattern at every sampling time. Cluster analysis indicated that calf-to-calf variation of *Bacteroides* spp. composition became the lowest at the termination of growing period (8 months). However, in *Clostridium* spp., the community showed the highest synchronization between individuals at 4 months (Figures 2). The other periods inferred the differences in composition among individual calves. Interestingly, in both breeds, *Lactobacillus* spp. community showed significant changes in diversity over the growing stage. This was demonstrated by three different groups at each sampling time, while maintaining synchronized composition between calves.

Bacterial populations during growing stage

The qPCR results of Japanese Black calves disclosed changes taken place in bacterial population. Total bacterial number tended to increase across weaning, whereas *Lactobacillus* spp. population decreased at the second sampling time (4 months) and reduced their size with advancement of age. Nevertheless, population size decreased in all cases at the last sampling (8 months). On the contrary, in Holstein heifers, bacterial population was relatively stable during growing stage for total bacteria, *Bacteroides* spp. and *Clostridium* spp. population. *Lactobacillus* spp. population again declined across weaning (Table 1).

Holstein heifers and Japan Black calves examined in this study were raised with different dietary regime and in different farm environments. Shanks *et al*. (2011) showed that bovine management played an integral role in fecal microbial community structure than the site-specific attributes, such as water source, elevation, humidity and particular geographic location. This could account for the similarity of bacterial structure between calves kept under the same management, which was proven by cluster analysis. Likewise, Holstein heifers and Japanese Black calves evidently possessed their own specific bands in their DGGE profiles regardless of *Bacteroides* spp., *Clostridium* spp., and *Lactobacillus* spp. communities. Meanwhile, although these two breeds were kept under different management, several common bacteria were seen occasionally or continuously over the growing stage. Species identification is performing to clarify the common bacteria in Holstein heifers and Japanese Black calves.

[Conclusion]

In this report, seven Holstein heifers and seven Japanese Black calves were monitored at different growing stages to examine dynamic changes in gut microbiota regarding to breed. Although changes due to growing were seen in common, the patterns were different between breeds and between *Bacteroides* spp., *Clostridium* spp., and *Lactobacillus* spp. communities. Combined analyses of DGGE and qPCR revealed that weaning period implicated greater on bacterial population than on bacterial composition in beef cattle, whereas contrary state was found in dairy cattle. *Lactobacillus* spp. community showed significant changes in composition over the growing stage regardless of breed, while maintaining the highest similarity between individual calves.

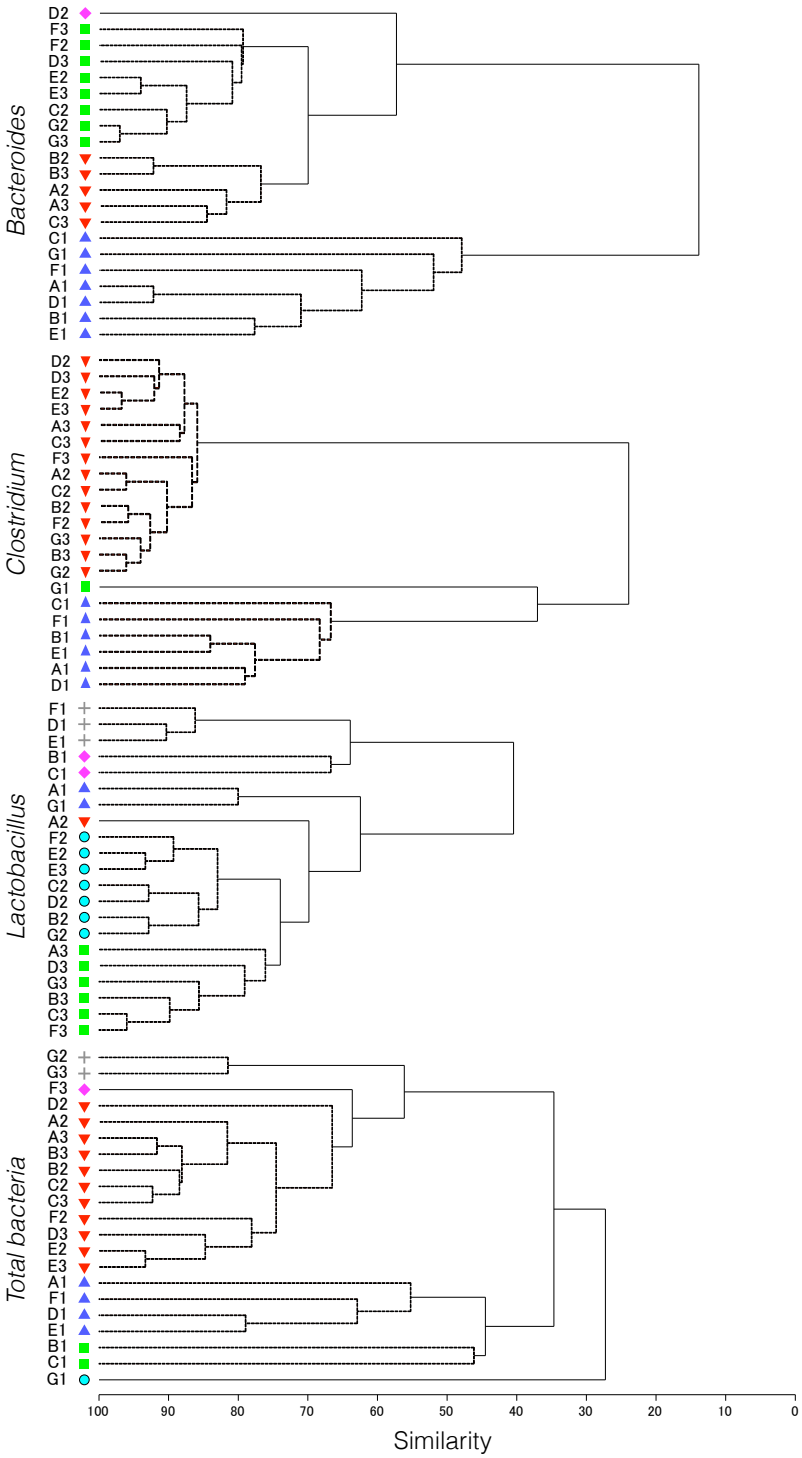

Figure 1. Cluster analysis results for *Bacteroides*, *Clostridium*, *Lactobacillus* and total bacterial community structure based on DGGE banding patterns of seven Holstein heifers during growing stage (1, 2, and 3 - 3 weeks, 4 months and 8 months after calving, respectively)

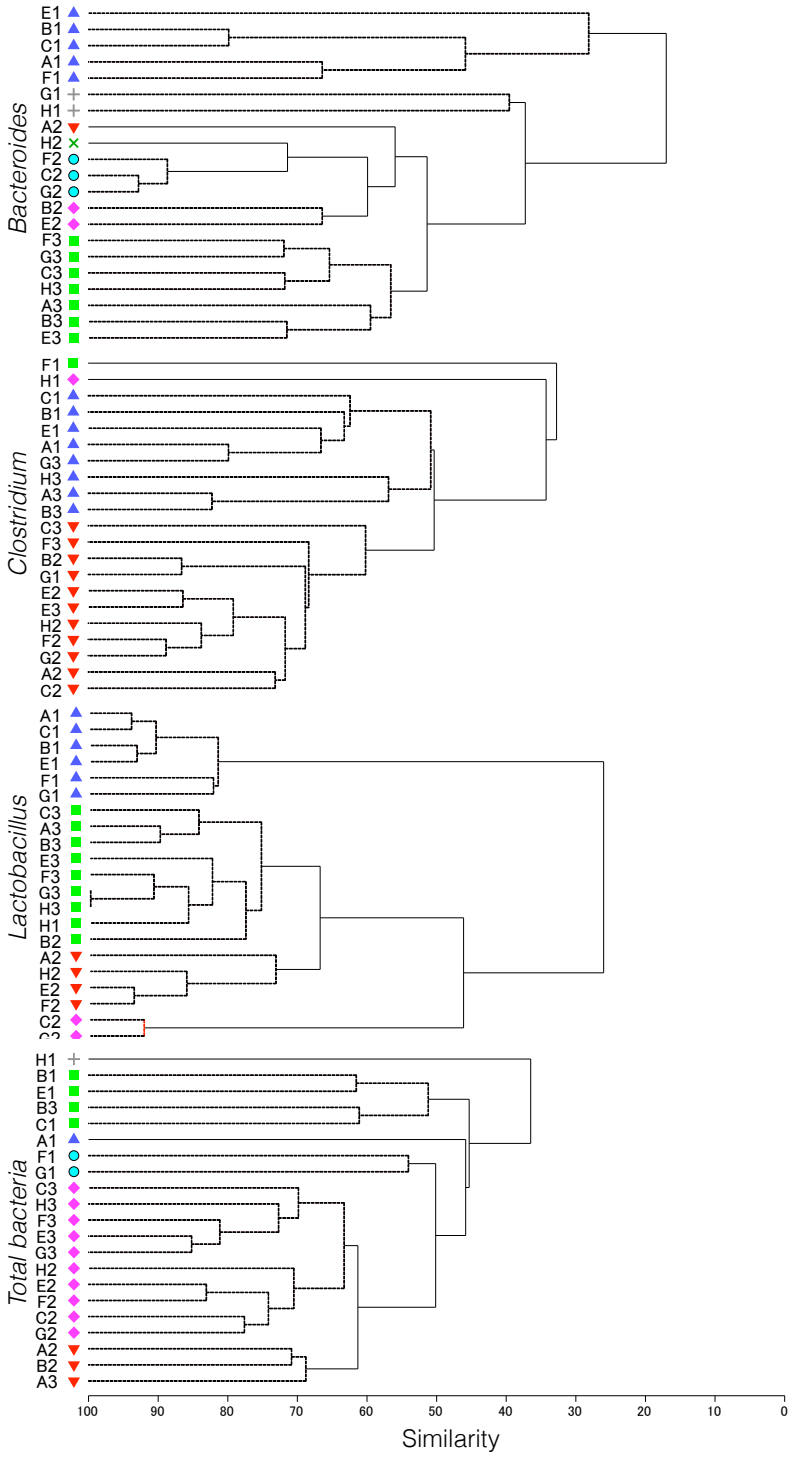

Figure 2. Cluster analysis results for *Bacteroides*, *Clostridium*, *Lactobacillus* and total bacterial community structure based on DGGE banding patterns of seven Japanese Black calves during growing stage (1, 2, and 3 - 3 weeks, 4 months and 8 months after calving, respectively)

Table 1. Quantification PCR results (log 16S rRNA gene copy number/g fresh matter) of total bacteria, *Bacteroides*, *Lactobacillus*, *Clostridium* population (Period 1, 2, and 3 - 3 weeks, 4 months and 8 months after calving, respectively)

		Total bacteria	*Bacteroides*	*Clostridium*	*Lactobacillus*
Japanese Black Calves	*Period 1*	10.3 ± 1.37[a]	8.59 ± 1.53[b]	5.11 ± 0.55[b]	7.87 ± 1.64[a]
	Period 2	11.4 ± 0.51[a]	9.91 ± 0.45[a]	7.21 ± 0.55[a]	6.25 ± 0.13[b]
	Period 3	8.51 ± 0.21[b]	7.53 ± 0.31[b]	5.11 ± 0.18[b]	5.39 ± 0.47[b]
Holstein Heifers	*Period 1*	10.7 ± 0.66[a]	10.1 ± 1.59[a]	6.19 ± 0.56[a]	6.28 ± 0.48[a]
	Period 2	10.6 ± 0.28[a]	9.91 ± 0.34[a]	6.17 ± 0.30[a]	5.39 ± 0.25[b]
	Period 3	11.1 ± 0.17[a]	10.5 ± 0.31[a]	6.02 ± 0.84[a]	5.67 ± 0.20[b]

Results are means ± standard deviation of seven samples in every sampling time. Samples that are labeled by different character (a, b, c) are considered statistically different.

References

Bartosch, S. et al., 2004. Characterization of Bacterial Communities in Feces from Healthy Elderly Volunteers and Hospitalized Elderly Patients by Using Real-Time PCR and Effects of Antibiotic Treatment on the Fecal Microbiota Characterization of Bacterial Communities in Feces from. *Applied and environmental microbiology*, 70(6), pp.3575–3581.

Guan, L.L. et al., 2008. Linkage of microbial ecology to phenotype: Correlation of rumen microbial ecology to cattle's feed efficiency. *FEMS Microbiology Letters*, 288(1), pp.85–91.

Heilig, H.G.H.J. et al., 2002. Molecular Diversity of Lactobacillus spp . and Other Lactic Acid Bacteria in the Human Intestine as Determined by Specific Amplification of 16S Ribosomal DNA Molecular Diversity of Lactobacillus spp . and Other Lactic Acid Bacteria in the Human Intestine. *Applied and environmental microbiology*, 68(1), pp.114–123.

Hung, C.H. et al., 2008. Application of Clostridium-specific PCR primers on the analysis of dark fermentation hydrogen-producing bacterial community. *International Journal of Hydrogen Energy*, 33(5), pp.1586–1592.

Marques, T.M. et al., 2010. Programming infant gut microbiota: influence of dietary and environmental factors. *Current Opinion in Biotechnology*, 21(2), pp.149–156.

McEwan, N.R. et al., 2005. Rumen microbial population dynamics in response to photoperiod. *Letters in applied microbiology*, 41(1), pp.97–101.

Mujico, J.R. et al., 2013. Changes in gut microbiota due to supplemented fatty acids in diet-induced obese mice. *The British journal of nutrition*, 110(4), pp.711–20.

Ng, S.C. et al., 2013. Effect of Probiotic Bacteria on the Intestinal Microbiota in Irritable Bowel Syndrome. *Journal of Gastroenterology and Hepatology*, p.n/a–n/a.

Penders, J. et al., 2006. Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. *Pediatrics*, 118(2), pp.511–521.

Rinttilä, T. et al., 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology*, 97(6), pp.1166–1177.

Rist, V.T.S. et al., 2012. Effect of feeding level on the composition of the intestinal microbiota in weaned piglets. Journal of Animal Science, 90, pp.19–21.

Scott, K.P. et al., 2013. The influence of diet on the gut microbiota. *Pharmacological research : the official journal of the Italian Pharmacological Society*, 69, pp.52–60.

Shanks, O.C. et al., 2011. Community Structures of Fecal Bacteria in Cattle from Different Animal Feeding Operations. *Applied and Environmental Microbiology*, 77(9), pp.2992–3001.

Todd R. Callaway, S.C.R., 2011. *Direct-Fed Microbials and Prebiotics for Animals*, Spinger.

Yu, Z. and Morrison, M., 2004. Improved extraction of PCR- quality community DNA from digesta and fecal samples. *BioTechniques* (36), pp.808–812.

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Effect of stabilizer on methane mitigation efficiency of encapsulated nitrate in rumen fermentation

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OBJECTIVE OF THE STUDY

The main objective of this study was to assess suitability of two emulsion matrices for coating nitrate. Matrices were studied for the rate of ruminal nitrate release, nitrite accumulation, and ammonia-N production, methane emission, and overall rumen fermentation in vitro.

MATERIAL AND METHODS

Pam oil (PO), isolate soy protein (ISP), gum arabic (GA), starch (ST) and nitrate (NO) were obtained from DAE JUNG Chemicals and Metals co., LTD, Siheung-si, Gyeonggi-do, Korea.

Design of Experiment

Treatments were: NEG, 5 mM NO coated in PO stabilized in ISP + GA; EG, PO without NO stabilized in ISP + GA (matrix only); NES, 5 mM NO coated in PO stabilized in ISP + ST; ES, PO without NO stabilized in ISP + ST (matrix only); FN, 5 mM un coated/free nitrate; and CON, control treatment with no supplement.

Nitrate Emulsion Preparation

For NEGA and NEST treatments, 20 g of ISP were blended with 93 g NO for 5 minutes but NO was not used for EGA and EST. Then 80 g of PO introduced into the blend to coat the oil with protein by homogenizing in a shear mixer (WF2211214, Wearing products division, Torrington CT USA) for two minutes. A stabilizer (20 g) added to the mixture before passing it through a high-speed blender for 3 min to form course emulsion. The course emulsion then introduced into 100 mL of aqueous phase (Double distilled water) in a high-speed shear mixer for five minutes to develop fine emulsion.

Inoculum, *in vitro* fermentation and sampling

Filtered rumen fluid were mixed with McDougall's buffer (McDougall, 1948) in 1:4 ratio, under anaerobic condition by bubbling oxygen free carbon dioxide (CO_2) gas and the temperature maintained at 39°C. 50 mL of buffered rumen fluid was dispensed under a stream of CO_2 into 125 mL serum bottles; containing 0.5 g (60:40 commercial concentrate to orchard grass (sized to 1 mm) of substrate plus additive. The bottles were immediately closed with rubber stoppers, sealed tightly with aluminium caps and incubated at 39°C. Sampling and analysis of rumen fermentation parameters was done according to Tilley and Terry (1963) at 0, 3, 6, 9, 12 and 24 h interval following incubation.

Rumen fermentation parameters

Total gas production was measured using a displacing glass syringe fitted with a needle, plunged through the rubber stopper into the headspace. Gas analysis was according to Lopez et al., (1999) using a gas chromatograph (GC-7890A, Agilent Technologies, Inc., Palo Alto, CA, USA) fitted with a thermal conductivity detector and a capillary column (Nukol Fused silca capillary column 30 m x 0.25 mm x 0.25 µm film thickness, supelco, Bellefonte, PA, USA). Ruminal pH was measured using a Mettler Toledo® pH meter. Then all rumen contents were centrifuged (3000 rpm for 15 min) and supernatant sampled and stored at -20ºC for volatile fatty acid (VFA), ammonia-N, and Nitrate/nitrogen and Nitrite analyses. Samples for VFA analyses were stabilized by adding 0.2 mL 25% (w/v) metaphosphoric acid into 1 mL of sample before storage. VFAs were analyzed according to Erwin et al., (1961) on GC fitted with a Flame Ionizing Detector (FID) and a capillary column. Nitrate-N and nitrite were analyzed according to Miranda et al., (2001) with slight modification using a spectrophotometer (Mecasys Optizen Pop, Daejeon, Korea). Ammonia-N was determined according to Chaney and Marbach, (1962) using a spectrophotometer and *in vitro* dry matter digestibility (IVDMD) was determined according to Moore, 1970.

Statistical analysis

Data were subjected to a two-way analysis of variance using the PROC general linear model of SAS, (SAS institute Inc., Cary, NC, USA. version 9.0 2002) for analysis of significance. The model: $Y_{ijk}= \mu + T_i + H_j + (TXH)_{ij} + e_{ijk}$ was used for all observed patterns, where Y_{ijk} is the response variable, μ is the overall mean common to all observations, Ti

the i^{th} treatment, H_j the j^{th} time and $(TXH)_{ij}$ the interaction between the i^{th} treatment and the j^{th} time while e^{ijk} is the residual error.

RESULTS AND DISCUSION

Rumen pH, gas production and feed digestibility

Treatments maintained within a normal ruminal pH range of 6.46 to 7.08 throughout the incubation period and pH was significantly ($p<0.05$) affected by treatments, time and their interaction. There was a significant ($p<0.05$) decrease in total gas production for NO treatments compared to CON and FN caused a significant ($p<0.05$) decrease in total gas production compared to NEGA and NEST (Table 1). This could be due to lower organic matter digestibility, normally associated with nitrate (Bozic et al., 2009). Methane production was significantly ($p<0.05$) lowered by NO treatments compared to control and no difference was observed between coated NO and FN (Fig. 1). Nitrate mitigates methane by diverting electrons away from methanogenesis, through direct inhibition of methanogenic archaea and through restricted organic matter digestibility (Iwamoto et al., 2002; Anderson et al., 2003). *.In vitro* dry matter digestibility of all treatments ranged from 35.40% to 40.71% after 24 h. Emulsion treatments significantly ($p<0.05$) increase IVDMD compared to control while FN was significantly ($p<0.05$) lower at 24 hour (Table 1). Palm oil could lower ruminal protozoa population, to cause a subsequent proliferation of ruminal bacteria and probably improved rumen fermentation and IVDMD (Abubakr et al., 2013; Dutta et al., 2008).

Nitrate, nitrite and ammonia-N concentration

The concentration of nitrate-N in the ruminal fluid was significantly ($p<0.05$) increased by NO treatments compared to CON. On average FN did not cause any significant increase compared to NEGA and NEST after 24 h, but it showed higher concentrations in the first 3 hours (Fig. 2). Nitrite concentration was significantly ($p<0.05$) higher in NO treatments compared to CON and matrices (Fig. 2 B). Free NO had significantly ($p<0.05$) higher nitrite accumulation compared to coated NO treatments. This indicates that FN poses a risk of acute/sub-acute or chronic nitrite toxicity depending on level of intake, while NEST and NEGA could ensure safe delivery of NO for effective methane mitigation (El-Zaiat et al., 2014; Lund et al., 2014). In addition, NEST had significantly ($p<0.05$) lower nitrite concentration compared to NEGA which demonstrates a more efficient coating (Fig. 3). For Ammonia-N concentration, NO treatments were significantly ($p<0.05$) higher compared to CON. Contrary to the observed trend in nitrite production, both NEGA and NEST produced significantly ($p<0.05$) higher ammonia-N compared to FN at 24h (Fig. 4). The matrix EG>ES both had significantly ($p<0.05$) higher ammonia-N relative to CON. High ammonia-N production in coated nitrite could be a result of both matrix material and gradual release (Ahmed 2015; Mamvura et al., 2014).

Volatile fatty acids

The molar concentrations of total volatile fatty acids (TVAFA) was significant ($p<0.05$) increased by EST and decreased by NEG compared to CON and other treatments had no effect (Table 1). Acetate was significantly increased by ES but was lowered by NEG and FN treatment especially at 24h ($p<0.05$). For Propionate production, there was a significantly ($p=0.05$) increased by ES while other treatments had no effect. NO treatments significantly ($p<0.05$) lowered the ratio of acetate to propionate and butyrate production at 24 h compared to CON while other treatments had no effect.

CONCLUSIONS

Coated NO and FN effectively abated methane production which confirms that, coating nitrate does not affect its affinity for electrons in the rumen. Coated nitrate further improved ammonia-N production, maintained efficient rumen fermentation and minimize nitrite accumulation compared to FN, an indicator of gradual release of nitrate from the matrix. In addition, NEST could be a better matrix choice to coat NO for methane mitigation, improved productivity without toxicity risks. The findings of this study suggest an efficient way to coat nitrate feed additive for domesticated ruminant animals

Table 1. Effect of treatments on *in vitro* rumen fermentation parameters over 24 h incubation

Variables	NEG	EG	NES	ES	FN	Control	Significance		
							Trt	Time	Trt* time
pH	6.82^{d}	6.84^{b}	6.83^{c}	6.83^{c}	6.84^{b}	6.86^{a}	0.98	**	**
Ammonia-N	4.86^{b}	1.79^{d}	5.26^{a}	1.59^{e}	4.61^{c}	1.24^{f}	**	**	**
IVDMD (%)	39.38^{bc}	40.52^{a}	38.85^{c}	40.71^{a}	36.01^{d}	35.38^{d}	0.87	**	**
Total gas (mL)	31.47^{d}	39.4^{a}	33.07^{c}	39.67^{a}	26.53^{e}	34.71^{b}	0.51	**	**
Hydrogen (mL)	0.23^{c}	0.04^{d}	0.31^{a}	0.05^{d}	0.28^{b}	0.04^{d}	**	**	**
Carbon dioxide (mL)	27.18^{d}	33.57^{a}	28.43^{c}	33.89^{a}	22.92^{e}	29.82^{b}	0.51	**	**
Methane (mL)	0.41^{c}	1.80^{ab}	0.35^{cd}	2.06^{a}	0.26^{d}	1.73^{b}	**	**	**
Nitrate (mM)	1.64^{c}	0.12^{d}	1.84^{b}	0.17^{d}	2.38^{a}	0.12^{d}	**	**	**
Nitrite (mM)	0.41^{b}	0.01^{d}	0.28^{c}	0.01^{d}	0.96^{a}	0.01^{d}	**	**	**
Acetate (mM)	21.12^{d}	21.83^{b}	21.56^{c}	22.65^{a}	22.30^{a}	21.82^{b}	1.00	**	**
Propionate (mM)	7.80^{c}	7.78^{c}	8.10^{b}	8.36^{a}	8.05^{b}	7.64^{c}	1.00	**	**
Isobutyrate (mM)	0.23^{bc}	0.26^{abc}	0.22^{c}	0.29^{a}	0.22^{c}	0.27^{ab}	0.43	**	**
Butyrate (mM)	4.16^{b}	5.14^{a}	4.49^{b}	5.32^{a}	4.36^{b}	5.15^{a}	0.40	**	**
Isovalerate (mM)	0.63^{ab}	0.65^{ab}	0.63^{ab}	0.67^{a}	0.63^{ab}	0.64^{ab}	0.98	**	**
n-Valerate (mM)	0.64^{c}	0.69^{ab}	0.67^{bc}	0.72^{a}	0.64^{c}	0.69^{ab}	0.98	**	**
TVFA (mM)	34.58^{c}	36.34^{b}	35.67^{b}	38.02^{a}	36.11^{b}	36.21^{b}	0.99	**	**
A:P	2.93^{ab}	3.00^{a}	2.88^{b}	2.88^{b}	3.00^{a}	2.98^{a}	0.95	**	**

NEG, 5mM nitrate-in-emulsion of palm oil stabilized with ISP + gam arabic; EG, emulsion of palm stabilized with ISP + gam arabic without nitrate; NES, 5mM nitrate-in-emulsion of palm oil stabilized with ISP + starch; ES, emulsion of palm stabilized with ISP + starch without nitrate; Trt5, 5mM free/ uncoated nitrate; Control, no additive

Trt, treatment; TVFA, total volatile fatty acids; IVDMD, *in vitro* dry matter digestibility; A/P, Acetate to Propionate ratio

$^{a, b, c,d,e,f}$, means differ significantly ** $p<0.05$,

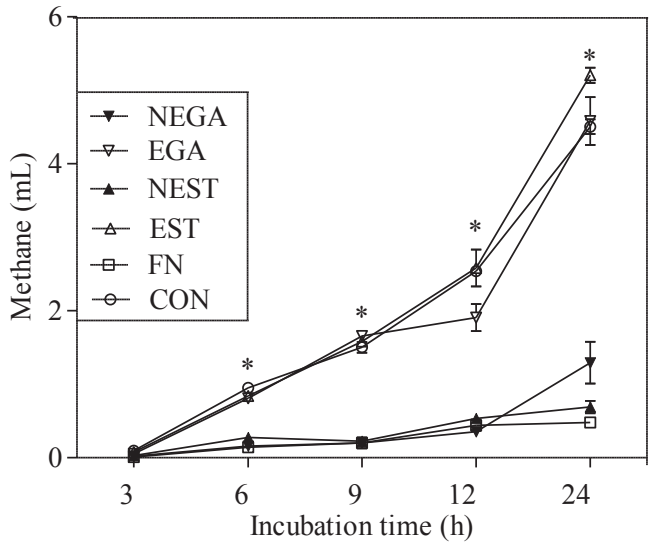

Figure 1, Effect of treatments on methane production over a 24 h fermentation period. * indicates significant difference between treatments ($p<0.05$).

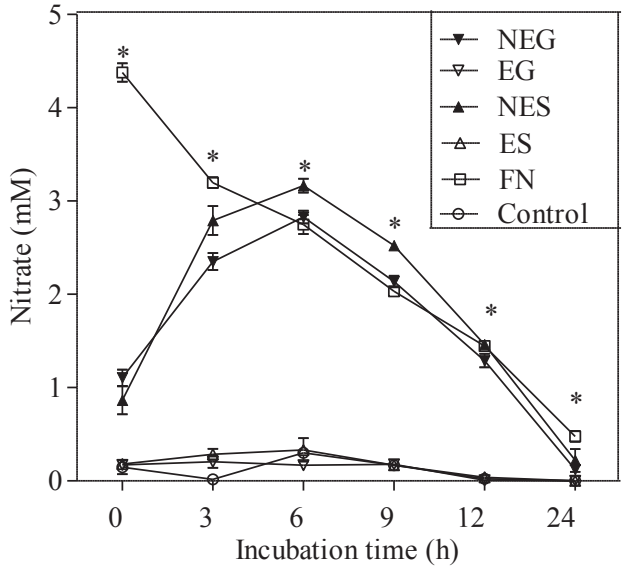

Figure 2, Effect of treatments on the rate of nitrate-N release in the ruminal fluid. * indicates significant difference between treatments ($p<0.05$).

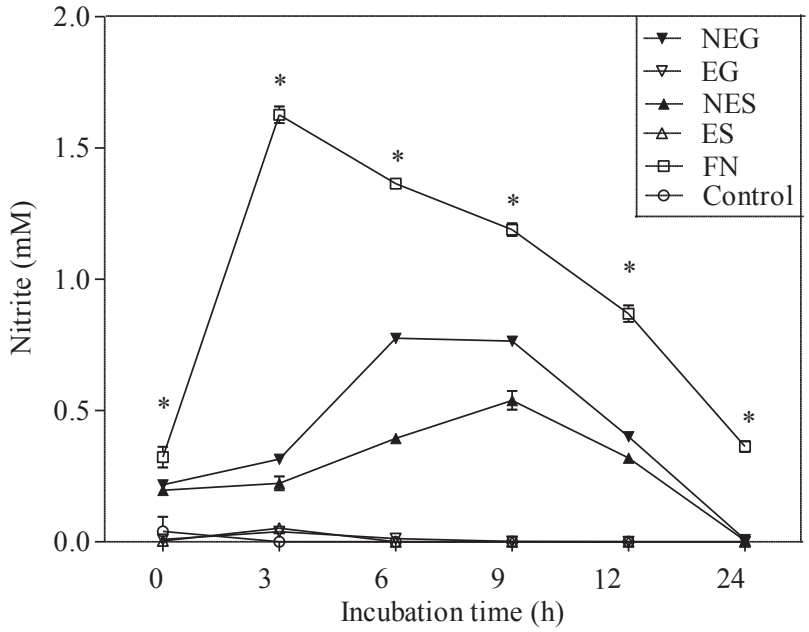

Figure 3, Effect of treatments on nitrite accumulation in the ruminal fluid over 24 h fermentation period. * indicates significant difference between treatments ($p<0.05$).

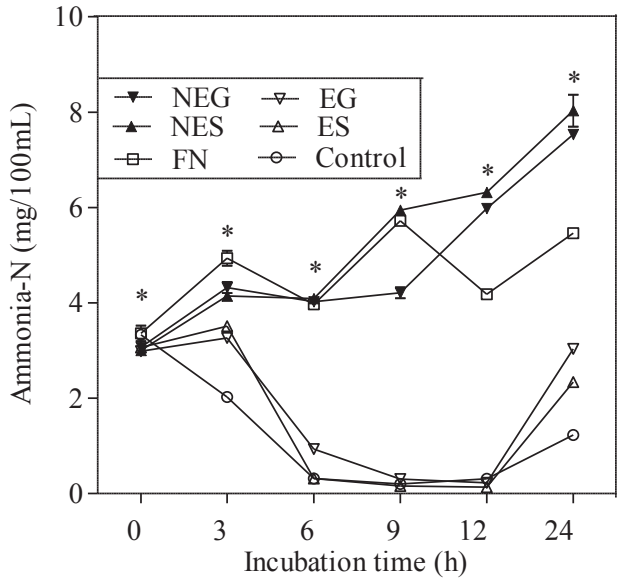

Figure 4, Effect of treatments on ammonia-N production in the ruminal fluid over 24 h fermentation period. * indicates significant difference between treatments ($p<0.05$).

REFERENCE

Abubakr, A., A. Alimon., H. Yaakub., N. Abdullah. and M. Ivan. 2013. Digestibility, rumen protozoa, and ruminal fermentation in goats receiving dietary palm oil by-products. J Saudi Soc Agric Sci 12**,** 147-154.

Ahmed, S. 2015. Effect of bacterial metabolites supplemented wi gum arabic on cell culture and effect of gum arabic as feed additive in small ruminants. (Doctoral dissertation, UOFK).

Anderson, R. C., T. R. Callaway., J. A. S. Van Kessel., Y. S. Jung., T. S. Edrington. and D. J. Nisbet. 2003. Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. Bioresource Technol 90**,** 59-63.

Božic, A., R. Anderson., G. Carstens., S. Ricke., T. Callaway., M. Yokoyama., J. Wang. and D. Nisbet, D. 2009. Effects of the methane-inhibitors nitrate, nitroethane, lauric acid, lauricidin® and the hawaiian marine algae chaetoceros on ruminal fermentation in vitro. Bioresource Technol. 100**,** 4017-4025.

Dutta, T., M. Agnihotri. and S. Rao. 2008. Effect of supplemental palm oil on nutrient utilization, feeding economics and carcass characteristics in post-weaned muzafarnagari lambs under feedlot condition. Small Ruminant Res. 78**,** 66-73.

El-Zaiat, H., R. Araujo., Y. Soltan., A. Morsy., H. Louvandini., A. Pires., H. Patino., P. Correa, and A. Abdalla. 2014. Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs. J Anim Sci. 92**,** 2214-2224.

Erwin, E., G. Marco. And E. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J Dairy Sci. 44**,** 1768-1771.

Iwamoto, M., N. Asanuma. And T. Hino. 2002. Ability of selenomonas ruminantium, veillonella parvula, and wolinella succinogenes to reduce nitrate and nitrite with special reference to the suppression of ruminal methanogenesis. Anaerobe. 8**,** 209-215.

Lund, P., R. Dahl., H. Yang., A. L. F. Hellwing., B. Cao. and M. R. Weisbjerg. 2014. The acute effect of addition of nitrate on in vitro and in vivo methane emission in dairy cows. Anim Prod Sci. 54**,** 1432-1435.

Mamvura, C. I., S. Cho., D. T. Mbiriri., H. –G. Lee. And N. –J. Choi. 2014. Effect of encapsulating nitrate in sesame gum on in vitro rumen fermentation parameters. Asian Austral J Anim Sci. 27**,** 1577.

Mcdougall, E. I. 1948. Studies on ruminant saliva. 1. the composition and output of sheep's saliva. Biochem. J. 99.

Tilley, J. and R. A. Terry. 1963. Two stage technique for the in vitro digestion of forage crops. Grass Forage sci. 18, 104-111 1

PO-03-40 Optimization of submerged fermentation of Bacillus subtilis for surfactin production

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Objective：Surfactin, an amphipathic cyclic lipopeptide, is secondary metabolites of *Bacillus subtilis* and exerts the anti-bacterial effects (Arima et al., 1968; Cladera-Olivera et al., 2004). Surfactin is able to inhibit pro-inflammatory cytokine and nitric oxide production in murine macrophages (Hwang *et al*., 2008). However, little is known about the effect of anti-bacterial substances produced from *Bacillus subtilis*, like surfactin on broilers. The purpose of this study was to investigate the optimal parameters for anti-bacterial substances production by *Bacillus subtilis* in submerged fermentation and the effect of anti-bacterial substances produced from *Bacillus subtilis* on *Clostridium perfringens* (*C. perfringens*)-induced necrotic enteritis in broilers.

Methodology：To establish the optimal parameters for anti-bacterial substances production by *Bacillus subtilis* in submerged fermentation, the availability of different carbon sources, including glucose, brown sugar and molasses were evaluated. Three nitrogen sources were also tested, including soybean meal, yeast extract and fishmeal. The sporulation percentage evaluated by heating at 80℃ for 10 minutes, acid-resistance at pH=2 and pH=3, bile-resistance by 0.2% and 0.3% bile salt treatment and anti-bacterial activity were also examined in the present study. To examine the effect of anti-bacterial substances produced from *Bacillus subtilis* on *Clostridium perfringens*-induced necrotic enteritis in broilers. Sixty broilers (avian) 1-day-old in age were randomly allocated to five different treatment groups (n=12 per group): (1) basal diet (control), (2) basal diet plus *C. perfringens* (CP), (3) basal diet plus 50 mg/kg of bacitracin methylenedisalicylic acid (BMD) and *C. perfringens*, (4) basal diet plus *C. perfringens* and 4 day fermentation product (4DF) and (5) basal diet plus *C. perfringens* and 6 day fermentation product (6DF). The basal diets were formulated based on National Research Council recommendations (NRC, 1998). Water and feed was provided ad libitum over the entire five-week experimental period. Total individual body weight, feed intake and feed conversion ratio was recorded every week. At the end of the experiment, broilers were sacrificed by cervical dislocation. The small intestine were excised and weighed. The results were expressed as the percentage subjected to logarithmic transformation prior to analysis of variance. Statistical significance among groups was determined by one-way analysis of variance. Duncan's new multiple range test was used to evaluate differences between means (SAS Institute, Cary, NC, USA). P values of less than 0.05 were considered statistically significant.

Result：In the first experiment, we examined the optimal parameters of submerged fermentation by *Bacillus subtilis* BCRC 14718 on carbon and nitrogen sources. We found that molasses for carbon source and yeast extract for nitrogen source in submerged fermentation showed the best viable bacteria count (Table 1 and 2). The total viable count, ability of sporulation, acid-resistance and bile salt-resistance were evaluated on 2, 4 and 6 fermentation days. Result showed that the 4 and 6 fermentation days have the best total viable count and resistance tests compared with 2 fermentation days (Table 3). Anti-bacterial activity results indicated that fermentation products harvested from 4 and 6 days were able to inhibit the growth of *S. aureus*, *C. perfringens*, *E. coli* and *S. typhimurium*. In the second experiment, the effect of anti-bacterial substances produced from *Bacillus subtilis* on *C. perfringens*-induced necrotic enteritis on growth performance, intestinal morphology in broilers was evaluated. On 21 day, the results of body weight in *Bacillus subtilis* fermentation product groups are significantly improved compared with *C. perfringens* alone group and in feed conversion ratio are also significantly improved compared with *C. perfringens* alone group (Table 4). Results from intestinal villi morphology showed *C. perfringens* challenge significantly destroyed intestinal villi morphology, while supplementation of *Bacillus subtilis* fermentation product in drinking water efficiently reversed the abnormal morphology of the small intestine in the *C. perfringens*-fed broilers (Table 5).

Conclusion：We established the optimal parameters for Bacillus subtilis growth in submerged fermentation and demonstrated that *Bacillus subtilis* fermentation product could improve the *Clostridium perfringens*-induced necrotic enteritis in broilers. These results provide a potential production platform of anti-bacterial substances, like surfactin in *Bacillus subtilis*.

Table 1

	Total viable count (CFU/mL)	
	Mean	SD
CTRL[1]	8.4×10^{7}	5.9×10^{7}
Glucose[1]	4.1×10^{7}	2.8×10^{7}
Brown sugar[1]	4.1×10^{8}	1.3×10^{8}
Molasses[1]	8.2×10^{8}	3.2×10^{8}

* Values are expressed as mean ± SD (n=3).

[1]The initial amount of bacteria is 2×10^{7} CFU/mL.

Table 2

	Total viable count (CFU/mL)	
	Mean	SD
CTRL[1]	1.5×10^{8}	8.1×10^{7}
Soybean meal [1]	1.1×10^{8}	4.0×10^{7}
Yeast[1]	8.0×10^{8}	2.7×10^{7}
Fish meal[1]	1.2×10^{8}	6.0×10^{7}

* Values are expressed as mean ± SD (n=3).

[1]The initial amount of bacteria is 2×10^{7} CFU/mL

Table 3

Group[1]	2DF		4DF		6DF	
	Mean	SD	Mean	SD	Mean	SD
Total viable count	4.8×10^{8}	6.0×10^{7}	5.4×10^{9}	5.8×10^{8}	7.5×10^{9}	7.9×10^{8}
Initial	4.8×10^{8}	6.0×10^{7}	5.4×10^{8}	5.8×10^{7}	7.5×10^{8}	7.9×10^{7}
Sporulation						
After heating	$6.2\times10^{6\,b}$	6.5×10^{5}	$3.9\times10^{8\,a}$	6.5×10^{7}	$4.2\times10^{8\,a}$	3.7×10^{7}
Acid resistance						
pH=3	4.9×10^{8}	3.1×10^{7}	3.3×10^{8}	1.2×10^{7}	3.8×10^{8}	3.1×10^{7}
pH=2	$4.1\times10^{7\,b}$	7.0×10^{6}	$2.4\times10^{8\,a}$	6.2×10^{6}	$2.5\times10^{8\,a}$	4.5×10^{7}
Bile resistance						
0.1% Bile	3.6×10^{8}	3.7×10^{7}	2.4×10^{8}	1.1×10^{7}	1.7×10^{8}	1.0×10^{7}
0.3% Bile	$2.9\times10^{7\,b}$	1.0×10^{6}	$1.5\times10^{8\,a}$	5.0×10^{7}	$1.4\times10^{8\,a}$	7.8×10^{7}

* Values are expressed as mean ± SD (n=3).

[a-b]Means in the same superscript followed by different letters are significantly different. ($P < 0.05$).

[1]The unit is CFU/mL.

Table 4

Items	CTRL (n=12)		CP (n=12)		BMD (n=12)		4DF (n=12)		6DF (n=12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (g)										
1d	46.00	0.00	46.67	0.58	46.00	0.00	46.67	0.58	46.00	0.00
21d	917.00^{ab}	16.37	858.67^{c}	80.79	897.00^{b}	52.37	963.67^{a}	46.76	934.33^{ab}	21.82
35d	1903.33	98.66	1766.67	5.77	2096.67	123.52	1993.33	5.77	1938.33	286.02
Daily gain (g/d)										
1~21d	41.33^{a}	0.58	38.67^{b}	4.16	40.33^{a}	2.08	43.67^{a}	2.08	42.00^{a}	1.00
21~35d	70.67	6.66	65.00	5.29	75.00	5.00	73.33	2.52	72.67	21.50
1~35d	53.00	2.65	49.00	0.00	58.68	3.51	56.00	1.73	54.00	8.19
Feed intake (g/d)										
1~21d	57.81	3.28	54.22	1.27	55.25	3.56	55.39	2.97	56.96	0.99
21~35d	127.89	9.00	130.77	10.73	129.65	8.33	128.34	8.99	126.65	7.01
1~35d	93.45	7.89	87.22	6.58	98.58	0.88	91.84	6.02	90.72	2.12
FCR (feed/gain)										
1~21d	1.39^{bc}	0.06	1.40^{c}	0.07	1.37^{b}	0.06	1.32^{a}	0.09	1.36^{ab}	0.02
21~35d	1.80	0.55	2.01	0.13	1.71	1.09	1.75	0.05	1.74	0.86
1~35d	1.75	0.08	1.77	0.08	1.68	0.09	1.64	0.06	1.68	0.09
Water intake (ml/d)										
1~21d	313.90	22.46	323.26	16.38	325.78	22.02	360.85	23.44	340.48	21.89
21~35d	624.86	114.68	695.48	101.03	685.19	78.16	781.47	44.45	684.47	63.58
1~35d	938.76	134.57	1018.74	111.76	1010.98	68.61	1142.32	64.28	1024.95	79.08

* Values are expressed as mean ± SD.

[a-c]Means in the same superscript followed by different letters are significantly different. ($P < 0.05$).

Table 5

Items	CTRL (n=12)		CP (n=12)		BMD (n=12)		4DF (n=12)		6DF (n=12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Duodenum										
21d										
Villus height (μm)	998.25^{b}	4.44	693.25^{c}	65.97	1079.20^{ab}	90.36	1211.39^{ab}	85.82	1261.90^{a}	89.09
Crypt depth (μm)	120.85	10.39	165.11	99.85	137.35	40.09	127.62	74.07	99.85	9.12
Villus height:crypt depth	9.52^{a}	0.87	5.70^{b}	0.28	8.30^{a}	1.41	8.82^{a}	0.10	8.72^{a}	0.10
35d										
Villus height (μm)	1037.30	327.10	851.30	327.10	1272.2	376.74	987.9	7.49	917.55	224.08
Crypt depth (μm)	121.83	25.78	158.63	20.55	135.72	7.95	116.97	53.63	79.35	34.30
Villus height:crypt depth	10.69	0.43	5.45	1.34	8.80	3.11	8.50	2.82	12.10	2.40
Jejunum										
21d										
Villus height (μm)	1014.48^{a}	20.53	515.55^{b}	100.62	1001.90^{a}	8.76	1212.65^{a}	123.53	1079.20^{a}	171.89
Crypt depth (μm)	114.9	103.37	198.25	28.21	119.3	17.39	137.30	71.70	125.05	122.68
Villus height:crypt depth	7.25^{a}	0.07	3.89^{b}	0.97	7.40^{a}	0.14	7.05^{a}	0.07	8.00^{a}	1.55
35d										
Villus height (μm)	647.39	14.15	596.10	29.98	833.38	132.34	1089.45	451.06	982.60	516.61
Crypt depth (μm)	82.49	28.14	139.91	5.95	110.61	5.35	108.55	24.67	98.85	5.02
Villus height:crypt depth	7.58	2.24	5.85	0.21	7.50	0.63	9.85	1.90	10.05	5.72

* Values are expressed as mean ± SD.

[a-c]Means in the same superscript followed by different letters are significantly different. ($P < 0.05$).

Referemce

Arima, K., A. Kakinuma and G. Tamura. 1968. Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation. Biochem. Biophys. Res. Commun. 31: 488-494.

Cladera-Olivera, F., G. R. Caron, and A. Brandelli. 2004. Bacteriocin-like substanceproduction by Bacillus licheniformis strain P40. Lett. Appl. Microbiol. 38(4):251-256.

Hwang, M. H., Z. Q. Chang, E. H. Kang, J. H. Lim, H. I. Yun, M. H. Rhee, K. S. Jeong, and S. C. Park. 2008. Surfactin C inhibits Mycoplasma hyopneumoniae-induced transcription of proinflammatory cytokines and nitric oxide production in murine RAW 264.7 cells. Biotechnol. Lett. 30(2):229-233.

PO-03-41 Applying fermented juice of epiphytic lactic acid bacteria (FJLB) powder as probiotics in weaned pig

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Introduction

The weaning process for piglets was remain one of the most challenging periods in a pig's survival. At the age of four weeks or less, the digestive tract is not that developed, which makes young pigs more susceptible to challenge from outside such as bacterial pressure leading to intestinal disorders. Common way to prevent disease, there were used antibiotics. However, it has been prohibited in the European Union since January 2006 (Regulation 1831/2003/EC). Natural feed additives that do not endanger the environment with residues in wastes should be developed. There has been normal used the inclusion of probiotics in weaner pig diet because of their can kill or inhibit undesirable organisms, stimulation of the growth, and/or increased digestibility. Bureenok et al., 2005 found methodology for growing and increasing LAB population from grass and used to be additive in silage making process. Moreover, there was found that LAB from grass has probiotics property. This study was conducted to evaluate the effect of dried lactic bacteria (FJLBP) on live performance, and fecal bacteria of weaning pigs.

Materials and Methods

The experiment was performed at the Experimental Unit of the Rajamangala University of Technology Isan and received prior approval from the Animal Protocol Review Committee of the Institution. A total of 40 weaning pigs excluded from receiving feed, 5.0 + 0.8 kg BW, were divided into 4 groups in an environmentally open room. Fermented juice of epiphytic lactic acid bacteria powder (FJLBP) was prepared according to Vasupen et al. (2015). Experimental treatments were as follows: control diet (commercial diet), commercial diet + 0.2% FJLBP, commercial diet + 0.5% FJLBP, and commercial diet + 1.0% FJLBP. Feeding regimen, Controls and Sampling according to standard methods. For 21 day, the animals were allowed ad libitum access to feed and water, and growth performance was monitored weekly to evaluate average daily gain (ADG), dry matter feed intake (DMFI), feed conversion ratio (FCR) and collecting fecal samples. The E. coli from fecal sampling were series diluted and plated on LB agar and incubated at 35°C for 3 d, after which viable colony-forming unit (cfu) were determined.
Data were analyzed as a complete randomized design. All means presented are least squares means. Significance was determined at $P<0.05$.

Results

From a nutritional point of view, it is shown that the relationship between FJLBP level and ADG was nonlinear. Supplemented FJLB at 0.2 and 0.5% in pig diets were higher growth rate than pigs fed control diets and diet with 1.0% FJLB. The *E. coli* number in fecal were not significant different in all treatment. However, the number of *E.coli* in fecal from numeric data were tend to decline when used FJLB increase in the diet.

Conclusion

The rearing of weaning pigs under this experiment, adding FJLBP in the diets have been clearly shown to influence growth performance. However, the relationship between FJLBP level and ADG was nonlinear. Mixing FJLBP in the feed at 0.2% 0.5% and 1.0% had reduced dry matter feed intake (DMFI) and effected on number of *Escherichia coli* (*E. coli*) in fecal compared to those fed control diet. Dietary supplementation of 0.2% and 0.5% FJLBP showed the improvement in term of ADG and FCR of pigs. The optimal FJLBP level for weaned pigs are 0.2% and 0.5%, which it would be conceivable for small-holder farmer.

Table 1. Effect of FJLBP on performance and number of *E. coli* in fecal of weaned pigs.

	control	FJLBP 0.2%	FJLBP 0.5%	FJLBP 1.0%	P-value
Initial Wt. (kg)	5.0±0.5	4.9±0.8	5.1±0.8	4.9±0.4	0.741
Final Wt. (kg)	8.1±1.3[a]	9.2±1.0[b]	9.5±1.2[b]	7.7±0.7[a]	0.002
ADG (g)	146.2±44.4[b]	203.8±23.9[a]	207.1±25.9[a]	137.6±15.8[b]	0.000
DMFI (g)	274.1±35.4	260.0±32.1	259.9±33.6	255.6±23.2	0.585
FCR	2.0±0.4[b]	1.3±0.2[a]	1.3±0.1[a]	1.9±0.1[b]	0.000
E.coli (log_{10} cfu/g)	8.68±0.30	8.66±0.45	8.59±0.67	8.22±0.56	0.578

[a, b] Means within the same row with difference superscripts differ significantly (P<0.05)

Reference

Bureenok, S., T. Namihira, Y. Kawamoto and T. Nakada. 2005. Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guineagrass (*Panicum maximum* Jacq.) silage. Grassland Science. 51: 243-248.

Vasupen, K., B. Saenmahayak, E. Vasupen and S. Bureenok. 2015. Effect of microbial mixture on survival of fermented juice of epiphytic lactic acid bacteria (FJLB). 5th SAADC2015 Proceedings, p.100-101.

Yang, F., C. Hou, X. Zeng and S. Oiao. 2015. The Use of Lactic Acid Bacteria as a Probiotic in Swine Diets. Pathogens (4) 34-45.

PO-03-56

The improvement of growth performance and antioxidant ability in broilers using the additives with herbs and fungal complexes in diets

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Introduction

High-stock intensive chicken farming has been applied to reduce the cost, However, such process may reduce the immune capability and physiological conditions and lead chicken more susceptible to microbial infection. Different fungal b-glucan sources have been investigated to improve the immune ability in chicken. Such changes were fungus dependent and include the change in phagocytic activity and lymphocyte proliferation (Gao et al., 2003), an increase in CD8 and T cell receptor (TCR) cells in chicks (Chae et al., 2006), up-regulation of splenocyte proliferation and IL-2 production in broilers (Chen et al., 2003), and enhancement of macrophage chemotaxis by 1,3-1,6- β glucan from *Schezophyllum commune* (Cheng et al., 2004) and the bactericidal capability of macrophages by interacting directly with receptors of macrophages (Chen et al., 2008).

Recently, we reported that feeding additives with fungal b-glucan can enhance the immune capability of young and mature chicken against *Salmonella* infection depending on fungal species (Chen et al, 2016). b-glucans of *Schizophyllum commune* and *Ganoderma tsugae* (GT) increased the IgM level, while b-glucans of *Ganoderma, Taiwanofungus camphoratus,* and *Botryosphaeria rhodina* increased the IgG level. In this study, we investigated the effects of farming conditions and the additive with different fungal glucans in high stocking farming on the growth performance and antioxidant ability.

Materials and methods

Experimental design

Totally, 360 0-day-old Arbor Acres broiler were used with equal sex and average weight of the chicks was 36 ± 1.3 g. After chicken grew on floor and reached 2 kg in weight. Chicken were randomly distribute into two groups: Low-stocking group with 12 chicken per 0.94 m^2 and high-stockingy group with 12 chicken per 0.64 m^2. In the later, the chicken were separated into six groups, that included the control, addtives with fungal fermented product compound (FFPC), *Ganoderma tsugae* (GT), *Cordyceps militaris* (CM), *Antrodia camphorate* (AC), and antibiotic, respectively. Four repeats in each group and 12 chicken in each repeat were performed. The experimental period was 35 days.

Growth performance measurement

Feed intake (FI) and weight (WG) was measured each week and survival rate was calculated. Food conversion rate was determined by ratio of FI and WG. Furthermore, performance efficiency factor (PEF) was calculated as the following.

PEF (%) = [(survival rate x weight)/(age x feed conversion rate)] x 100%

Measurement of antioxidant and related enzyme activity

Blood was collected from through wing vein of each chicken and stored in tube with anticoagulant EDTA. Then, the serum and red blood cells (RBC) were separated for the following research. In serum, the level of antioxidants malondialdehyde (MDA), glutathione (GSH), and nitric oxide were measured. In red blood cells (RBC), activities of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) and amounts of GSH and MDA were analyzed.

Statistical analysis

Differences among treatments were first analyzed by general linear model (GLM) of SAS (2013) and then Duncan's new multiple range test were determined among treatments.

Results and Discussion

Growth performanceIn general, high-stocking farming reduced the growth performance than the low-stocking

farming (Table 1). In high-stocking farming, antibiotic addition could reduce the death and improved the growth performance; however, it causes some problems such as induction of antibiotic resistant bacteria and antibiotic residuals in chicken. Such disadvantage could be improved by addition of FFPC or single fungus with 0.01% herb. Among six treatments in high-stocking farming, supplementation with CM and 0,01% herb gave the best performance. Furthermore, GT, CM, and AC groups increased the body weight (>7%), FC (>6%) and PEF (>35%), while compared to H control. These results demonstrated that additives can improve the growth performance and then we examined their effects on antioxidant ability.

Effect of supplementation and chicken density on antioxidant abilityTo determine the effect of additives on the antioxidant ability, we evaluated the level of antioxidants MDA, GSH and NO in serum and activity of CAT, GR, GPx, SOD and level of MDA and GSH in RBC cells. In serum, the level of antioxidant substances differed between two controls with lower MDA and NO and higher GSH in low-stocking group, while compared to high-stocking group (Table 2). Among five treatments in high-stocking group, antibiotic group reduced the MDA, GSH and NO compared to control group. In other groups, FFPC treatment resulted in the highest MDA and the lowest GSH and AC treatments exhibited the lowest MDA and NO and the highest GSH. Other treatments did cause any significant change.

In RBC cells, all examined enzymes, except SOD, and antioxidant substances were lower in low-stocking group than high-density group (Table 2), indicating the stress could increase these enzyme activity and antioxidant substances in RBC. In high-density group, antibiotic treatment increased the level of CAT, GR, GSH and SOD and did not vary the level of GPx and MDA. Additives with single fungus plus 0.01% herb varied the enzyme activity and antioxidant level; for examples, the highest activity of CAT and GPx was observed in FFPC treatment, the lowest GR activity in GT treatment, the lowest activity of CAT and GPx and the lowest level of SOD and MDA in CM treatment, and the highest activity of GR and SOD and the highest level of SOD and MDA in AC treatment.

Stress in chicken can be increase by density and stimulate the change in immune ability and other physiological change such as antaioxidant enzymes and substance. Early, we reported that addition of fungus and herb could change the IgG and IgM level (Chen et al., 2016). Indeed, supplementation could change the growth performance and antioxidant activity depending on fungal species. Based on PFP, antioxidant activity, and additive cost, FFPC is recommended as additive to use in high-stocking farming.

Table 1. Nutrient content (DM, OM, CP, and CF) of feed ingredients and complete feeds used in each treatment

Samples	Nutrient content			
	DM (%)	OM (%DM)	CP (%DM)	CF (%DM)
Feed Ingredients :				
Elephant grass	22.45	85.92	9.30	33.81
Concentrate feed	89.46	87.65	17.93	13.05
Soybean seed epidermis	93.42	97.52	13.74	37.59
Complete feed :				
Treatment P0	89.28	87.29	13.72	23.42
Treatment P1	88.96	89.10	13.61	25.88
Treatment P2	89.01	89.48	12.29	28.33
Treatment P3	89.77	90.46	11.37	30.79

Tabel 2. Average CP degradability, gas production, microbial N production, rumen fermentable organic matter (RFOM) and efficiency of microbial protein synthesis (EMPS) at 48 incubation period

Treatment	CP Degradability (%)	Gas Production (ml/500mg DM)	Microbial N production (g)	RFOM (g)	ESPM (g microbial N/kg RFOM)
P_0	45.46^{c}	67.20^{a}	10.62^{a}	260.74^{c}	40.75^{c}
P_1	43.47^{b}	74.50^{a}	10.61^{a}	313.62^{b}	33.82^{b}
P_2	36.25^{b}	83.00^{a}	10.52^{a}	336.52^{b}	31.26^{b}
P_3	29.92^{a}	84.33^{a}	9.79^{a}	356.56^{a}	27.31^{a}

Reference

Chuzaemi, S. dan Hartutik. 1989. Ilmu Kimia Makanan Ternak. Fakultas Peternakan. Universitas Brawijaya. Malang

Elihasridas. 2000. Estimasi Sintetis Protein Mikroba dalam Rumen Sapi yang Diberi Ransum Ampas Sagu Urea Kompleks. Jurnal Peternakan dan Lingkungan. vol.6, No. 01

Iriyani, N. 2001. Pengaruh penggunaan kulit biji kedelai sebagai pengganti jagung dalam ransum terhadap kecernaan energi, protein dan kinerja domba. Animal Production. Journal Produksi Ternak.

Nelwida. 2011. Pengaruh Pemberian Kulit Ari Biji Kedelai Hasil Fermentasi dengan Aspergillus niger dalam Ransum terhadap Bobot Karkas Ayam Pedaging. Jurnal Ilmiah Ilmu-Ilmu Peternakan. Vol.14, No.1

PO-03-58

Effects of dietary resources in high antioxidant activity on broiler chickens.

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Introduction

Yamanashi prefecture (in Japan) is a producing center of a grape and a wine factory is often seen. Grape pomace is used as feed of beef, but most of grape pomace is used as a fertilizer. However, Grape pomace has polyphenol containing antioxidant activity, and chicken meat preservation-related improvement is expected using grape pomace as feed of the chickens. On the other hand, Quinoa native to South America is cultivated as one of the agriculture promotion in Yamanashi. Hirose *et al.* (2010) reports that antioxidant activity of the quinoa seed is high, but antioxidant activity not only the seed but also the straws and leaves are high. It is made good use of resources by making straws and leaves of the quinoa having low utility value feed. There is the expectation of the adipose oxidation suppressant effect by broilers intake rice (red rice and black rice). Therefore I measured the antioxidant activity about local resources in Yamanashi prefecture and investigated influence to preservation characteristics when used with chicken feed it.

Materials and methods

1) Antioxidant activity levels

In first experiment was investigated antioxidant activity of each local source. It was made feed for broiler finisher feed (basal diet), quinoa straws and leaves, grape pomace and black rice as it indicated resources in table 1. Survey item made the phenol level, the flavonoid level, the proanthocyanidin level, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

2) Animal, Diets and Housing

In second experiment, one hundred twelves female broiler chicks (ROSS strain) of 21-day old obtained from commercial hatchery were reared on floor pens in windowless house. There were 16 birds in each group. Basal diet was formulated to contain most essential nutrient at recommended levels (Japanese Feeding Standard for Poultry, 2011). Basal diet contained with 10IU Vitamin E. Chick were provided for 51 days with one of seven experimental diets: (1)Basal diet (CON) (2)Quinoa seed (QS): a diet of with 5% quinoa seeds (3)Crush quinoa straws and leaves (QL): a diet of with 5% quinoa straws and leaves in crasher (4) Grape pomace (GP): a diet of with 5% grape pomace (5)Red rice (RR): a diet of with 5% red rice (6)Black rice(BR): a diet of with 5% black rice (7)Vitamin E(VE): a diet of with 500IU vitamin E. Feed and water were provided *ad libitum* throughout the experimental periods and light was provided 24 hours a day.

3) Measure of Abdominal fat weight and ratio

At the end of the second experiment, 5birds from each group that had body weight close to the grope mean were chosen. The birds were killed by cutting the carotid artery and the feather were removed by soaking the killed birds in warm water (62℃).

The carcasses without feather were chilled for 24 hours before separation of abdominal fat. The percentage ratio of abdominal fat was calculated on the basis of the carcass weight.

4) Measurement of thiobarbituric acid reactive substance (TBARS) value in breast meat

In second experiment, at 51 days of age, 6 birds in each dietary group were used for the determination of breast meat of TBARS value. The right breast meat which I offered for the dismantling was stored at 4°C. The breast meat stored at 4°C was considered to be a mince at 1st day, 4th day and 7th day later. I handled a vacuum with a bag made of aluminum and stored a mince of 20g until analysis at -80°C. TBARS value was determined by improved procedure of Salih *et al.* (1987). The provided data performed a regression analysis. The thing that linear regression did not become meaningful performed quadratic regression or exponential regression.

Results

1) Antioxidant activity levels (Experiment 1)

Table 1 show result of antioxidant activity levels by local resources. Water content showed no differences due to

resources. Local resources (Quinoa straws and leaves, Grape pomace and Black rice) were higher total phenol and total flavonoid than basic diet.

The proanthocyanidin level of grape pomace exceeded 27mgE/100gFW of basic diet. Quinoa straws and leaves and black rice were below the measuring limit. The DPPH radical scavenging activity that was an antioxidant active index showed the value that quinoa, black rice and grape pomace were high in and showed activity of 4-6 folds of the basal diet.

2) Growth performance, Feed conversion and Abdominal Fat weight and ratio (Experiment 2)

Table 2 show results of body weight, feed conversion and abdominal fat weight and ratio in 51days as influenced by supplementation of local resources. There was no significant difference in growth performance and abdominal fat weight and abdominal fat ratio. But body weight in chick fed on quinoa straws and leaves and grape pomace were lower than basal diet.

3) Change of TBARS value in breast meat (Experiment 2)

The results regarding thiobarbituric acid reactive substance (TBRAS) value of breast meat showed Figure1 and Figure2. No significant differences were observed TBARS value in 1st day. TBARS value of breast meat of 7th day preservation was shown in CON as 0.5043mg of MDA/100g meat. TBARS levels of the breast meat increased by the progress of the preservation days, and the increment had most control group. In 7th day, QS and VE was significantly lower than control. It showed the value that was lower than CON which did not become the difference of the significantly about other resources. Therefore, the possibility that the meats chicken diet which had high antioxidant activity contributed to preservation-related improvement was suggested.

Table 3 showed the result of the liner regression that assumed in TBARS level (x) and the preservation days (y) .

A liner regression was significantly difference in CON, RR and BR. TBARS levels increased 0.0521 in control ward as it passed on 1st day. In addition, it was shown that a red rice over 0.0191 and a black rice over 0.0226.

On the other hand, about a quinoa straws and leaves, the grape pomace, a result of the liner regression did not significantly difference. Therefore I performed the quadratic regression analysis or involution analysis and showed the exponential regression of the coefficient of correlation in table 3. The quadratic regression ($y = -0.0103x2 + 0.1005x + 0.0538$) was shown about a quinoa straws and leaves. A grape pomace increased with the progress on the date was recognized, and about the Quinoa seed, red rice, black rice and vitamin E, it was shown in the quinoa straws and leaves and grape pomace by the progress in the preservation days that the rise of the value was remarkable whereas TBARS level rose approximately linearly with the progress in the preservation days.

Discussion

Chicken meat was preservation-related low food that was contained a lot of unsaturated fatty acid. Expiry date is around 3-4 days.

It is wished that distribute local special product chickens such as Koshujidori and Koshuhootoshidori as fresh in Kansai area or Kyushu area. Because the circulation of the wide area with fresh is high-risk in quality maintenance, it is the situation supporting by the frozen circulation at the expense of taste. On the other hand, Saura-Calixto F. *et al.* (2003) reports that 35-61% of the wine polyphenol bind to the fiber was not only activate a intestine, but also contribute to antioxidation. In addition, quinoa, red rice and black rice include a lot of polyphenol, too. A quinoa straws and leaves, grape pomace, black rice was showed the value that the DPPH radical scavenging activity was high. DPPH radical scavenging activity of the broiler diet was 524nmolE/100gFW. Because it was the value that quinoa straws and leaves 5% addition was 23% high, and grape pomace 5% addition was14% high, it was thought that the use was possible enough as antioxidant resources. The preservation characteristics of the chicken assumed TBARS level. It is a method to let the content develop in thiobarbituric acid (TBA) because lipid in chicken oxidizes the TBARS level and changes into malondialdehyde (MDA). All resources were superior to control group in preservation in the final examination for seven days. In addition, control group, the imported red rice, black rice and VE group rise linearly with the progress of the days in preservation. This showed that the effect varied according to local resources, and it was suggested that the mechanism was different.

There are various reports about the grape pomace. Goni *et al*. (2007) Bernes *et al.* (2008) was reported that there was not influence to growth performance, the digestibility of the crude protein (CP), abdominal fat weight, liver weight, pancreatic weight, splenic weight and the small intestinal length as diet added 6% of grape pomace condensates in broiler. In addition, there were reported the diachronic change of the MDA content in breast meat. These results showed that addition of grape pomace is lower MDA content than control group. It was similar to a present result. Pasko *et al.* (2010) was investigated MDA (plasma, heart, liver, pancreas, lungs and spleen) that

give a quinoa seed of the rat took in high fructose diet. As a result, plasma MDA content increased that didn't take quinoa seed in rat, but it took quinoa seed was decreased. In this way, because the reduction effect of the oxidation stress is reported, I think that it is necessary to investigate polyphenol and relations with oxidation stress and the immune reactivity more.

Conclusion

Six local resources (quinoa seed, quinoa straws and leaves, grape pomace, red rice, black rice and vitamin E) investigated that effect on growth performance and meat preservation characteristics (TBARS value). As a result, it became clear that these local resources has effects to lower the TBARS level, however, quinoa straws and leaves, grape pomace were decreased final body weight than basal diet. Therefore, the quinoa straws and leaves and grape pomace were shown not to be practical to cause a down of the growth weight. The development of the method to let feed put local resources to practical use will be necessary in future.

Table 1 Antioxdant activity of local resources

Resources	moisture	Total Phenol	Total Flavonoid	DPPH radical scavenging activity	Proanthocyanidin
	(%)	mgE／100gFW	mgE／100gFW	nmolE／100gFW	mgE／100gFW
Basal diet	11.6±0.1	194±1	131±9	524±9	27±2
Quinoa straws and leaves	8.1±0.4	847±22	517±3	2,999±24	N.D.*1
Black rice	11.3±0.2	490±15	280±4	2,629±26	N.D.*1
Grape pomace	8.0±0.2	389±19	407±9	1,958±46	838±29

※Mean ± standard diviation (n=4)

*1 : No Detection

Table 2 Effect of dietary local resources on growth performance, feed conversion and Abdominal fat ratio

Diet	Body weight (g)		Feed conversion		Abdominal fat	
	21days	51days	21-51d	0-51d	weight(g)*1	ratio(%)*1
Basal Diet (Control)	1,016	3,511	2.10	1.86	92 ± 23	2.89 ± 0.71
Quinoa seed	968	3,540	2.15	1.93	88 ± 11	2.76 ± 0.33
Quinoa straws and leaves	978	3,444	2.30	2.02	87 ± 9	2.51 ± 0.28
Grape pomace	956	3,347	2.25	1.99	97 ± 29	3.21 ± 0.96
Red rice	996	3,626	2.11	1.90	93 ± 16	2.83 ± 0.42
Black rice	997	3,581	2.15	1.92	109 ± 22	3.35 ± 0.70
Vitamin E	999	3,555	2.24	1.98	87 ± 22	2.81 ± 0.63

※Abdominal fat ratio (% of carcass in 51days)

*1 : Mean ± standard diviation (n=5)

Table 3 TBARS value regression of breast meat (Liner or quadratic or exponential regression)

Diet	Liner regression			Quadratic or exponential regression	
	Liner regression	Correlation coefficient	P-value	Quadratic or exponential regression	Correlation coefficient
Basal Diet (Control)	y = 0.0521x + 0.1300	0.841	0.010	–	–
Quinoa seed	y = 0.0118x + 0.1879	0.195	0.300	$y = -0.0103x^2 + 0.1005x + 0.0538$	0.677
Quinoa straws and leaves	y = 0.0208x + 0.1495	0.516	0.100	$y = 0.1499x^{0.3303}$	0.744
Grape pomace	y = 0.0476x + 0.1637	0.502	0.110	$y = 0.1709x^{0.5358}$	0.710
Red rice	y = 0.0191x + 0.1572	0.800	0.016	–	–
Black rice	y = 0.0226x + 0.1327	0.817	0.013	–	–
Vitamin E	y = 0.0199x + 0.0956	0.705	0.036	–	–

※ y: TBARS value (mg MDA/kg) x: Preservation days in 4℃ (Days)

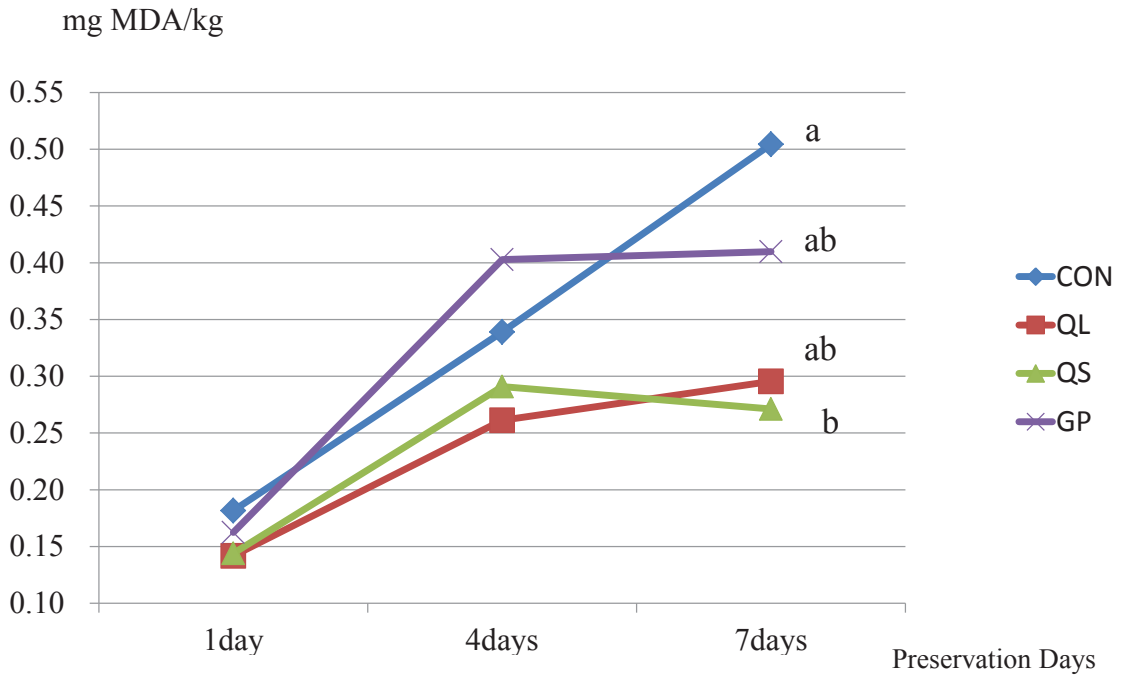

Fig 1 Change of TBARS value by the preservation at 4℃ (Local sources)

a,b Means within the same rows with no common superscript are significantly different ($p<0.05$)

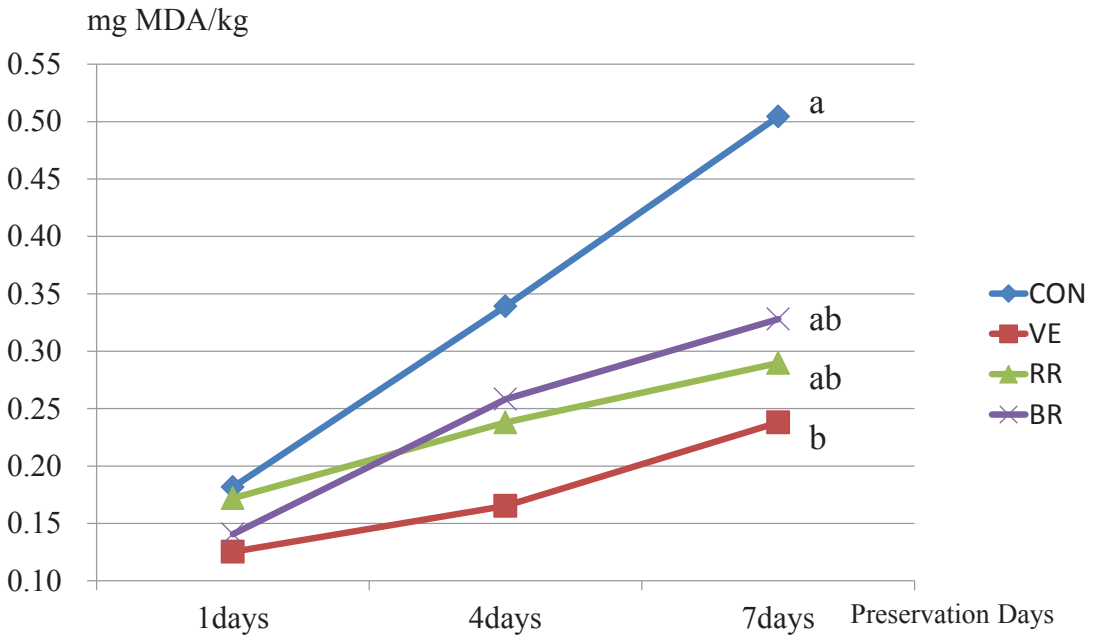

Fig 2 Change of TBARS value by the preservation at 4℃ (Rice and Vitamin E)

a,b Means within the same rows with no common superscript are significantly different ($p<0.05$)

References

Brenes A., A. Viveros, I. Goni, C. Centeno, S. G. Sayago-Ayerdy, I. Arija and F. Saura-Calixto : Effect of Grape Pomace Concentrate and Vitamin E on Digestibility of Polyphenols and Antioxidant Activity in Chickens., Poult. Sci., Vol.87, 307-316, 2008

Goni I., A. Brenes, C. Centeno, A. Viveros, F. Saura-Calixto, A. Rebole, I.Arija and R. Estevez : Effect of Dietary Pomace and Vitamin E on Growth Performance, Nutrient Digestibility, and Susceptibility to Meat Lipid Oxidation in Chickens., Poult. Sci., Vol.86, 508-516, 2007

Hirose Y., T. Fujita, T. Ishii and N. Ueno : Antioxidative properties and flavonoid composition of Chenopodium quinoa seeds cultivated in Japan, Food Chemistry, Vol.119, 1300-1306, 2010

Pasko P., H. Barton, P. Zagorodzki, A. Izewska, M. Krosniak, M. Gawlik, M. Gawlik and S. Gorinstein : Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats., Plant Foods Hum. Nutr., Vol.65, 146-151, 2010

Salih A.M., D.M. Smith, J.F. Price and L.E. Dawson : Modified extraction 2-thiobarbituric acid method for measureing lipid oxidation in poultry., Poult. Sci. vol.66, 1483-1488, 1987

Saura-Calixto F. and M. E. Diaz-Rubio : Polyphenols associated with dietary fiber in wine. A wine polyphenol gap? , Food Res. Int. Vol.40, 613-619, 2003

PO-03-62

Effect of radioactive cesium on Japanese black cows grazing on decontaminated pastures

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Introduction

The accident at the Fukushima Daiichi Nuclear Power Plant on March 11, 2011 released a large amount of radioactive substances into the environment. Radioactive cesium released from this site polluted the soil, plants and animal products in extensive regions of eastern Japan. Since the levels of radioactive cesium contamination in grass decreased owing to pasture renovation, renovated grass, whose radioactive cesium concentration is determined to be below the provisional regulation value, which could be permitted for cattle grazing. We previously found a correlation between count rate on the body surface of live -cattle (surface monitoring) and radioactive cesium concentration in the skeletal muscle (neck) of cattle. However, information about the transfer of radioactive cesium in the body of cattle grazing on decontaminated pastures is lacking. This study aimed to estimate the radioactive cesium concentrations in the muscle by measuring those in the body and peripheral blood of cattle grazing on decontaminated pastures.

Materials and Methods

The pasture used in this study was decontaminated by pasture renovation with plow cultivation and seeding on December 3, 2013. The experiment was performed using four Japanese black cows that were allowed to graze in the decontaminated pasture (1.5 ha) in Miyagi Prefecture from May 26 to October 21 in 2014. Count rate on the surface monitoring was measured at the cow neck by using a NaI (TI) scintillation survey meter of a portable detector (SAM 940; Berkeley Nucleonics Co., USA). The radioactive cesium (^{137}Cs + ^{134}Cs) concentrations in the peripheral blood, the grass and soil were determined using gamma-ray spectrometry by high-purity germanium detectors (Ortec Co., USA).

Results and Discussion

The radioactive cesium (^{137}Cs + ^{134}Cs) concentrations of the decontaminated pasture were higher in the grass roots (24.9–1159.8 Bq/kg) or soil (33.0–586.0 Bq/kg) than in the grass (5.5–103.6 Bq/kg). They differed at each sampling point in the pasture. Count rate on the surface monitoring was significantly ($P < 0.05$) higher at 5, 7, 9, 13, 15, 17, 19, and 21 weeks after grazing than before grazing. The radioactive cesium (^{137}Cs + ^{134}Cs) concentration in the peripheral blood was significantly ($P < 0.05$) higher at 1, 3, 7, 9, 13, 15, 17, 19, and 21 weeks after grazing than before grazing.

Count rate on the surface monitoring and the radioactive cesium concentrations in peripheral blood of grazing cows increased by 9.9 cps and 1.0 Bq/kg more than those before grazing, respectively. Fukuda et al. (2015) reported a linear correlation between blood and muscle cesium concentrations at low radioactivity level ($Y = 26.0X$, $R^2 = 0.738$), indicating that cesium radioactivity in the muscle can be estimated from that in the blood. We also obtained similar results in the past study. Based on the relationship between the body surface and blood and muscle cesium radioactivity, we estimated that the radioactive cesium concentration in the muscle was around 26 Bq/kg.

Conclusion

The radioactive cesium concentration in the muscle of cows grazing in decontaminated pastures was around 26 Bq/kg.

References

Fukuda T, Kino Y, Abe Y, Yamashiro H, Kobayashi J, Shimizu Y, et al. 2015. Cesium radioactivity in peripheral blood is linearly correlated to that in skeletal muscle: Analyses of cattle within the evacuation zone of the Fukushima Daiichi Nuclear Power Plant. *Animal Science Journal* 86, 120–124.

PO-03-63

Anatomy and morphology of hyperostosis in Genus Caranx and Genus Alectis, from Anadaman sea, Thailand

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INTRODUCTION

The hyperotosis usually describes as a thickening of special bones that takes a swollen aspect. It is characterized by an increase of the periosteal ossification that combined to resorption of the bone tissue (Meunier *et al*.,2010; Smith-Vaniz *et al*.,1995; Smith-Vaniz and Carpenter, 2007).

From previous report, researchers likely discuss the nomenclature linked to these forms as the focal (osteoma), the regional (pachyostosis) benign bone tumour (Capsso, 2005). The focal hyperostosis known as osteoma that were documented in comparative pathology (Gervais,1865).

Moreover, the comparative pathology of cancer has been reported in serveral species of vertebrates,starting from jawless fish.In amphibians and wild birds seen to be extremely rare.The prevalence of neoplasms is particularly higher in both bony fish and mammals(Capasso,2005). In addtion, hereditary osteochondroma has been common documemted in some other mammals such as domestic horses, cats and dogs (Owen and Bostock,1971;Capasso,2005). In mammal, focal hyperostosis has also been reported mandibular swelling in dogs (Thornburg,1979). Capasso (1997) have reported the phylogenetic relationship between focal hyperostosis in fish and the osteoma in modern humans.

In teleost, the hyperostosis is found in different bones such as supraoccipital, cleithrum, pterygiophores, ribs, skulls, claviculae, haemal and neural spines (Smith-Vaniz *et al*.,1995;Smith-Vaniz and Carpenter, 2007; Meunier *et al*., 2010; Giarratana *et al*., 2012). However, The cause of hyperostosis is in bone still unknown.

Up to present day, in the seas at the Southeast Asia Peninsula, the prevalence of hyperostosis bone in marine fish is no reported. Therefore, the aim of this research is ducumented as evident citation of hyperotosis bone in genus Caranx and genus Alectis in this region including Andaman sea, Southern Thailand.

MATERIALS AND METHODS

In present study, the specimens of Genus Caranx (*Caranx iqnobilis)* and the genus Alectis (*Alectis indica and Alectis ciliaris*) were captured from Andaman sea,the southern coast of Thailand during March to June 2016. The fish was identified using Nelson guide (2006). The specimens of hyperostosis were measured by vernier caliper, including the position, shape and number of neural spine were recorded. Moreover, the hyperostosis of specimens was compared among the genus by radiological examination. The X-ray radiography was made using Fuji Computed Radiography (FCR) digital model. In addition,the gross anatomy of the hyperostotic bones only the part of the column that showed the hyperostosis was described and photographed by using digital camera and FCR capsular V view program.

All measurements were in millimeters (mm); standard length was measured from the tip of the snout to the midbase of the caudal fin. Fork length was measured from the front of the upper lip to the tip of shortest median caudal-fin ray. Body depth was measured from the greatest vertical distance across the body. Eye diameter was defined by the orbital rims. Lengths of the dorsal and anal-fin bases were straight-line measurements from both the dorsal and anal-fin to the posterior base of the terminal fin ray of the respective fin.

RESULTS AND CONCLUSION

Anatomical measurements

The morphological characteristics of the *Caranx ignobilis* is determined the species, following the key by Nelson (2006). The one specimen, the following measurements are recorded from x-ray digital program in different proportions; The standard length of the fish is 693.31 mm; head length, 187.02 mm; eye diameter, 41.26 mm; body depth, 228.58 mm; fork lenght, 727.3mm; dorsal fin length, 255.17 mm; anal fin length, 211.82 mm.

There are found the hyperostotic bone, occurring in *C. ignobilis* on specimen from kuraburi, Andaman sea. The hyperostotic regions of the spine are located in the first third part of the neural and haemal spine. The hyperostosis on the neural spine and ribs found varies in size and shape in different proportions; on the neural

spines of the 11 th to 16 th vertebra, on the haemal spines of the 10 th to 16 th vertebra as show in Figure1. The largest hyperostotic bone is the haemal spine of the 12th vertebra (8.38 mm in maximum size width).

Measurements (*Alectis indica* Rüppell,1830): The standard length of the fish is (844.45 mm in maximum size and 810 mm in minimum) of three specimens observed; head length (214.1 mm in maximum size and 189.54mm in minimum), eye diameter (43.18 mm in maximum size and 40.57 mm in minimum), body depth (348 mm in maximum size and 340 mm in minimum).

This study founded hyperostosis bone in supraoccipital area *of Alectis indica*. The hyperostotic bone located on four regions; on the supraoccipital area being the largest size; the dorsal pterygiophore;on the postcleithrum and the ribs being smallest size. The largest hyperostotic bone found in supraoccipital area with oval borders (125.82 mm in maximum length and 55.67 mm in width), the smallest hyperostotic bone found in supraoccipital area (113.09 mm in lenght and 54.17 mm in width) but hyperostosis in haemal spines and neural spines are absent.

The hyperostotic region of the dorsal pterygiophore is situated in the top third part of the neural spine. Both of sizes and shapes are varies as irregular-spherical and spherical elongated, for the 1st , 2rd and 3th dorsal pterygiophore respectively, while on postcleithrum is located in the first third with spindle-shaped. The sizes of ribs ranged between 4.47 mm in maximum width and 3.97 mm in minimum width). The largest pterygiophore is the 1st dorsal (47.99 mm in maximum length and 27.89 mm in width) on the other hand, the smallest hyperostotic bone in the 3th (9.53 mm in lenght and 5.30 mm in width) as show in Figure 3.

Measurements (*Alectis ciliaris* Bloch,1788): Three specimen of *Alectis ciliaris* are recorded in different proportions; The standard length of the fish is (720.39 mm in maximum size and 628.29 mm in minimum), head length (176.94 mm in maximum size and 162.16 mm in minimum), eye diameter(46.01 mm in maximum size and 42.85 mm in minimum), body depth (277.70 mm in maximum size and 256.29mm in minimum).The hyperostotic bone are absent on the supraoccipital, dorsal pterygiophore,postcleithrum, ribs, neural and haemal spines. The specimen of *Alectis ciliaris* is hyperossified at the centrum of caudal vertebrae between proximal and dital of caudal peduncle as show in Figure 4.

Measurements (*Alectis ciliaris* Bloch,1788): Three specimens of *Alectis ciliaris* are recorded in different proportions; The standard length of the fish is (720.39 mm in maximum size and 628.29 mm in minimum),head length (176.94 mm in maximum size and 162.16 mm in minimum), eye diameter(46.01 mm in maximum size and 42.85 mm in minimum), body depth (277.70 mm in maximum size and 256.29mm in minimum).The hyperostotic bone are absent on the supraoccipital, dorsal pterygiophore, postcleithrum, ribs, neural and haemal spines. The specimen of *Alectis ciliaris* is hyperossified at the centrum of caudal vertebrae between proximal and distal of caudal peduncle as show in Figure 4.

For the first time, a case of hyperostotic bone is first reported from the marine water in Thailand. This case is described the hyperostotic bone in the three species teleost from Genus Caranx and Genus Alectis captured from the Andaman sea, Thailand. The hyperostotic bone were present on the supraoccipital, dorsal pterygiophore, postcleithrum, ribs, neural and haemal spines. The size, shape and position of hyperostotic bones showed the variation in neural spines with this case. In addion, new characteristics of the hyperostotic bone found in *Alectis indica* that located on postcleithrum as spindle shaped. In the specimen of *Alectis ciliaris* is hyperossified at the centrum of caudal vertebrae between proximal and distal of caudal peduncle.

Because only a single specimen from Genus Caranx (*Caranx iqnobillis*) and six specimen from Genus Alectis (*Alectis indica* and *Alectis ciliaris)* were obtain. So far,it was needed more data to comfirmation hyperostotic hypothesis (Bazzini *et al*.,1986 and capasso,2005).

Therefore,further study will be necessary to certify previous hypothesis to fill the research gap in morphological and histological data in order to understand the systematic distribution of the different types of hyperostosis.

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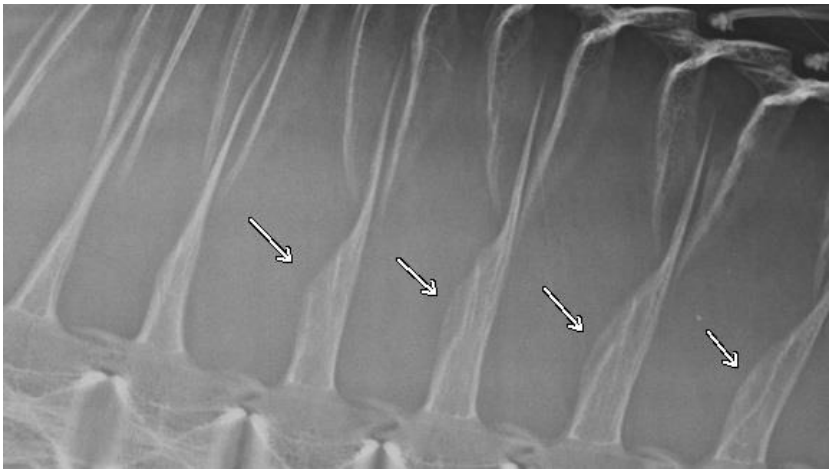

Figure 1. Radiographs of the *Caranx ignobilis* exhibiting hyperostosis bone (white arrow) and the normal structure of neural spines as show on the left

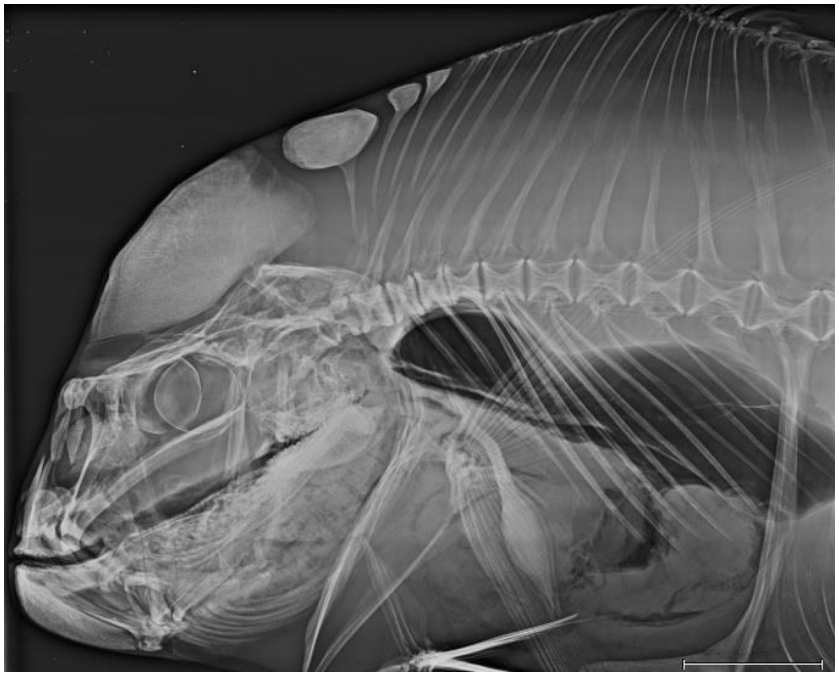

Figure 2. Radiographs of *Alectis indica* with presence of hyperostosis (scale bar:70 mm)

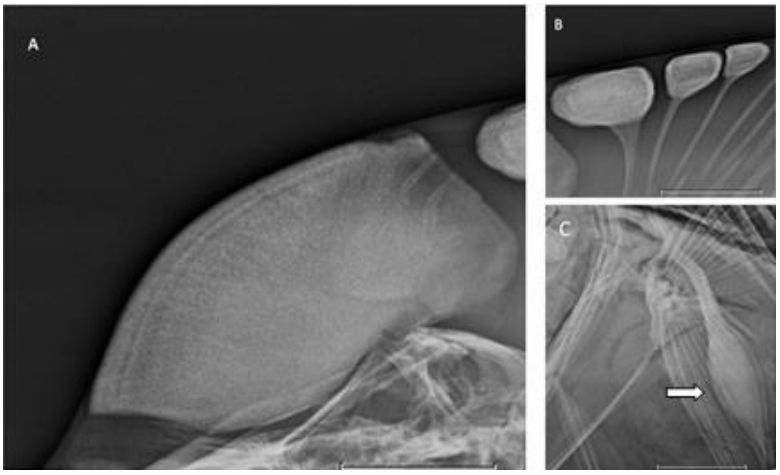

Figure 3. Radiographs of *Alectis indica* with presence regions with hypoostosis A.Supraoccipital B.dorsal pterygiophore C. postcleithrum (white arrow) scale bar: 40 mm

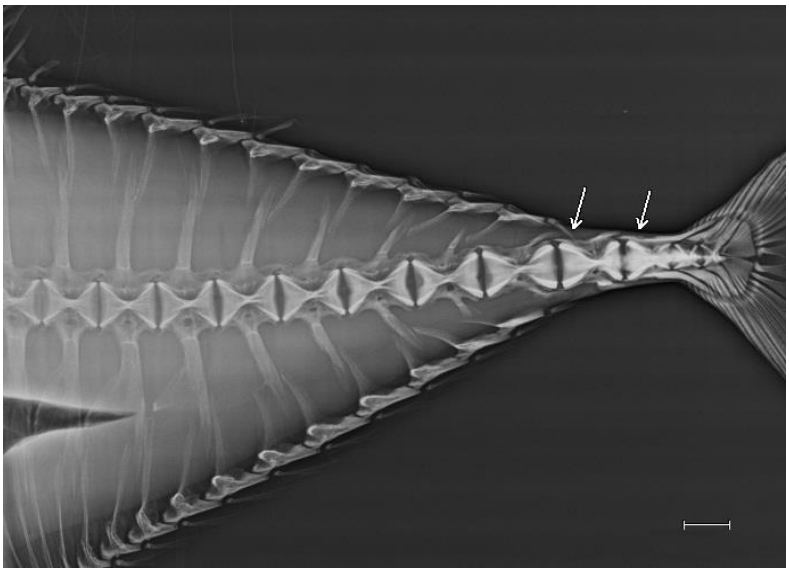

Figure 4. Radiographs of *Alectis ciliaris* with hyperossified at the centrum of caudal vertebrae (arrows), collected from Andaman Sea, Thailand.(scale bar:20 mm)

REFEREANCES

Bazzini, M.D., Reynold, J.E III. and Essman, R.A.1986. Erythropoiesis and granu-antiquity of cancer lopoiesis in the West Indian manatee. *Am J Clin Pathol*. 83:457–62.

Capasso, L.L.2005.Antiquity of cancer. *Int. J. Cancer*.113: 2–13.

Capasso, L.L.1997.Osteoma: paleopathology and phylogeny. *Int J Osteoarch*.7:615–20.

Gervais, P.1875. De l'hyperostose chez l'homme et chez les animaux. I et II parties. *Journ Zoologie*. 4:272–84 and 445–62.

Giarratana,F.,Ruolo,A.,Muscolino,D.et al.2012.Occurrence of hyperostotic pterygiophores in the silver scabbardfish, *Lepidopus caudatus* (Actinopterygii: Perciformes, Trichiuridae). *Acta Ichthyologica et Piscatoria*.42(3): 233-237.

Holland, K.N., C.G. Lowe and B.M. Wetherbee. 1996. Movements and dispersal patterns of blue trevally (*Caranx melampygus*) in a fisheries conservation zone.*Fisheries Research*. 279-292.

JAWAD, L.A. 2013. Hyperostosis in three fish species collected from the Sea of Oman. *The Anatomical Record*, 296(8): 1145-1147.

Kimura, S., Satapoomin, U., and Matsuura, K. 2009. Fishes of the Andaman Sea west coast of southern Thailand.National Museum of Nature and Science, Tokyo. 346 pp.

Kranenburg, H.C., Meij,B.P. and Hazewinkel,H.W. 2013. Spinal hyperostosis in humana and companion animals.*Veterinary Sciences Tomorrow*.1-15.

Mata, S., Fortin, P.R., Fitzcharles, M.A., et al.1997. A controlled study of diffuse idiopathic skeletal hyperostosis: Clinical features and functional status. Medicine .76(2):104-117.

Meunier,F.J.,Gaudant,J. and Bonelli,E.2010. Morphological and histological study of the hyperostoses of Lepidopus albyi (Sauvage, 1870), a fossil Trichiurideae from the Tortonian (Upper Miocene) of Piedmont(Italy). *Cybium*.34: 293-301.

Nelson, J.S.2006. Fishes of the World. 4thed.John Wiley and Sons,New York.601 p.

Owen,L.N. and Bostock,D.E.1971. Multiple cartilaginous exostoses with development of a metastasizing osteosarcoma in a Shetland Sheepdog. *J. small Anim. Pract*. 12:507-512.

Satapoomin, U. 2011. The fishes of southwestern Thailand, the Andaman Sea a review of research and a provisional checklist of species. *Phuket Marine Biological Center Research Bulletin*. 70: 29–77.

Smith-Vaniz,W. and Carpenter, K.E.2007.Review of the crevalle jacks, *Caranx hippos* complex (Teleostei:Carangidae),with a description of a new species from West Africa.*Fish. Bull*.105: 207–233.

Thornburg, L.P.1979. Craniomandibular osteopathy in the Canidae. *Am J Pathol*. 95:575– 8.

PO-03-66 Zinc sulfate pretreatment impacts the testicular antioxidant capacity and the acrosome biogenesis in Bama miniature pig under high ambient temperature

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Objective

The acrosome is a specialized membranous organelle that covers over the anterior part of the sperm nucleus and plays an important in the process of fertilization (Berruti et al., 2011; Jin et al., 2011). High environmental temperature could cause the increased value in abnormal acrosome rate in mammals (Aydilek et al., 2015; Cabezón et al., 2016). Acrosome biogenesis begins with the initial phase of spermatid development and ends until the spermatogenesis is finished with the production of mature sperm (Kang-Decker et al., 2001). There are four phases described as follows: the Golgi phase, the cap phase, the acrosome and maturation phases (Kang-Decker et al., 2001). It is unclear whether high environmental temperature impact the acrosomal biogenesis. The molecular mechanism underlying the heat-induced abnormal acrosome is still unknown.

Zinc (Zn) is an indispensable element in reproduction and required for the maintenance of germ cells, progression of spermatogenesis and regulation of sperm motility (Zhao et al., 2011). Dietary Zn supplementation might enhance the activity of antioxidant pathways via promoting the expression of Cu-Zn superoxide dismutase (Cu-Zn SOD) (Cao et al., 2015). Our recent studies reported that dietary Zn alleviated the decline of serum Zn during periods of high ambient temperatures, but decreased the testis weight and impacted epididymal sperm counts (Li et al., 2015). Taken together, the present study also studied the acrosome biogenesis to determine whether dietary Zn affect the spermatogenesis.

The aim of this study was to investigate the effect of Zn sulfate pretreatment on the acrosome biogenesis and testicular antioxidative potential in heat-stressed Bama miniature pigs.

Methodology

Bama miniature pigs (male; 6-mo old; BW = 10.79 ± 0.06 kg; n=24) were randomly allotted to 4 groups and fed a basal diet or the basal diet supplemented with 1500 mg of Zn ($ZnSO_4 \cdot H_2O$)/kg diet for 38 d. At 7 mo of age (d 30), the thermal neutral (TN) groups remained at 25 ºC, whereas the heat treatment (HT) groups were exposed to ambient temperature at 40 ºC for 5 h daily for 8 consecutive days. Pigs in 4 groups were sacrificed on d 38.

Small pieces of testes were fixed for 1 h at 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Preparations were washed in the same buffer and postfixed for 1 h in cold 1% osmium tetroxide in cacodylate buffer. After dehydration in graded series of ethanol solutions, the preparations were embedded in Araldite (EPON 812, Emicron, Shanghai, China). Ultra-thin sections were stained with saturated uranyl acetate in 50% ethanol and lead citrate, and examined by transmission electron microscopy (JEM,1230, JEOL Company, Tokyo, Japan).

Testicular malondialdehyde (MDA), Glutathione peroxidase (GSH-PX), hydrogen peroxide (H_2O_2), Cu-Zn SOD and γ-glutamyl cysteine synthetase (γ-GCS) were measured using commercial kits in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Proteins were extracted with protein extraction reagents (Thermo Fisher Scientific Inc., USA). Proteins (30 μg) were separated by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to apolyvinylidene difluoride (PVDF) membrane (BioRad, Hercules, CA, USA). Membranes were blocked and then incubated with p62 primary antibody (ab56416). Pig β-actin antibody (Sigma) was used for protein loading control. After primary antibody incubation, membranes were washed, incubated with alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgG antibodies (Promega, Madison, WI, USA), and quantified and digitally analyzed using the image J program (NIH).

Total RNA was isolated from frozen small intestine using TRIzol reagent (Invitrogen, Carlsbad, CA, US) and treated with DNase I (RNase-free) (TaKaRa, Dalian, China) to remove genomic DNA. The total RNA concentration and purity were determined by a spectrophotometer (SMOIF, Shanghai, China). For each sample, 5 μg of total RNA was reverse transcribed to cDNA with M-MLV reverse transcriptase (TaKaRa, Dalian, China) and oligonucleotide primers.

Targets genes and the housekeeping gene *beta actin* (*β-actin*) were quantified by real-time PCR on an ABI 7300 system using a commercial kit (SYBR Premix Ex Taq, TaKaRa, Dalian, China). Primers were designed with Primer

5.0 according to the gene sequence of pig (http://www.ncbi.nlm.nih.gov/pubmed) to produce an amplification product. The primer sets used were shown as follows: *β-actin* (F: 5'-CGTTGACATCCGTAAAGACC-3'; R: 5'-GGAGCCAGGCAGTAATCT-3'); *Nrf2* (F: 5'-TTTGGAGGCAAGACATAAG-3'; R: 5'-TGGGCAACCTGGAGTA-3'); *HO-1* (F: 5'-TTCACCTTCCCGAGCAT-3'; R: 5'-GCCTCTTCTGTCACCCTGT-3'); *NQO1* (F: 5'-CATCATTTGGGCAAGTCC-3'; R: 5'-TCACAGCTGGCAGAAC-3'); *GCLC* (F: 5'-ATCAGTAAGTCTCGGTATG-3'; R: 5'-ATGTCACGCACGATTT-3'). PCR reactions (consisting of SYBR Premix Ex Taq, ROX Reference Dye, 200 nM primer, and 100 ng cDNA template) were run in triplicates in a 20-μl total reaction volume. The amplification conditions were as follows: DNA polymerase activation at 95°C for 30 sec, followed by 42 amplification cycles of denaturation at 95°C for 5 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. The specificity of the PCR product was verified with a melting curve and by agarose gel electrophoresis. The relative mRNA concentration was calculated using the $2^{-\Delta\Delta Ct}$ method. All samples were measured in triplicate. The values were normalized using ACTB as the endogenous standard.

Two-way ANOVA was used to determine the effects of dietary Zn and temperature and their interaction using the GLM procedure of Statistical Production and Service Solution software (version 16.0; SPSS, Inc., Chicago, IL). Data are expressed as the mean ± standard error of the mean. Probability values less than 0.05 were considered statistically significant.

Results

High environmental temperature significantly increased the Cu-Zn SOD levels (TN0: 2.789 ± 0.405; HT0: 3.981 ± 0.452; TN1500: 2.629 ± 0.319; HT1500: 3.257 ± 0.277; P_t = 0.008; P_{Zn} = 0.521; $P_t \times_{Zn}$ = 2.30). There is no significant difference in the *P*-values of P_t, P_{Zn}, and $P_t \times_{Zn}$ in MDA concentration, H_2O_2 level, and the activities of GSH-PX and γ-GCS.

High environmental temperature significantly increased the mRNA expression of *NQO1* (TN0: 1.000 ± 0.561; HT0: 3.224 ± 0.145; TN1500: 1.621 ± 0.312; HT1500: 3.900 ± 1.196; P_t = 0.003; P_{Zn} = 0.322; $P_t \times_{Zn}$ = 0.965), and *GCLC* (TN0: 1.500 ± 0.236; HT0: 21.180 ± 3.714; TN1500: 2.454 ± 0.439; HT1500: 9.623 ± 4.818; P_t = 0.001; P_{Zn} = 0.01; $P_t \times_{Zn}$ = 0.001). Additional Zn decreased the mRNA expression of *Nrf2* (TN0: 1.000 ± 0.473; HT0: 1.864 ± 0.113; TN1500: 0.846 ± 0.113; HT1500: 0.886 ± 0.048; P_t = 0.055; P_{Zn} = 0.041; $P_t \times_{Zn}$ = 0.124), *HO-1* (TN0: 1.000 ± 0.302; HT0: 1.236 ± 0.613; TN1500: 0.166 ± 0.020; HT1500: 0.337 ± 0.056; P_t = 0.574; P_{Zn} = 0.030; $P_t \times_{Zn}$ = 0.915) and *GCLC* (P_{Zn} = 0.01).

Both high environmental temperature and Zn supplementation increased testicular p62 protein levels (P_t = 0.001; P_{Zn} = 0.001; $P_t \times_{Zn}$ = 0.055). TEM images showed many autolysosome accumulated and the acrosomal vesicles were failure to recruit to the nucleus-associated acrosome in spermatids in pigs of the group fed with supplemental Zn diet.

Conclusion

Mammalian spermatogenesis is temperature dependent, and a scrotal temperature above the normal range causes the decreased semen quality and infertility (Li et al., 2014). There are several mechanisms underlying the heat-induced poor semen quality, such as oxidative stress, autophagy and apoptosis. The present study determined the relations between acrosome biogenesis and the oxidative stress and autophagy in heat-exposed Bama miniature pigs fed with dietary Zn diet.

Oxidative stress has been shown to be widely involved in apoptosis and germ cell death, and in the pathophysiology of male infertility. Heat-induced testicular oxidative stress occurs mainly via the lipid peroxidation of the cellular membrane and the mitochondria-derived reactive oxygen species (ROS) (Circu and Aw, 2010). MDA, a product of lipid peroxidation, has been always used as an popular marker of testicular oxidative stress (Shiraishi, 2012). The testicular MDA levels were significantly increased under oxidative conditions (Abarikwu et al., 2010; Jelodar et al., 2013). ROS play an important role in the induction of apoptosis under physiologic and pathologic conditions (Simon et al., 2000). Testicular oxidative stress is impacted by the balance between ROS production and this scavenging system. When cellular ROS production overwhelms the scavenging system, antioxidant enzymes are damaged and cellular lipid peroxidation increase (Amara et al., 2008). Previous studies reported that oxidative stress occurred, accompanying with elevated MDA and decreased GPX activities (Wang et al., 2011; Das et al., 2012). In the present study, There is no significant difference in MDA concentration, H_2O_2 level, and the activities of GSH-PX and γ-GCS, implying that testicular oxidative stress did not occur and the testicular cells maintained the balance between the scavenging system and ROS production.

Germ cells contain a battery of ROS scavengers, including antioxidative enzymes such as SOD, glutathione peroxidase/reductase system and catalase (de Lamirande et al., 1997). The SODs is more powerful oxidants and have the capability to initiate free radical chain oxidations (Celino et al., 2011). High levels of Cu-Zn SOD and Zn

could increase spermatogonia resistance to ROS and avoid oxidative stress (Ishii et al., 2005). Over-expression of Cu-Zn SOD could decrease ROS levels, inhibit lipid peroxidation in the liver, brain, and testes, and extend life span (Koksal et al., 2000; Orr and Sohal, 2003; Celino et al., 2011). Furthermore, Zn ion could induce the expression of Cu-Zn SOD and increase the activity of Cu-Zn SOD (Mei et al., 2013). In this study, high environmental temperature significantly increased the Cu-Zn SOD levels, indicating that high environmental temperature could activate the testicular antioxidative scavenger Cu-Zn SOD.

Nrf2-antioxidative response play an important role in scavenging ROS and ameliorate oxidative stress via up-regulating the Nrf2-regulated antioxidants HO-1, NQO1 and γ-GCS translated by *GCLC* gene (Wang et al., 2013). Under normal conditions, newly synthesized Nrf2 protein is captured by Kelch-like ECH-associated protein 1 (Keap1) and constitutively degraded via the cytoplasmic ubiquitin-proteasome pathway (Kobayashi et al., 2004). The presence of excess ROS, Zn, nitric oxide and alkenals could modify cystein residues of Keap1, thereby inactivating it (McMahon et al., 2010; Taguchi et al., 2012). Therefore, cytoplasmic Nrf2 is stabilized and translocate into the nucleus to promote the expression of Nrf2-regulated antioxidative genes (Kobayashi et al., 2004). In the present study, high environmental temperature significantly increased the mRNA expression of *NQO1* and *GCLC,* indicating high environmental temperature activate the Nrf2-antioxidant response. However, the decreased the mRNA expression of *Nrf2*, *HO-1* and *GCLC*, in pigs of Zn-supplemented groups suggested dietary Zn inhibited the Nrf2-antioxidant response.

The p62 protein, also called sequestosome 1 (SQSTM1), binds directly to LC3 and is degraded by autophagy (Jain et al., 2015). Since the p62 accumulates when autophagy is inhibited, and decreased levels can be observed when autophagy is induced, p62 is always used as a marker to determine autophagy occurrence (Bjøkøy et al., 2009). The Nrf2-Keap1 pathway provides the cellular defense against oxidative stress by promoting the expression of the downstream target antioxidant genes. Recent research reported sestrins could protect cells from oxidative stress via interacting with the Nrf2 suppressor Keap1, the autophagy substrate p62, and activate Nrf2-antioxidant response in a manner reliant on p62-dependent autophagic degradation of Keap1 (Bae et al., 2013). p62 interacts with the Nrf2-binding site on Keap1, and overproduction of p62 or a deficiency in autophagy increase cellular p62 levels and competes with interaction between Nrf2 and Keap1, resulting in stabilization of Nrf2 and transcriptional activation of Nrf2 target genes, which implied p62 accumulation could cause hyperactivation of Nrf2 (Komatsu et al., 2010). In the present study showed both high environmental temperature and Zn supplementation increased testicular p62 protein levels, indicating both heat exposure and Zn inhibited testicular autophagy, and the increased p62 protein levels might activate the Nrf2-antioxidant response in heat-exposed Bama miniature pig, but not in pigs of Zn-supplemented groups. The decreased the mRNA expression of *Nrf2*, *HO-1* and *GCLC*, was not consistent with the increased p62 protein levels in pigs testes of Zn-supplemented groups, suggesting an opposite example against the molecular mechanism underlying p62 accumulation activating Nrf2-antioxidant system. The possible reason was that Zn might inhibit the Nrf2-related antioxidative system via disturbing the binding between p62 and Keap1, or by damaging the binding between Nrf2 and antioxidant response elements in nucleus.

Spermatids were most readily affected if the degree of testicular heating was increased in pigs (Li et al., 2015). Injection of autophagy or lysosome inhibitors into testis made the proacrosomal vesicles failed to fuse into a single acrosomal vesicle during the Golgi phase, which finally resulted in abnormal acrosome and irregular round-headed spermatozoa (Wang et al., 2014). Acrosome biogenesis begins with the initial phase of spermatid development (Kang-Decker et al., 2001). The present study showed dietary Zn induced many autolysosome accumulated and the acrosomal vesicles were failure to recruit to the nucleus-associated acrosome in spermatids in the groups fed with supplemental Zn diet, indicating Zn inhibited autophagy and impacted acrosome biogenesis in the Golgi phase in spermatids in Bama miniature pig under high ambient temperature.

In conclusion, high environmental temperature could activate the testicular antioxidative response. Zinc supplementation weakened the testicular Nrf2-antioxidant response, inhibited autophagy, and caused the malformed acrosome via damaging the acrosome biogenesis in Bama miniature pig under high ambient temperature.

References

Abarikwu et al. Changes in sperm characteristics and induction of oxidativestress in the testis and epididymis of experimental rats by a her-bicide, atrazine. Arch Environ Contam Toxicol 2010;58:874-882.

Amara et al. Preventive effect of zinc against cadmium-induced oxida-tive stress in the rat testis. J Reprod Dev 2008;54:129-134.

Aydilek et al. Effect of dietary restriction on sperm characteristic and oxidative status on testicular tissue in young rats exposed to long-term heat stress. Andrologia 2015; 47(9):1055-1061.

Bae et al. Sestrins Activate Nrf2 by p62-Dependent Autophagic Degradation of Keap1 and Prevent Oxidative Liver Damage. Nat Cell Biol 2010; 12, 213-223.

Berruti et al. Acrosome biogenesis: revisiting old questions to yield new insights. Spermatogenesis 2011; 1:95-98.

Bjøkøy et al. Monitoring autophagic degradation of p62/SQSTM1. Methods Enzymol 2009; 452:181-197.

Cabezón et al. Effect of natural betaine on estimates of semen quality in mature AI boars during summer heat stress. Anim Reprod Sci 2016; 170:25-37

Cao et al. Dietary zinc may attenuate heat-induced testicular oxidative stress in mice via up-regulation of Cu-Zn SOD. Genet Mol Res 2015; 14 (4): 16616-16626.

Celino et al. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. PloS One 2011; 6: e16938.

Circu and Aw. Reactive oxygen species, cellular redox systems,and apoptosis. Free Radic Biol Med 2010;48:749-762.

Das et al. Taurine protects rat testes againstdoxorubicin-induced oxidative stress as well as p53, Fas andcaspase 12-mediated apoptosis. Amino Acids 2012; 42:1839-1855.

de Lamirande et al. Reactive oxygen species and sperm physiology. Rev Reprod 1997; 2: 48-54.

Ishii et al. Accelerated impairment of spermatogenic cells in SOD1-knockout mice under heat stress. Free Radic. Res 2005; 39: 697-705.

Jain et al. p62/sequestosome-1, autophagy-related gene 8, and autophagy in Drosophila are regulated by nuclear factor erythroid 2-related factor 2 (NRF2), independent of Transcription Factor TFEB. J Biol Chem 2015; 290, 14945-14962.

Jelodar et al. The prophylactic effect of vitaminC on induced oxidative stress in rat testis following exposureto 900 MHz radio frequency wave generated by a BTS antennamodel. Electromagn Biol Med 2013;32:409-416.

Jin et al. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc Natl Acad Sci USA 2011; 108:4892-4896.

Kang-Decker et al. Lack of acrosome formation in Hrb-deficient mice. Science 2001; 294:1531-1533.

Kobayashi et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol 2004; 24:7130-7139.

Koksal et al. role of reactive oxygen species in testicular dysfunction associated with varicocele. BJU Int 2000; 86: 549-552.

Komatsu et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol 2010; 12(3):213-223.

Li et al. Declining semen quality and steadying seminal plasma ions in heat-stressed boar model. Reprod Med Biol, 2015; 14, 171-177.

Li et al. Effects of zinc sulfate pretreatment on heat tolerance of Bama miniature pig under high ambient temperature . J Anim Sci 2015; 93:3421-3430.

Li et al. Scrotal heat induced the Nrf2-driven antioxidant response duringoxidative stress and apoptosis in the mouse testis. Acta Histochemica 2014; 116: 883-890.

McMahon et al. Keap1 per-ceives stress via three sensors for the endogenous signalingmolecules nitric oxide, zinc, and alkenals. Proc Natl Acad Sci USA 2010; 107:18838-18843.

Mei et al. Zinc (II)-curcumin accelerates the healing of acetic acid-induced chronic gastric ulcers in rats by decreasing oxidative stress and downregulation of matrix metalloproteinase-9. Food Chem Toxicol 2013; 60: 448-454.

Orr and Sohal. Does overexpression of Cu,Zn-SOD extend life span in Drosophila melanogaster? Exp Gerontol 2003; 38: 227-230.

Shiraishi. Heat and oxidative stress in the germ line. In: AgarwalA, John Aitken R, Alvarez JG, editors. Studies on men's health andfertility. New Jersey: Humana Press; 2012. p. 149-178.

Simon et al. Role of reactive oxygenspecies (ROS) in apoptosis induction. Apoptosis 2000;5:415-418.

Taguchi et al. Keap1 degradation by autophagy for the mainte-nance of redox homeostasis. Proc Natl Acad Sci USA 2012; 109:13561-13566.

Wang et al. Atg7 is required for acrosome biogenesis during spermatogenesis in mice. Cell Res 2014; 24:852-869.

Wang et al. Cadmium-induced oxidative stress and apoptotic changes inthe testis of freshwater crab, Sinopotamon henanense. PLoS ONE 2011; 6:e27853.

Wang et al. Seaweed extracts and unsaturated fattyacid constituents from the green alga Ulva lactuca as activatorsof the cytoprotective Nrf2-ARE pathway. Free Radic Biol Med 2013; 3:141-153.

Zhao et al. Exacerbation of diabetes-induced testicular apoptosis by zinc deficiency is most likely associated with oxidative stress, p38 MAPK activation, and p53 activation

PO-03-70 Weeding Effect of Free-Ranging Pigs in an Abandoned Paddy Field Dominated by Solidago altissima L.

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Introduction

Solidago altissima L. is a very common wildflower that reaches 1–2 m in height. It has excellent reproductive capabilities with respect to pollen dispersion, seed dispersion, and the development of the root system (Fukuda, 1982), and can form dense colonies on roadsides, in open fields, and on abandoned agricultural land where there is full to partial sun and humid to dry conditions. Recently, the number of abandoned fields has increased in Japan. In 2010, 396,000 ha of agricultural land were found to be abandoned, representing 10.1% of the country's entire arable area (MAFF, 2015). Because *S.altissima* is one of the serious rhizomatous perennial weeds that overgrow abandoned agricultural lands, methods to control the plant by ecological vegetation management, e.g. by grazing animals, are urgently needed.

Pigs are classified as omnivores that eat roots, grasses, and some animals such as earthworms (Fraser, 1974). The snout of the pig is a highly developed sense organ, and outdoor pigs often dig up earth with their mobile snouts—a behaviour called rooting. Rooting is certainly the salient feature of the ingestive behaviour in pigs. Imura et al. (2005) reported that the vegetation coverage of a deteriorated pasture, including *Pennisetum alopecuroides* and *Pteridium aquilinum* var. *latiusculum*, was almost eliminated by free-ranging pigs over the course of 74 days. However, there is little information on the efficacy of free-ranging pigs for weed control in abandoned agricultural land. Moreover, the effects of free-ranging pigs on *S.altissima* populations have not been elucidated in detail.

We therefore investigated the effectiveness of pigs for sustainable management of vegetation on unused agricultural land. In particular, we aimed to determine whether an abandoned paddy field dominated by the weed *S.altissima* could be managed using free-ranging sows.

Materials and Methods

Our experiment was conducted in an abandoned paddy field (15.5a) at Kagoshima-shi, Japan, from July 7 to October 7 (93days) during 2015. Two treatments were used in the paddy field: 1) with two free-ranging sows (average body weight, 212kg; age, 5–6 years; experimental plot, 14a), and 2) without pigs (control plot, 1.5a). The experimental plot was surrounded by electric and net fences and the pigs were provided with 1.0–1.6 kg of grain-based formula feed every morning (TDN: 73%, CP: 15%), an amount that is 50–80% of the standard nutritional requirement.

Measurements of herbage, including *S.altissima*, were taken on July 7, August 5, September 4, and October 6, 2015. Density, coverage, and height of *S.altissima* were measured at fixed points (1 × 1 m) in the control (n = 6) and experimental (n = 16) plots. Herbage mass was randomly sampled using a quadrat (1 × 1 m) in the control (n = 4) and experimental (n = 8) plots. The herbage was cut at 3–5 cm in height, and oven-dried at 60 °C for 48 h to determine dry matter.

Maintenance behaviour of the free-ranging pigs was observed on July 15, August 5, September 8, and September 29, 2015, and it was classified into grazing, feeding, rooting, resting, walking, body care, drinking, eliminative, and other. Scan sampling was made at 1-min intervals, resulting in 720 observations each day over a 12 h period (6:00–18:00). Ambient temperature and relative humidity were measured using an automatic recorder during the experimental period, and then the effective temperature (ET = (0.65 × dry-bulb temperature) + (0.35 × wet-bulb temperature)) of the pig was calculated (Notsuke and Yamamoto, 1991; Ueda, 2000).

The growth of *S.altissima* and the herbage mass were compared between control and experimental plots by *t*-test.

Results and Discussion

The maintenance behaviour time budget of the free-ranging sows is shown in Table 1. Resting behaviour ranged from 74.4 to 81.2% on the three measurement dates of July 15, August 5, and September 8. On the same three dates, grazing behaviour and rooting behaviour ranged from 1.7 to 14.7% and 5.2 to 12.9%, respectively. All

animals have a zone of comfort called the thermo-neutral zone (TNZ). This is the temperature range where they are most comfortable and productive. Generally, the feed intake of pigs gradually decreases as ambient temperature increases (Hata et al., 1983; Le Dividich, et al., 1987), whereas thermoregulatory lying behaviour (huddling and wallowing) increases (Huynh et al., 2005). Pigs feel "most comfortable" between 18 and 20 °C (Tanaka, 2001). In this study, the effective temperatures of the sows on July 15, August 5, and September 8 were 29.9, 30.6, and 26.4 °C, respectively. This suggests that an ET over 25 °C results in frequent resting behaviour. However, a flattening of the shoots of *S.altissima* was observed whenever the sows engaged in resting behaviour, and this contributed to the pigs' weeding effect.

In contrast, the ET of the sows on September 29 was 20.8 °C, and on that date, the resting behaviour of the sows was lower and rooting behaviour was higher (Table 1). Feeding, walking, body care, and drinking behaviour of the sows changed slightly throughout the experimental period, with values ranging from 3.0 to 6.1, 1.0 to 2.4, 0.2 to 0.7, and 0.1 to 0.8%, respectively. Rooting behaviour is certainly the salient feature of the ingestive behaviour of free-ranging pigs outdoors. Takayama et al. (2012) reported that free-ranging pigs spent approximately 50% of the day rooting on abandoned forest land, and their behaviour was helpful for weeding. The stem of *S.altissima* stands straight and the rhizome creeps underground. In this study, we observed that the grazing and resting behaviour of the free-ranging sows caused defoliation and flattening of *S.altissima* shoots. In addition, the rooting behaviour of the sows may have resulted in removal of the plants' roots.

The density of *S.altissima* in the abandoned paddy field is shown in Fig. 1. Density was significantly lower in the experimental plot than in the control plot by Day 30 ($P<0.05$). At the end of the experiment (Day 85), density continued to be significantly different between the two treatments ($P<0.05$), with an average of 26 plants/m^2 in the control plot and 2 plants/m^2 in the experimental plot. Similar results were observed for coverage and height ($P<0.05$) (Fig. 2 and 3). Herbage mass in the abandoned paddy field is shown in Fig. 4. Herbage mass was significantly lower in the experimental plot than in the control plot by Day 30 ($P<0.05$). At the end of the experiment (Day 85), herbage mass also exhibited large and significant differences between the two treatments ($P<0.05$), with an average of 649 kg DM/10 a and 20 kg DM/10 a in the control and experimental plots, respectively.

Several previous studies of weeding control by animals in abandoned paddy fields suggested that grazing was one of the most effective measures. Most studies focused on ruminants, e.g. cattle, goats, and sheep. Chikara et al. (2014) reported that grazing goats were effective for weed control in abandoned paddy fields, and their findings are in agreement with those obtained in our study.

The growing period of *S.altissima* is from April to November. The species has a strong capacity for vegetative reproduction and grows densely in various habitats (Nakashima et al., 2000). Cutting the stems by hand does not exterminate the plant, and they can reproduce through the root system the next year. It is, therefore, expected that grazing of only the stems and leaves by ruminants causes regrowth the next year. In contrast, free-ranging sows were effective weeders of *S.altissima*. The grazing and resting behaviour of the sows flattened the shoots of *S.altissima*, and rooting behaviour removed the root systems, thereby decreasing plant density, height, and coverage in the experimental plot.

In summary, free-ranging pigs did have a weeding effect in an abandoned paddy field dominated by *S.altissima*.

Table 1. Time budget of maintenance behaviour of free-ranging sows in an abandoned paddy field.

Behaviour	Days after grazing (Date)			
	9 (July 15, 2015)	30 (Aug. 5)	64 (Sep. 8)	85 (Sep. 29)
	- % -			
Grazing	6.8	1.7	14.7	1.6
Feeding	3.0	6.1	2.4	4.1
Rooting	5.2	12.9	6.4	64.6
Resting	81.2	74.4	76.0	28.0
Walking	2.3	2.4	1.0	1.1
Body care	0.7	0.3	0.2	0.4
Drinking	0.8	0.6	0.1	0.3
Eliminative	0.1	0.1	0.1	0

n=2

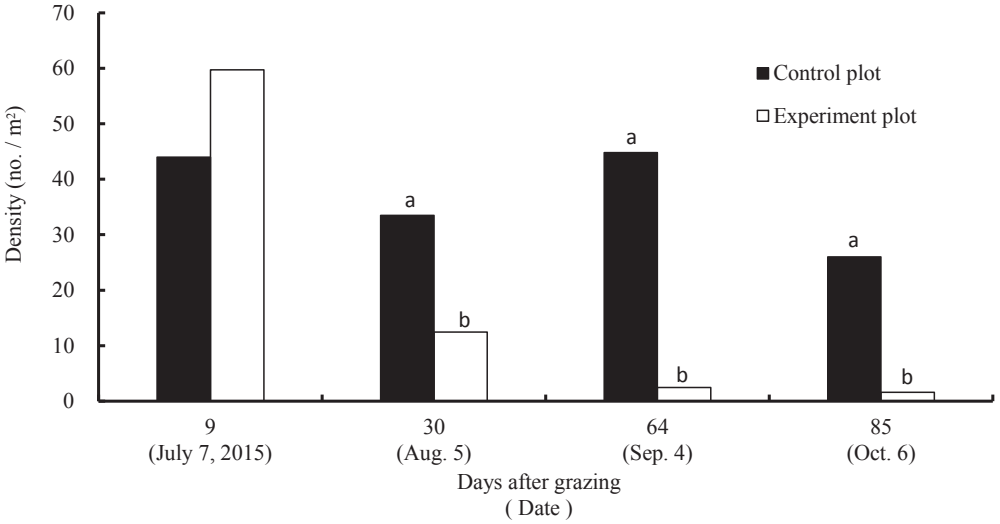

Fig. 1. Effect of grazing sows on density of *Solidago altissima* L. in abandoned paddy fields

a, b: Means with different superscripts in same date differ significantly ($P < 0.05$).

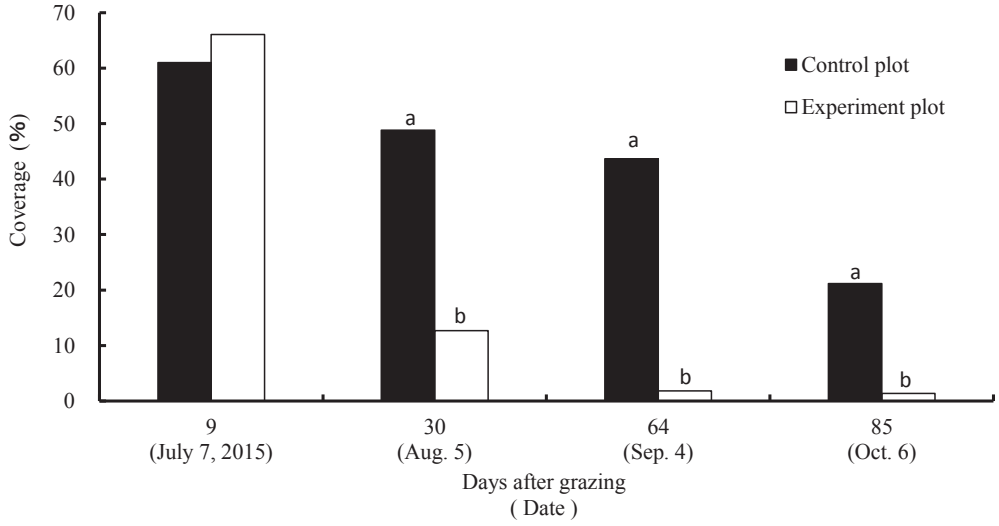

Fig. 2. Effect of free-ranging sows on coverage of *Solidago altissima* L. in abandoned paddy fields.

a, b: Means with different superscripts in same date differ significantly ($P < 0.05$).

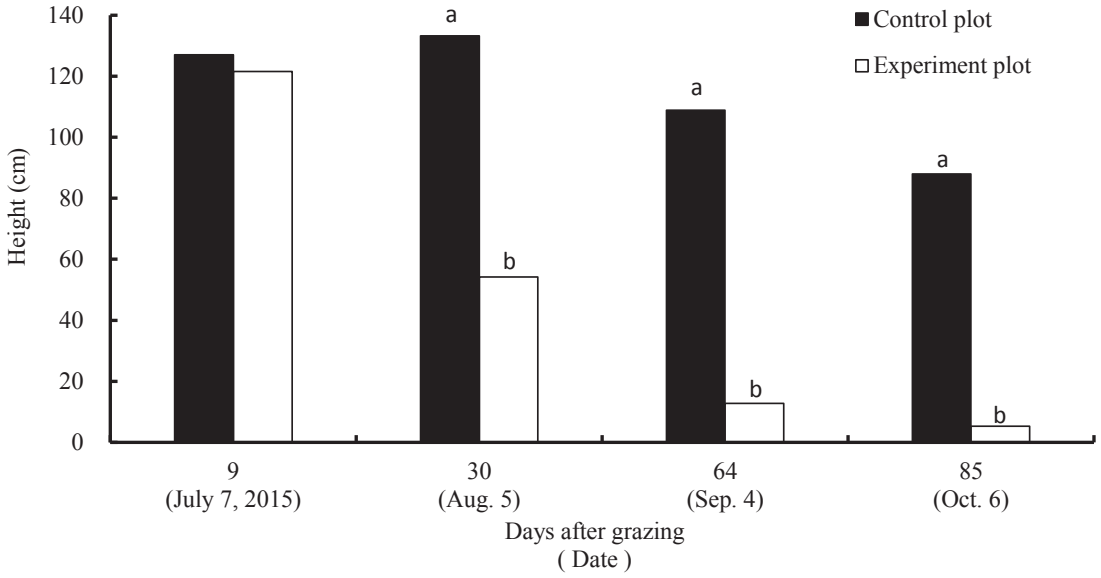

Fig. 3. Effect of free-ranging sows on height of *Solidago altissim*a L. in abandoned paddy fields

a, b: Means with different superscripts in same date differ significantly (P＜0.05).

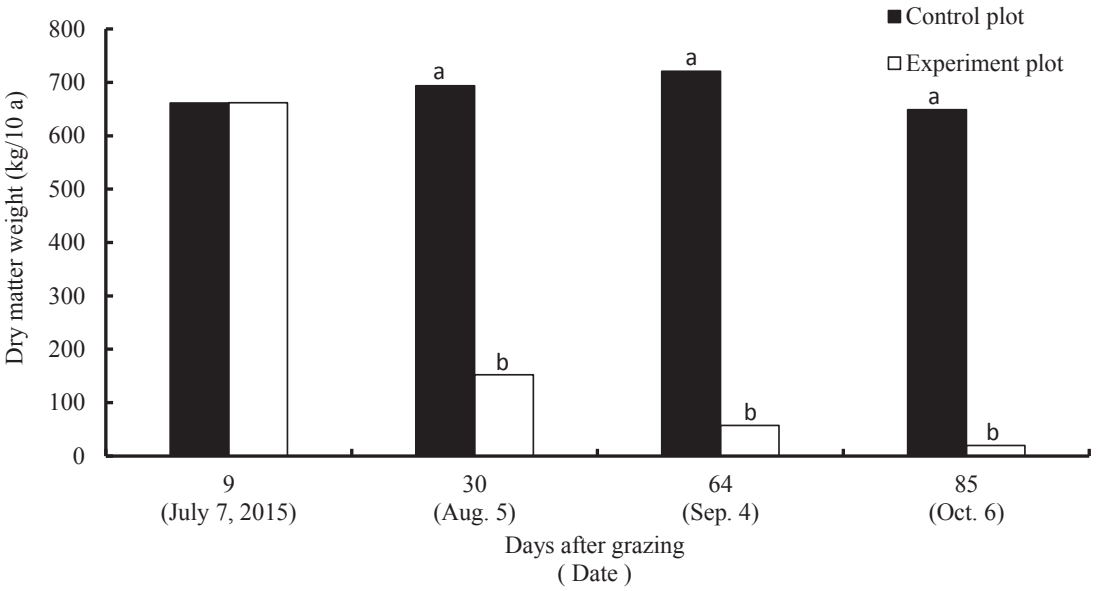

Fig. 4. Effect of free-ranging sows on herbage mass in abandoned paddy fields.

a, b: Means with different superscripts in same date differ significantly (P＜0.05).

References

Chikara, Y., D. Uchitomi, Y. Mizoguchi, D. Fukunaga, I. Oshima, K. Takayama and Y. Nakanishi. 2014. Plant preference and weeding effect by goats grazes in an abandoned paddy field dominated by *Solidago altissima* L.. Journal of the Japanese Society of Agricultural Technology Management. 21(2): 41-47. (in Japanese with English abstract)

Fraser, A. F. 1974. Farm Animal Behaviour. 179 - 187. Baillere Tindall, London.

Fukuda, I. 1982. Distributions and population structures of North American plants, *Solidago altissima* L. and *S. gigantea* AIT., introduced into the Japanese islands. Science reports of Tokyo Woman's Christian University. 32(3): 675-690. (in Japanese with English abstract)

Hata, H., S. Kajino, K. Tokoro and Y. Asahida. 1983. The effects of housing temperature and its seasonal change on feed intake, rate of gain and feed conversion rate in growing finishing pigs fed ad-libitum under practical conditions. Japanese Journal of Livestock Management. 19(2): 35-42. (in Japanese with English abstract)

Huynh T.T.T., A.J.A. Aarninka, W.J.J. Gerritsc, M.J.H. Heetkampd, T.T. Canhe, H.A.M. Spoolderf, B. Kempd and M.W.A. Verstegenc. Thermal behaviour of growing pigs in response to high temperature and humidity. Applied Animal Behaviour Science. 91: 1-16.

Imura, Y., Y. Ando, A. Nishitani, T. Uchimura and Y. Nakanishi. 2005. Effects of level of concentrate on performance of free-ranging fattening pigs and vegetation of pasture. Japanese Journal of Grassland Science. 51: 34-39. (in Japanese with English abstract)

Le Dividich, J., J. Noblet, J and T. Bikawa. 1987. Effect of environmental temperature and dietary energy concentration on the performance and carcass characteristics of growing-finishing pigs fed to equal rate of gain. Livestock Production Science. 17: 235-246.

Ministry of Agriculture, Forestry and Fisheries. 2015. Chapter 2: Effort for creating strong, aggressive agriculture. In: *FY2014 Annual Report on Food, Agriculture and Rural Areas in Japan*. p.92-116. (in Japanese)

Nakashima, K., K. Nehira and N. Nakagoshi. 2000. Effects of mowing to *Solidago altissima* populations. Memoirs of the Faculty of Integrated Arts and Sciences, Hiroshima University. IV, Science reports: studies of fundamental and environmental sciences. 26: 81-94. (in Japanese with English abstract)

Notsuke I., S. Yamamoto. Animal management. pp.262. Buneido, Tokyo. (Translated from Japanese by the present authors)

Takayama K., K. Tsukano, Y. Mizoguchi, Y. Chikara, I. Oshima and Y. Nakanishi. 2012. Grazing pigs for weed control in abandoned forest land. Japanese Journal of Organic Agriculture Science. 4:89-96. (in Japanese with English abstract)

Tanaka T. 2001. Zoology pig (Animal Science). pp.180. University of Tokyo Press, Tokyo. (in Japanese)

Ueda M. 2000. Humidity and Evaporation. pp.164. Corona publishing, Tokyo. (in Japanese)

PO-03-73

Molecular Detection Of Anaplasma maginale In Cows In The Eastern Border Area

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Introduction

Anaplasma marginale is a rickettsial organism causing bovine anaplasmosis with significant economic losses in tropical and subtropical regions worldwide. Anaplasmosis in cattle have both *A. centrale* and *A. marginale. A. central* infection causes mild, inapparent disease in cattle. On the other hand, *A. marginale* causes severe debility, anemia, jaundice and abortion in adult cattle (Radostits *et al.*,1994). It invades the erythrocyte and leads to extravascular hemolysis. Ticks are biological vectors of *A. marginale* but the pathogen is often transmitted mechanically to susceptible cattle by blood-contaminated mouthparts of biting flies or fomites. These obligate intracellular organism replicates in membrane-bound parasitophorous vacuoles in bovine erythrocytes or tick cells. Both cattle and ticks become persistently infected with *A. marginale* and thus serve as reservoirs of infection (Kocan et al., 2004).

Many geographic strains of *A. marginale* have been identified, which differ in biology, genetic characteristics and transmissibility by ticks. The genetic diversity of *A. marginale* strains have been characterized using major surface protein (MSP) genes involved in interactions with vertebrate host cells (de la Fuente et al.,2005). These genes may have evolved more rapidly than other genes because of selective pressures exerted by the host immune system (de la Fuente et al.,2006).

Sa Kaeo province of Thailand located in the eastern border of Thailand, facing Cambodia at Aranyaprathet approximately 165 kilometres. Generally, the area varies from plains to highlands. Highlands and mountains are in the north where Pang Sida National Park is located, while the south is covered with wavy plains and hills. Also, there are evergreen forests along the Chanthaburi mountain range. In the central, there are plains and hills, with Amphoe Watthana Nakhon as the highest area when compared to the other Amphoe Mueang in the west, and Amphoe Aranyaprathet in the east.

At the Thai border in Amphoe Aranyaprathet, Sa Kaeo serves as a gateway to Cambodia, connecting international commercial transport and tourism at a hectic border crossing, as a result of the massive influx of goods and people passing back and forth. Livestock production in the region includes buffaloes, swine, beef cattle, dairy cows and poultry. The objective of this study was to assess the prevalence of anaplasmosis of beef cattle in Sa Kaoe province.

MATERIALS AND METHODS

A total of 190 blood samples of cows collected from 4 districts in Sa Kaeo province (Figure 1) including Aranyaprathet, Khlong Hat, Khok Sung and Ta Phraya were investigated. Blood was collected into separate sterile tubes with anticoagulant (EDTA) and maintained at 4-8 °C until processed. The *A. marginale* msp4 gene was amplified by PCR as reported previously (de la Fuente et al., 2002). Briefly DNA was extracted from 0.5 ml of blood following the method of Sambrook et al. (1989). A fragment of msp4 gene from each sample was amplified by PCR using msp4 primers; MSP4 F 5' GGG AGC TCC TAT GAA TTA CAG AGA GAA TTG TTT AC 3' and MSP4 R 5' CCG GAT CCT TAG CTG AAC AGG AAT CTT GC 3' (de la Fuente et al., 2002). PCR amplification was performed in a reaction of 25 μl containing 1X Taq buffer $(NH_4)_2SO_4$, 0.4 μM each primer, 200 μM dNTP and 0.25 U of Taq DNA polymerase (Fermentas). The PCR was carried out for 35 cycles in MyCyclerTM thermal cycler (Bio-rad). The following thermal profile was used for the PCR: denaturation at 94°C for 45 sec, primer annealing at 58.2° C for 1 min and extension at 72°C for 1 min. The final cycle included an extension for 10 min at 72°C to ensure full extension of the products. The amplified PCR products were then separated by electrophoresis in 1.0% (w/v) agarose gel.

RESULTS AND DISCUSSIONS

The *A. maginale* infection prevalence was determined as the percentage of animals positive for the pathogen DNA

detected by PCR.

The results demonstrated that the PCR positive of *A. marginale* was 867 bp in length (Figure 2). The observed prevalence of *A. marginale* from 4 districts in Sa Kaeo provinces was 45.78 % (87/190). Aranyaprathet districts had the highest prevalence at 74.07 (40/54) while the estimated incidence for the adjacent Khlong Hat, Khok Sung and Ta Phraya districts was 47.80% (22/46), 34.04% (16/47) and 20.93% (9/43) respectively (Table 1 and Figure 3).

The differences in *A. maginale* infection prevalence between districts in Sa Kaeo provinces may result from differences in husbandry practices, wildlife reservoir hosts, and/or habitat suitability for ticks. For treatment, the blood parasite disease should be diagnosed at early stages in the cattle.

As soon as cattle farmers see any problem with their cattle, they apply to veterinarian or the animal hospital for treatment and reduce morbidity of cattle in farms from blood parasite diseases. Probable reasons behind highest disease prevalence in poorly hygienic farms, un-cemented floor pattern and closed housing system include: (a) in the absence of sunlight with unhygienic measures, heaps of dung cakes and stacks of bricks in the closed houses will provide breeding places for the vector ticks (Iqbal, 2013) and (b) higher humidity in closed housing system which provides favorable environment for the ovipositioning of female ticks in the cracks and crevices of the walls, roof and floor of house (Muhammad, 2008).

CONCLUSIONS

The results reported herein have important implications for the control of *Anaplasma* spp. in Sa Kaeo. The information about pathogen prevalence helps the establishment of surveillance and control programs for these pathogens. These results may be used to construct models to predict the risks for tick-borne pathogens in tropical ecosystems. Therefore, further studies are required to investigate the genetic diversity of *A. marginale* strains.

ACKNOWLEDGEMENTS

We thank The Graduate Study and Research in Agricultural Biotechnology Project, Faculty of Agricultural Technology. King Mongkut's Institute of Technology Ladkrabang (KMITL) for materialand equipment support.

Figure 1 Map of Thailand highlighting Sa Kaeo Province (http://www.german-thai-link.de)

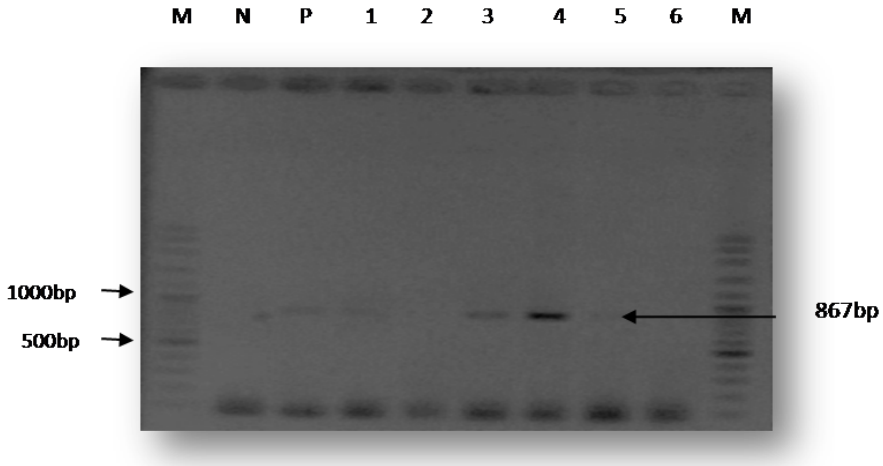

Figure 2 PCR amplification of MSP 4 gene in cattle blood sample (Lane 1-6) using the MSP 4 primers. Lane M is 100-bp DNA ladder, lane N is negative control and lane P is positive control.

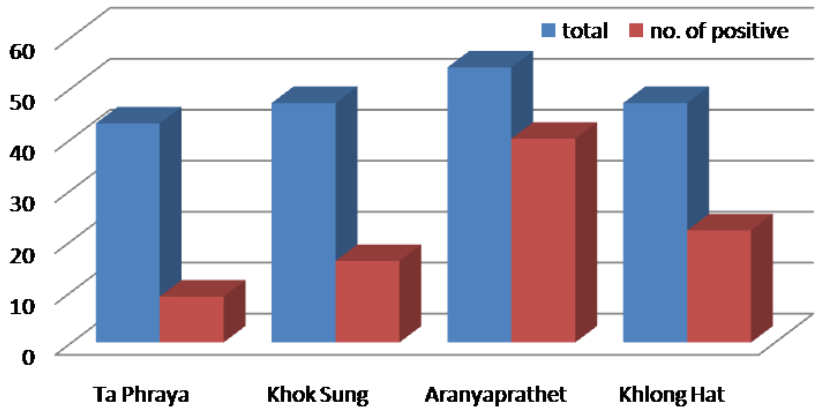

Figure 3 The observed prevalence of *A. marginale* in cows.

Table1 The observed prevalence of *A. marginale* in cows.

Originating area	Total number of cows	No. of positive	Prevalence (%)
Ta Phraya	43	9	20.93
Khok Sung	47	16	34.04
Aranyaprathet	54	40	74.07
Khlong Hat	47	22	46.80

REFERENCES

Bacanelli , G.M., C.A.N. Ramos and F.R. Araujo. 2014. Molecular diagnosis of *Anaplasma marginale* in cattle : quantitative evaluation of a real-time PCR (Polymerase Chain Reaction) based on *msp5* gene. Pesquisa Veterinária Brasileira. 34(1): 29-33.

de la Fuente, J., R.F Massung S.J Wong, F.K. Chu, H. Lutz, M. Meli, et al. 2005.Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. J Clin Microbiol. 43:1309-1317.

de la Fuente, J., P. Rubial, M.S. Mtshali, V. Naranjo,L. Shuqing A.J. Mangold , et al. 2006. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequence. Vet Microbiol. 119:382-390.

Iqbal,A., M.S. Sajid, M.N. Khan and M.K. Khan. 2013. Frequency distribution of hard ticks (Acari:Ixodidae) infesting bubaline population of districtToba Tek Singh, Punjab, Pakistan. Parasitol Research. 112: 535-541.

Kocan, K.M., J. de la Fuente, E.F. Blouin and J.C. Garcia-Garcia. 2004. *Anaplasma marginale* (Rickettsiales: Anaplsmataceae): recent advances in defining host-pathogen adaptations of a tick borne rickettsia. Parasitol. 129:285-300.

Muhammad, G., A. Naureen, S. Firyal and M. Saqib. 2008. Tick control strategies in dairy production medicine. Pakistan Vet. J. 28: 43-50.

Noaman, V., P. Shayan and N. Amininia. 2009. Molecular Diagnostic of *Anaplasma marginale.* Ticks and Tick-borne Disease. 5(2): 100–104.

Radostits, O.M., D.C. Blood, and C.C. Gay. 1994. Veterinary Medicine. A textbook of the Diseases of Cattle, Sheep, Pigs, Goat and Horses. 8th Bailliere Tindall, London. pp 1146-1150, 1171-1222.

Radwan, M.E.I., Ali, A.E.F. and Hamied, O.A.E. 2013. Epidemiological studies, molecular Reaction based on *msp5* gene. Pesq. Vet. Bras. 34(1) : 29-33.

Sambrook J. and D. Russell, *Molecular Cloning a Laboratory Manual*. 2001.3rd. U.S.A: Cold Spring Harbor Laboratory Press.

Silva, J. B., Vinhote W. M.S., Oliveira C. M.C., André. M. R., Rosangela Z. M., HENRIQUE. D. F.A. and JOSÉ. D. B. 2014. Molecular and serological prevalence of *Anaplasma marginale* in water buffaloes in northern Brazil. Kaliobia Governorate. Med Sci. 2(2) : 41-46.

Smisek, D.L. and D.A. Hoagland. 1989. Agarose gel electrophoresis high molecular weight, synthetic polyelectrolytes. Macromolecules. 22(5):2270-2277.

Soulsby, E.J.L., 1982. Helminths, Arthropods and Protozoa of Domesticated Animals, Ist Ed. Bailliere Tindall, London, UK.

Thrusfield, M., 2007. Veterinary Epidemiology. 3 Ed, Blackwell Science, London, pp: 231-232.

PO-03-76

Prevalence of Trypanosoma evansi Infection among Cattle and Buffalo from Sa Kaeo Province, Eastern Border Area of Thailand-Cambodia

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Introduction

Trypanosoma evansi, a protozoon blood parasite in livestock animal, causes a trypanosomosis or surra disease (Webster and MacDonald, 1995) which has a wide distribution in Asia. The main host species varies with the geographical region. Horses, cattle, and buffalo are most often affected in South-East Asia. The surra symptoms are high fever, loss of appetite, paralysis and some animal have neurological symptoms, immunodeficiency, and death may occure in 2 weeks to 4 months. In addition, T. *evansi* is also the leading cause of animal birth abortion in late stage of pregnancy (Chobjit et al., 2006). These pathogens are spread by blood-sucking insects such as flies, tabanid, which are vectors of disease. Sa Kaeo province that has an area of the eastern border with Cambodia consists of Ta Phraya, Aranyaprathet, Khok Sung, and Khlong Hat district. Most of the population is farmer, crop production and livestock that the important livestock are dairy cattle, beef cattle and buffalo. The animal production system is pasture or forest grazing that it is poor management and care of animal. Therefore, it is necessary to check the epidemiology of *T. evansi* of cattle and buffalo. The objective of this research was to investigate prevalence of *Trypanosoma evansi* infection among cattle and buffalo from Sa Kaeo province, eastern border area of Thailand-Cambodia in order to local people benefits about the academic data of diseases transmitted from animals to animals and humans (zoonosis) which leads to the prevention of disease and the development of the animal production system.

Materials and Methods

Study design and Blood samples

Sa Kaeo province was divided into 9 regions consisting of the following 4 districts adjacent to the Cambodian border: (1) Ta Phraya; (2) Khok Sung; (3) Aranyaprathet; (4) Khlong Hat. A total of 191 cattles and 71 buffaloes were randomly sampled from four out of nine districts of Sa Kaeo province (Figure 1). Blood samples were collected from the jugular vein and kept in the EDTA tubes to block clotting of the blood, for PCR examinations. Whole blood was stored at -20°C until processing.

DNA extraction and PCR amplification

Total genomic DNA was extracted from whole blood by the acid phenol-chloroform method (Chomczynsky and Sacchi, 1987). The resulting DNA was amplified by polymerase chain reaction (PCR) using a specific forward primer TBR(F) 5'- GAATATTAAACAATGCGC AG -3' and a specific reverse primer TBR(R) 5'- CCATTTATTAGCTTTGTTGC -3'. Polymerase chain reaction was performed for 35 cycles at 94 ^{O}C for 1 min, 60 ^{O}C for 30 sec, and 72 ^{O}C for 30 sec and final extension at 72 ^{O}C for 2 min in a Primus 96 plus thermocycler. The PCR products were checked by gel electrophoresis technique.

Statistical analysis

The PCR-positive prevalence was calculated as the percentage of positive results out of the total number of the cattle and buffalo sampled. The percentage was calculated for the individual district of Sa Kaeo province, eastern border area of Thailand-Cambodia. Reporting for each region included the number and percentage of animal species with positive results.

Results and Discussions

Previously, Masiga et al., (1992) and Moser et al., (1989) had checked *Trypanosoma evansi* by molecular technique using TBR primer for amplification of trypanozoon DNA. The PCR product was 164 bps in size. In this study, total DNAs were extracted from whole blood of cattle and buffalo and amplified by Polymerase Chain Reaction (PCR) using the primers TBR1 and TBR2. Gel electrophoresis was used to check PCR product in each district. The PCR products of the satellite gene of T. *evansi* are approximately 164 bps in length (Figure 2 and

3.) that the results are similar to previous reports. From this result, PCR condition can be used to determine the prevalence and genetic diversity of T. *evansi* in cattle and buffalo.

In this report, prevalence of *Trypanosome evansi* was assessed in the blood of cattle and buffalo from Sa Kaeo province, eastern border area of Thailand-Cambodia. Polymerase chain reaction (PCR) method was evaluated for detection of T. *evansi* DNA in cattle and buffalo of each border district in Sa Kaeo province. The results demonstrated that the prevalence of T. *evansi* infection in cattle of Ta Phraya, Khok Sung, Aranyaprathet and Khlong Hat district was 20.93%(9/43), 41.67%(20/48), 46.00%(23/50), 28.00%(14/50), and prevalence of T. *evansi* infection in buffalo was 16.67%(3/18), 31.82%(7/22), 40.91%(18/44), 23.08%(3/13), respectively. The percentage of T. *evansi* infected cattle and buffalo were found in Aranyaprathet and Khok Sung district higher than other districts might be due to the number of livestock animal higher than other districts. The animal production system of border area is pasture or forest grazing. This feeding is difficult to get rid of insects that are disease vectors such as tabanidae, flies, as well as mosquitoes and also easy to spread or transmits trypanosome. Additionally, the animals were transported in and out all the day at the border area which might be result in the prevalence of T. *evansi* infection increased (Table 1).

Conclusions

The PCR product of trypanozoon DNA was approximately 164 bps in length. The prevalence of T. *evansi* infection in cattle and buffalo in Ta Phraya, Khok Sung, Aranyaprathet and Khlong Hat district in Sa Kaeo province was 20.93%(9/43) 16.67%(3/18), 41.67%(20/48) 31.82%(7/22), 46.00%(23/50) 40.91%(18/44) and 28.00%(14/50) 23.08%(3/13), respectively. The overall prevalence of T. *evansi* infection in cattle and buffalo in four border area district was 33.68%(97/288). The high percentage of trypanosome prevalence in Khok Sung and Aranyaprathet district might be due to a higher number of the live stock-producing areas, local livestock markets, and border crossing point than other districts. In addition, forest grazing system might be effect to spread or transmit trypanosome.

Figure 1 The four districts border area of Sa Kaeo province, eastern border area of Thailand-Cambodia.

Figure 2 Analysis of PCR products of satellite gene of T. *evansi* from cattle using 1% agarose gel electrophoresis. A 10 ml of PCR mixture was loaded onto each lane of agarose gel. Lane 1 = DNA marker (100 bp), Lane 2, 5-18 = PCR product of satellite gene from each district area and lane 3 = Negative control.

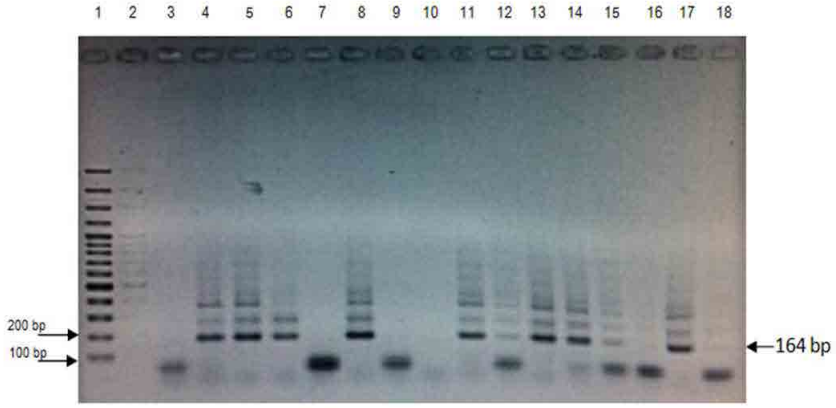

Figure 3 Analysis of PCR products of satellite gene of T. *evansi* from buffalo using 1% agarose gel electrophoresis. A 10 ml of PCR mixture was loaded onto each lane of agarose gel. Lane 1 = DNA marker (100 bp), Lane 2 = Positive control, Lane 3 = Negative control and Lane 4-18 = PCR product of satellite gene from each district area.

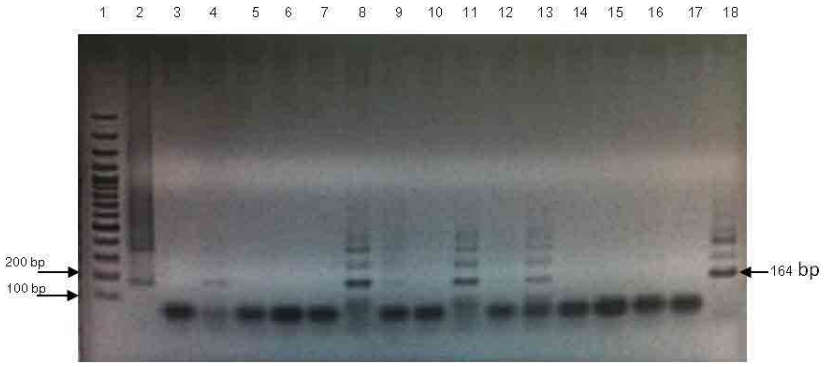

Table 1 Prevalence of *Trypanosoma evansi* in cattle and buffalo blood from each district of Sa Kaeo province

District	Sample (n)	TBR primer (positive)	Prevalence (%)
Ta Phraya			
cattle	43	9	20.93
Buffalo	18	3	16.67
Khok Sung			
cattle	48	20	41.67
Buffalo	22	7	31.82
Aranyaprathet			
cattle	50	23	46.00
Buffalo	44	18	40.91
Khlong Hat			
cattle	50	14	28.00
Buffalo	13	3	23.08
Total	**288**	**97**	**33.68**

References

Chobjit, S., Kheowsri S., Rodtian P., Jongchansitto P. and Neungmek A. 2006. Serra outbreak in cattle – cattle at Ban Mae Mueang Luang, Pongsa sub-district, Pai district, Mae Hong Son Province, northern Thailand. Chiang Mai Veterinary Journal. 4(2):127-136.

Chomczynsky, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol choroform extraction. Anal. Biochem. 162: 156-159.

Masiga D.K., Smyth A.J., Hayes P., Bromidge T.J. and Gibson W.C. 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int. J. Parasitol. 22: 909–918.

Moser D.R., Cook G.A., Ochs D.E., Bailey C.P., McKane M.R. and Donelson J.E. 1989. Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. Parasitology. 99: 57–66.

Webster, J.P. and MacDonald D.W. 1995. Parasites of wild brown rats (Rattus norvegicus) on UK farms. Parasitology (111): 247–255.

PO-04-7

Improvement in the post-thaw qualities of Okinawan native Agu pig spermatozoa treated with skim milk and Trolox prior to cryopreservation

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The technical establishment of boar sperm cryopreservation is indispensable for effective breeding of the scarce Okinawan native Agu pig. The objective of the present study was to elucidate the protective effect of skim milk (SM) added to semen diluents (BTS) and Trolox added to BTS and the freezing extender (BF5) on the maintenance of sperm cryotolerance during cryopreservation.

Semen samples from Agu were immediately diluted with equal volumes of BTS, and transported to the laboratory within 1.5 h at 30°C, as the semen standing incubation. In Experiment 1, the semen diluent was supplemented with SM at a final concentration of 0, 0.25, 0.5, 1 and 2%. In experiment 2, sperm were diluted BTS containing 0.5% SM during the standing incubation, and treated without (control) or with 50 μM Trolox (TL) in only BTS, only BF5, and both solutions (TL: none, TL: BTS/-, TL: -/BF5, and TL: BTS/BF5, respectively) prior to the cryopreservation.

The addition of 0.5% SM during the semen standing incubation had the beneficial effects on post-thaw sperm motility and plasmalemma integrity, compared with those of other groups ($P<0.05$). Likewise, treatment with Trolox in groups of TL: BTS/- and TL: BTS/BF5 significantly increased sperm motility, and the integrity of plasmalemma and mitochondria, and decreased the amount of lipoperoxidation, whereas these phenomena were not obviously found in sperm treated with Trolox only during the freezing procedure (TL: -/BF5). The potential resistance to cell damage from cryoinjury was enhanced in sperm treated with SM and Trolox during the semen standing incubation followed by the addition of Trolox to the frozen extender (TL: BTS/BF5): caspase activity was markedly lower, and DNA integrity was higher than those in untreated sperm treated ($P<0.05$). Furthermore, higher sperm penetrability to matured oocytes in vitro was maintained in post-thaw sperm treated with SM and Trolox after the beginning of semen standing incubation ($P<0.05$).

These findings indicate that the addition of SM and Trolox to BTS during the semen standing incubation was essential for improvement in the post-thaw qualities of Agu sperm by protecting the reduction of sperm cryotolerance prior to cryopreservation.

INTRODUCTION

The male reproductive ability (i.e., semen volume, sperm quality, and sperm concentration) of the Okinawan native Agu pig has markedly decreased because of repeated inbreeding within a minority of the closed population for the past 25 years. The technical establishment of long-term preservation and storage of Agu sperm available for artificial insemination has consequently become a subject of interest.

Boar sperm are more sensitive to the detrimental effects of cryoinjury than human and bull sperm (Johnson et al., 1981; Watson 1981). In general, the bulk of seminal plasma proteins before freezing are conventionally removed in boar sperm cryopreservation protocols. Ejaculated seminal plasma was shown to contain some factor(s) that negatively affected the freezability of frozen-thawed miniature pig sperm (Kawano et al., 2004), which raised the possibility that it may be necessary to segregate sperm from seminal plasma just after semen collection. Boar sperm present in the first 10 ml of the sperm-rich fraction of the ejaculate containing a small amount of seminal plasma proteins were shown to be more resilient to cryopreservation than sperm immersed in the rest of the ejaculate (Rodríguez-Martínez et al., 2008; García et al., 2009). However, Agu sperm characteristics and cryoresistance were readily deteriorated by seminal plasma proteins during the semen standing periods before the freezing procedure because Agu semen do not have as definite a sperm-rich fraction as that of commercial pigs. Therefore, establishing a method that protects sperm functions from the detrimental actions of seminal plasma proteins during the semen transportation is needed to efficiently use Agu pigs bred widespread.

Whole milk or skim milk (SM) is commonly used as the diluent for sperm storage or cryopreservation of mammalian semen (Salamon and Maxwell 2000; Purdy 2006), and casein, the major protein in milk, is the most important constituent responsible for sperm protection (Batellier et al., 1997; Pagl et al., 2006). Bergeron et al. (2007) reported that casein decreased binding of the major proteins of bull seminal plasma proteins to sperm and

prevented the loss of cholesterol and choline phospholipids from sperm membranes during the storage of semen. On the other hand, boar seminal plasma possessed the higher level of scavenging activity derived from superoxide dismutase (SOD), but the activities of catalase and glutathione peroxidase (GPx) were very low, suggesting that the prevention of sperm from oxidative stress is essential for retention of sperm quality and viability during the semen standing periods (Brezińska-Ślebodzińska et al., 1995).

However, to the best of our knowledge, no reports have demonstrated whether the addition of SM and Trolox, bioavailable α-tocopherol derivative, to semen diluents could preserve the optimal sperm performance by reducing the detrimental effects of seminal plasma proteins and oxidative stress, respectively, on the post-thaw qualities of boar sperm. Therefore, the objective of the present study was to elucidate the protective effect of SM added to semen diluents (Beltsville thawing solution; BTS) and Trolox added to BTS and the freezing extender (Beltsville F5 extender; BF5) on the maintenance of sperm cryotolerance during cryopreservation in Okinawan native Agu pig.

MATERIALS AND METHODS

Semen collection and sperm cryopreservation

Boars were kept under uniform feeding and handling conditions in the Experimental Farm of Hokubu Agricultural and Forestry High School, Okinawa, Japan. Whole semen from eight mature Agu pigs with proven fertility for each experiment was collected by a glove-hand technique and filtered through a gauze at 1-week intervals. Only samples with more than 70% motile sperm and more than 80% morphologically normal sperm were used for this experiment. Just after collection, semen samples were diluted with equal volumes of BTS and transported to the laboratory within 1.5 h at 30°C, as the semen standing incubation. After transportation, the suspension was centrifuged to remove the seminal plasma. The sperm sediments were diluted in BF5 at a concentration of 10×10^8 sperm/ml. Sperm were cooled from 25 to 5°C over a 1.5-h interval. After standing for 2 h at 5°C, the sperm suspension was mixed with the same volume of BF5 containing 5% glycerol to obtain a final concentration of 5×10^8 sperm/ml, and were quickly frozen in 0.1-ml pellets on dry ice and stored in liquid nitrogen for more than 2 weeks. Each pellet of frozen sperm was thawed in 2.5 ml of modified Tyrode's solution at 39°C.

Analysis of parameters in the post-thaw sperm

The judgments in post-thaw sperm characteristics (sperm motility, sperm plasmalemma integrity, sperm mitochondrial integrity, lipoperoxidation content, as indicated by MDA concentration, intracellular caspase activity, DNA damage, acrosomal proteolytic activity, and in vitro fertilization capacity) were carried out according to the methods reported by Yoshimoto et al. (2009) and Shimokawa et al. (2012).

Experimental design

In Experiment 1, the optimum concentration of SM in the diluents during the semen standing incubation (semen transportation) for improving sperm motility and the integrity of plasmalemma in post-thaw sperm were determined. Each ejaculated semen sample was immediately split into five aliquots and diluted with equal volumes of BTS supplemented with 0, 0.5, 1.0, 2.0, and 4.0% SM sodium to obtain a final concentration of 0 (control), 0.25, 0.5, 1.0, and 2.0%, respectively. After semen transportation, diluted sperm were cryopreserved using procedures previously described.

Experiment 2 was designed to elucidate the effects of Trolox added to the semen diluents and/or the freezing procedure on sperm motility, sperm plasmalemma integrity, sperm mitochondrial integrity, lipoperoxidation content (MDA concentration), intracellular caspase activity, DNA damage, acrosomal proteolytic activity, and in vitro fertilization capacity in post-thaw sperm. Each ejaculated semen sample was immediately split into two aliquots and diluted with equal volumes of BTS containing 1.0% SM in the absence or presence of 100 μM Trolox to obtain a final concentration 0.5% SM and 0 or 50 μM Trolox, respectively. After semen transportation and removal of seminal plasma by centrifugation, a portion of sperm was suspended to BF5 supplemented without Trolox, and another was suspended to BF5 supplemented with 50 μM Trolox; that is, sperm were diluted BTS containing 0.5% SM during the standing incubation, and treated without (control) or with 50 μM Trolox (TL) in only BTS, only BF5, and both solutions (TL: none, TL: BTS/-, TL: -/BF5, and TL: BTS/BF5, respectively) prior to the cryopreservation.

RESULTS AND DISCUSSION

In Experiment 1, the optimum concentration of SM in the diluents during the semen standing period for

improving sperm motility and the integrity of plasmalemma in post-thaw sperm were determined. The effect of treatment with SM during semen transportation on the post-thaw sperm motility is shown in Table 1. A significant interaction of individuals and boars × incubation times was observed in sperm motility parameters. The percentage of total motile sperm (TMS) at 3 h after incubation was significantly ($P<0.05$) higher in sperm treated with 0.5% SM during semen transportation than sperm treated without SM (control) in three of four pigs. Furthermore, the presence of 0.5% SM during semen transportation significantly increased ($P<0.05$) the incidence of post-thaw sperm with the intact plasmalemma in three pigs (Figure 1). However, the diluents supplemented with higher concentrations (1.0 and 2.0%) of SM during semen transportation did not result in further increases in motile sperm or plasmalemma integrity in post-thaw sperm; these were reduced to the same levels as those of the control. These data suggest that an appropriate concentration of 0.5% SM prevents the qualities in post-thaw sperm from the detrimental effects of cryoinjury derived from seminal plasma during the semen standing period before the onset of freezing procedure.

In Experiment 2, treatment with Trolox in group TL: BTS/- and TL: BTS/BF5 significantly increased the sperm motility (TMS and rapid progressive motility sperm; RPMS), and the integrity of plasmalemma and mitochondria, and decreased the amount of lipoperoxidation, whereas these phenomena were not obviously found in sperm treated with Trolox only during the freezing procedure (TL: -/BF5) (Figure 2). The same results were reported that the addition of antioxidants (catalase and Na-pyruvate) to the freezing extender could not improve the post-thaw sperm characteristics in boar, and the effect of supplementation of antioxidants to the extender on sperm quality was related to types and concentration of antioxidants, sperm fraction of the ejaculate, semen preservation protocols and the methodology used to evaluate sperm function (Buranaamnuay et al., 2011). The present results are the first to demonstrate that the presence of Trolox during the semen standing period plays an essential role in protecting sperm against oxidative stress responsible for cryoinjury. Furthermore, these findings raise the interesting possibility that treatment with antioxidant agents during the semen standing period is more significant than antioxidant treatment during the freezing procedure.

The potential resistance to cell damage from cryoinjury was enhanced in sperm treated with SM and Trolox during semen standing incubation followed by the addition of Trolox to the frozen extender (TL: BTS/BF5): caspase activity was markedly lower, and DNA integrity was higher than those in untreated sperm treated ($P<0.05$). In addition, higher sperm penetrability to matured oocytes in vitro was maintained in post-thaw sperm treated with SM and Trolox after the beginning of semen standing incubation ($P<0.05$).

CONCLUSION

It could be concluded that the addition of SM and Trolox to BTS during semen standing incubation was essential for improvement in the post-thaw qualities of Agu sperm by protecting the reduction of sperm cryotolerance prior to cryopreservation.

Keywords: Okinawan native Agu pig, Spermatozoa, Cryopreservation, Skin milk, Trolox

Table 1. The percentage of total motile sperm (TMS) in post-thaw Agu sperm treated with various concentrations of SM during semen standing incubation.

Agu	Concentration of skim milk (%)	Incubation time after thawing (h)		
		0	1	3
A-59	0.0	44.1 ± 1.6 $^{a, x}$	42.8 ± 1.7 $^{a, x}$	23.1 ± 1.3 $^{a, y}$
	0.25	44.1 ± 1.6 $^{a, x}$	43.6 ± 1.6 $^{a, x}$	28.6 ± 1.4 $^{b, y}$
	0.5	44.0 ± 1.6 $^{a, x}$	44.4 ± 1.5 $^{a, x}$	30.5 ± 1.4 $^{b, y}$
	1.0	43.6 ± 1.6 $^{a, x}$	41.9 ± 1.6 $^{a, x}$	29.3 ± 1.5 $^{b, y}$
	2.0	36.8 ± 1.6 $^{b, x}$	36.1 ± 1.6 $^{b, x}$	17.9 ± 1.3 $^{c, y}$
A-71	0.0	49.9 ± 1.6 ^{x}	45.3 ± 1.6 $^{ab, x}$	26.6 ± 1.5 $^{a, y}$
	0.25	48.3 ± 1.6 ^{x}	42.6 ± 1.6 $^{a, y}$	28.5 ± 1.4 $^{ab, z}$
	0.5	53.9 ± 1.6 ^{x}	50.6 ± 1.7 $^{b, x}$	32.8 ± 1.5 $^{b, y}$
	1.0	49.9 ± 1.6 ^{x}	47.8 ± 1.5 $^{ab, x}$	32.4 ± 1.6 $^{b, y}$
	2.0	48.7 ± 1.6 ^{x}	45.6 ± 1.6 $^{ab, x}$	29.7 ± 1.4 $^{ab, y}$
A-87	0.0	48.6 ± 1.7 $^{a, x}$	41.7 ± 1.7 $^{a, y}$	25.3 ± 1.5 $^{a, z}$
	0.25	46.4 ± 1.6 $^{ab, x}$	43.6 ± 1.7 $^{ab, x}$	29.8 ± 1.5 $^{ab, y}$
	0.5	50.8 ± 1.6 $^{a, x}$	47.9 ± 1.7 $^{b, x}$	32.7 ± 1.5 $^{b, y}$
	1.0	48.3 ± 1.6 $^{a, x}$	40.9 ± 1.6 $^{a, y}$	22.4 ± 1.3 $^{c, z}$
	2.0	42.5 ± 1.6 $^{b, x}$	38.7 ± 1.6 $^{a, x}$	22.6 ± 1.4 $^{c, y}$
A-109	0.0	54.3 ± 1.6 ^{x}	50.7 ± 1.7 ^{x}	42.6 ± 1.6 ^{y}
	0.25	52.9 ± 1.7 ^{x}	49.9 ± 1.6 ^{x}	41.6 ± 1.5 ^{y}
	0.5	53.4 ± 1.6 ^{x}	52.3 ± 1.7 ^{x}	42.1 ± 1.7 ^{y}
	1.0	52.3 ± 1.6 ^{x}	49.4 ± 1.6 ^{x}	41.4 ± 1.6 ^{y}
	2.0	52.3 ± 1.6 ^{x}	50.5 ± 1.6 ^{x}	40.5 ± 1.6 ^{y}

Values are expressed as the mean ± SEM of four ejaculates from each Agu.

$^{a-c}$ Values with different superscripts in the same column are significantly different in the same Agu ($p<0.05$).

$^{x-z}$ Values with different superscripts in the same line are significantly different ($p<0.05$).

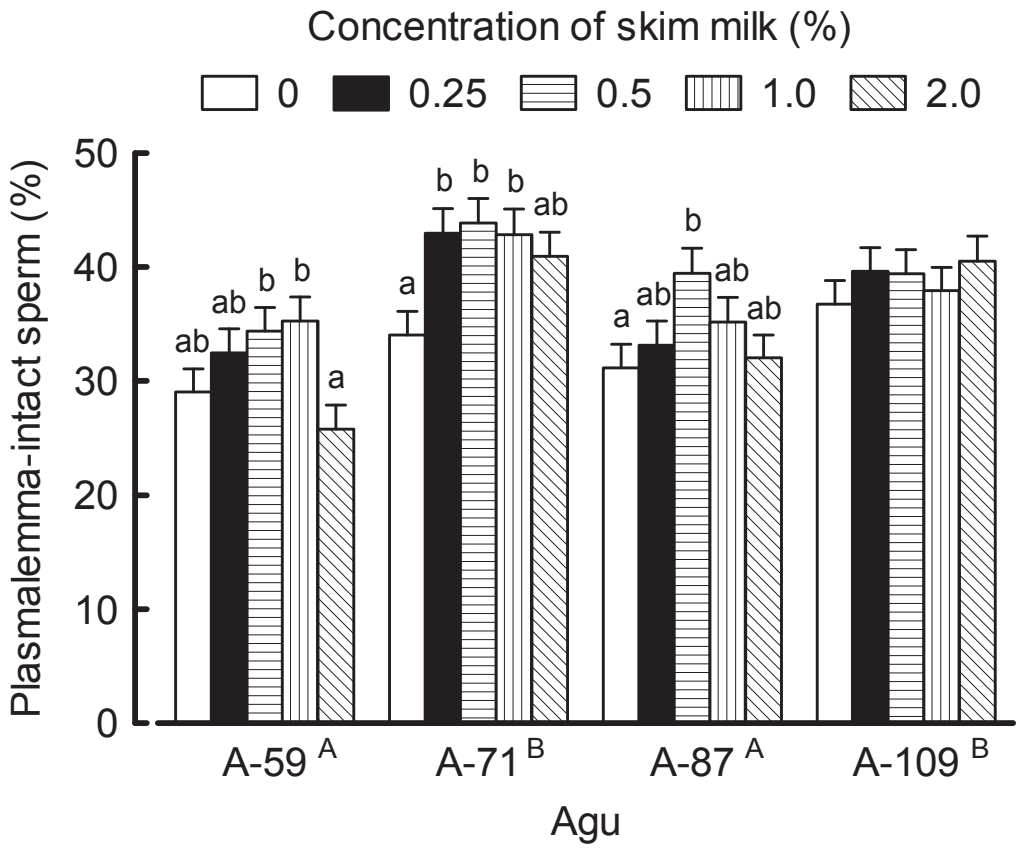

Figure 1. The integrities of the plasmalemma (A) in post-thaw Agu sperm treated with various concentrations of SM during semen standing incubation. Values are expressed as the mean ± SEM for four ejaculates from each of the four Agu pigs. The total number of sperm examined were 493 to 542 in each treatment group. [a, b] Significant differences were observed within the casein treatment groups in the same pigs as determined by a one-way ANOVA ($P<0.05$). [A, B] Significant differences were observed among pigs as determined by a two-way ANOVA ($P<0.05$).

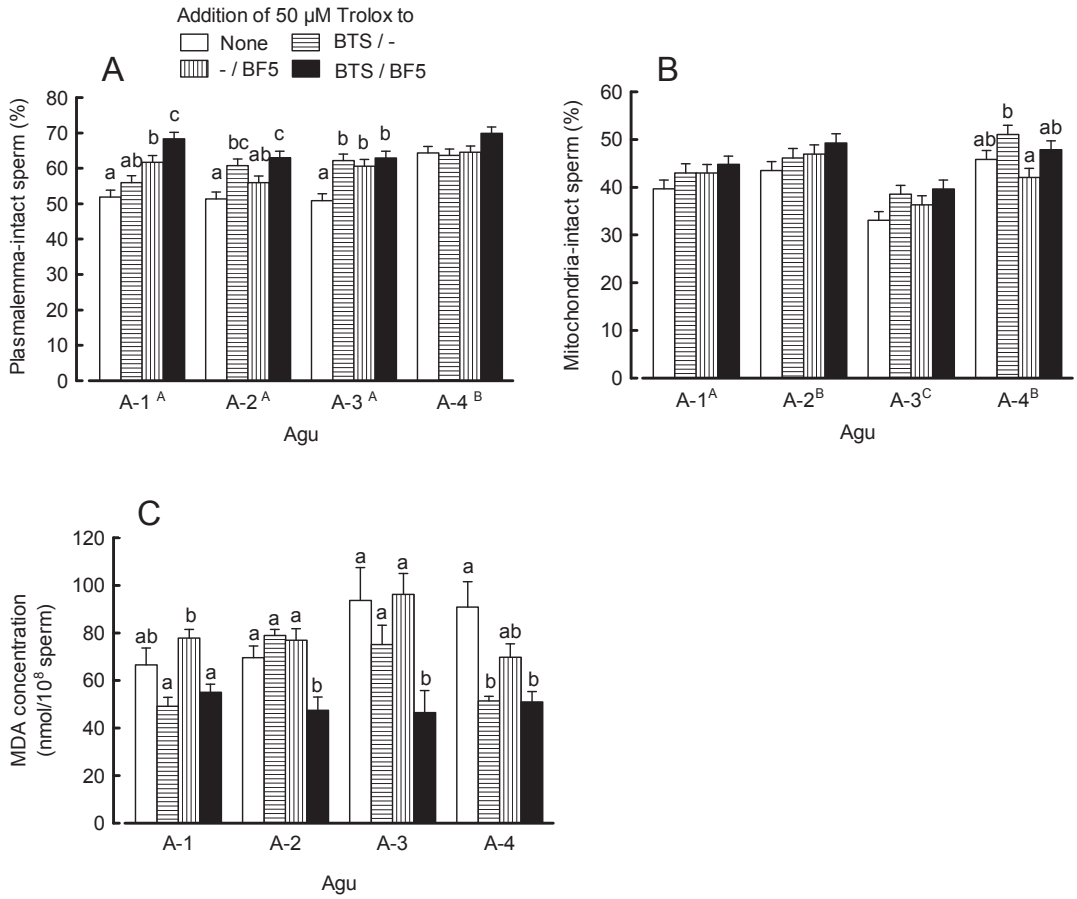

Figure 2. The integrities of plasmalemma (A) and mitochondria (B), and the generation of MDA in post-thaw Agu sperm treated with 50 μM Trolox during the semen standing incubation and/or the freezing procedure. Values are expressed as the mean ± SEM for four ejaculates from each of the four Agu pigs. The total number of sperm examined to determine plasmalemma integrity and mitochondrial integrity ranged from 633 to 719 and 650 to 839 in each treatment group, respectively. $^{a\text{-}c}$ Values are significant different between four treatments in the same individuals ($p<0.05$). $^{A\text{-}C}$ Values with different superscripts are significantly different among individuals ($p<0.05$).

REFERENCES

Batellier F, Magistrini M, Fauquant J, Palmer E. 1997. Effect of milk fractions on survival of equine spermatozoa. Theriogenology, 48: 391-410.

Bergeron A, Brindle Y, Blondin P, Manjunath P. 2007. Milk caseins decrease the binding of the major bovine seminal plasma proteins to sperm and prevent lipid loss from the sperm membrane during sperm storage. Biol. Reprod., 77: 120-126.

Brzezińska-Ślebodzińska E, Ślebodziński AB, Pietras B, Wieczorek G. 1995. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace. Elem. Res., 47: 69-74.

Buranaamnuay K, Grossfeld R, Struckmann C, Rath D. 2011. Influence of cryoprotectants glycerol and amides, combined with antioxidants on quality of frozen-thawed boar sperm. Anim. Reprod. Sci., 127: 56-61.

García EM, Calvete JJ, Sanz L, Roca J, Martínez EA, Vázquez JM. 2009. Distinct effects of boar seminal plasma fractions exhibiting different protein profiles on the functionality of highly diluted boar spermatozoa. Reprod. Domest. Anim., 44: 200-205.

Johnson LA, Aalbers JG, Willems CMT, Sybesma W. 1981. Use of boar spermatozoa for artificial insemination. I. Fertilizing capacity of fresh and frozen spermatozoa in sows on 36 farms. J. Anim. Sci., 52: 1130-1136.

Kawano N, Shimada M, Terada T. 2004. Motility and penetration competence of frozen-thawed miniature pig spermatozoa are substantially altered by exposure to seminal plasma before freezing. Theriogenology, 61: 351-364.

Pagl R, Aurich JE, Müller-Schlösser F, Kankofer M, Aurich C. 2006. Comparison of an extender containing defined milk protein fractions with a skim milk-based extender for storage of equine semen at 5˚C. Theriogenology, 66: 1115-1122.

Purdy PH. 2006. A review on goat sperm cryopreservation. Small Ruminant Res., 63: 215-225.

Rodríguez-Martínez H, Saravia F, Wallgren M, Roca J, Peña FJ. 2008. Influence of seminal plasma on the kinematics of boar spermatozoa during freezing. Theriogenology, 70: 1242-1250.

Salamon S, Maxwell WMC. 2000. Storage of ram semen. Anim. Reprod. Sci., 62: 77-111.

Shimokawa K, Oshiro R, Yamanaka K, Ashizawa K, Ohta S, Tatemoto H. 2012. Improvement of the post-thaw qualities of Okinawan native Agu pig sperm frozen in an extender supplemented with antiapoptotic PTD-FNK protein. Theriogenology, 78, 1446-1455.

Watoson PF. 1981. The effects of cold shock on sperm cell membrane. In: Morris GJ, Clarke A. (eds.), Effects of low temperatures on biological membranes, pp. 189-218. Academic Press, London.

Yoshimoto T, Nakamura S, Yamauchi S, Muto N, Nakada T, Ashizawa K, Tatemoto H. 2008. Improvement of the post-thaw qualities of Okinawan native pig spermatozoa frozen in an extender supplemented with ascorbic acid 2-*O*-α-glucoside. Cryobiology, 57, 30-36.

PO-04-9

Attempt on the establishment of somatic cell nuclear transfer method in Japanese quail

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Introduction

Number of endangered species in bird is increasing during the last decade. With the purpose of the preservation of endangered species in bird, the production of interspecies germline chimeras has been attempted, but which have not been developed as yet for practical application due to low efficiency (Han, 2009). In mammals, on the other hand, somatic cell nuclear transfer (SCNT) of which different kinds of somatic cell nucleus is transferred into enucleated egg has contributed to produce cloned offspring (Wakayama et al. 1998), but in birds no SCNT has been reported so far.

An enucleation of methaphase II chromatin of avian eggs, which is an essential step to produce cloned animals, is hampered due to opacity of their egg cytoplasm. It is reported that the egg undergoes an aging process referred to as "*egg aging*" in which they gradually lose fertilizing capability with the time after ovulation (Miao et al. 2009). Olszanska et al. (2002) have reported the egg aging process in birds that the avian egg undergoes degeneration and fragmentation of metaphase II chromatin by deoxyribonuclease I and II before the egg is expelled from oviduct, when the fertilization does not take place in the oviductal infundibulum after ovulation (Stepinska et al. 2003).

It is crucial to determine how long the fertilization capability of avian egg maintains after ovulation, because an enucleation process of the methaphase II chromatin could be omitted by means of the use of eggs with fragmented metaphase II chromatin. Furthermore, if eggs collected from shell gland (4 hours onward after ovulation) by abdominal massage or freshly laid eggs could be used for SCNT, this could save birds from being sacrificed for SCNT studies. Accordingly, the aim of the current study is to determine when the metaphase II chromatin of quail egg undergoes aging process after ovulation. Furthermore, the present studies were conducted 1) to establish a method of in vitro development of quail ova using SCNT followed by *ex vivo* culture; 2) to observe the effects of egg aging on the in vitro development of SCNT-egg; and 3) to test the competency of blastodermal cell and myoblast nuclei in development of reconstructed embryo.

Materials and Methods

Preparation of donor cell

Breast muscles cells taken from Japanese quail embryos at 11-day of incubation were dissociated mechanically by mincing and pipetting. After being washed twice in Eagle's minimum essential medium containing 15% horse serum, the cells were passed through lens paper layers. Blastodermal cells were obtained from stage X blastoderm. Briefly, the blastoderm was isolated from yolk, and the blastodermal cells were dispersed by trypsin treatment and washed in Dulbecco's modified Eagle's medium (DMEM). Following dispersal, myoblasts or blastodermal cells were transferred to DMEM containing 5% (w/v) polyvinylpyrrolidone.

Egg collection and intracytoplasmic sperm injection (ICSI)

Quail ova were collected from the oviduct at 2, 4, 8, 10, 14 and 20 hours after ovulation, and were subjected to ICSI test. ICSI procedures were performed as described by Mizushima et al. (2014). cRNA mixture solution containing 60 μg/ml PLCZ, 100 μg/ml AH and 100 μg/ml CS was first drawn into an injection micropipette under an inverted microscope, followed by a single ejaculated sperm in the same micropipette, and was microinjected into the central area of the germinal disc of quail egg under a stereomicroscope. Each egg was cultured for 24 hours in DMEM in a plastic cup at 41.5°C in an atmosphere containing 5% CO_2 (Ono et al., 1994).

Donor cell nucleus injection into egg and ex vivo culture

Donor cells were held with a holding pipette and their membranes were ruptured using an injection pipette under a Hoffman modulation contrast microscope using a piezo micromanipulator. Donor cell nucleus was aspirated in micropipette and was microinjected into the quail egg together with PLCZ, AH and CS cRNA solution. Injected

eggs were incubated in DMEM containing 5μg/ml cytochalasin B for 4-5 hours, and was subsequently cultured in DMEM at 41.5°C as described above. Furthermore, developing embryos were transferred to a large surrogate Japanese quail eggshell and were additionally cultured for 48 hours at 37.5°C and 70% relative humidity, with rocking at a 90° angle every 30 min. The incubation procedures were carried out according to the simple culture system described by Kato et al. (2014). Developmental stages of embryos were staged according to the criteria described previously by Eyal-Giladi and Kochav (EGK; 1976) or Hamburger and Hamilton (HH;1951).

Results and Discussion

When sperm and cRNA solution were co-injected into the egg collected 2 hours after ovulation, 70% of the egg developed to blastodermal stage. The same result was obtained when sperm was injected into the egg collected 4 hour after ovulation. On the other hand, the rate of blastodermal development decreased in the egg collected between 8 (25%) and 10 hours (11%) after ovulation when compared to those of the egg collected at 2 or 4 hours post ovulation, but these results indicate that even unfertilized eggs in the shell gland are capable of fertilizing in quail. In contrast, no egg collected 14 and 20 hours after ovulation or laid were fertilized after ICSI, suggesting that the quail egg lost its fertilizing capability at 14 hours post ovulation.

When quail eggs were collected 14 hours after ovulation and microinjected with a single nucleus from blastodermal cell, 8% of the eggs, which reached EGK stages III-VIII 24 hours after SCNT, were observed. It should be noted that one of these blastoderms developed up to HH stage 8 when the culture period was extended up to 72 hours. On the other hand, 5% of the eggs developed to EGK stages III-VII 24 hours after SCNT when the laid eggs were microinjected with blastodermal cell-nucleus and cRNA solution, but none of them reached to HH stage (EGK stages XII) despite extending the culture period up to 72 hours. In addition, neither the egg collected 14 hours after ovulation nor laid egg that were microinjected with nucleus from myoblast developed. These results indicate that blastodermal cell, but not myoblast from 11-day old quail embryo, could be reprogrammed in quail egg using nuclear transfer to produce viable embryo. Although it remains unknown at present why the development of the SCNT-generated embryo are arrested at early HH stage, the present nuclear injection method of blastodermal cell into egg collected 14 hours after ovulation will be helpful to establish a cloning technique of endangered avian species.

Conclusion

The present study is the first report demonstrating the embryo development of avian egg microinjected with somatic cell nucleus. Although much work is yet to done comparing development from different kinds of cells, this study has important implications for the study of cell selection for nuclear transfer, and further investigations should be pursued for the synchronization of the cell cycle between donor nucleus and egg cytoplasm following egg activation. Kuhholzer et al. (2001) reported that use of nuclei in cell cycle S and G2 phases for nuclear transfer damages to the reconstituted chromatin and/or aneuploidy, whereas nuclear transfer of somatic cells that are arrested at G0 or G1 phase may facilitate reprogramming of the nucleus to produce reconstituted porcine embryo. Therefore, it should be possible to establish a novel method to overcome the current issue in future practical application in birds (Figure 1).

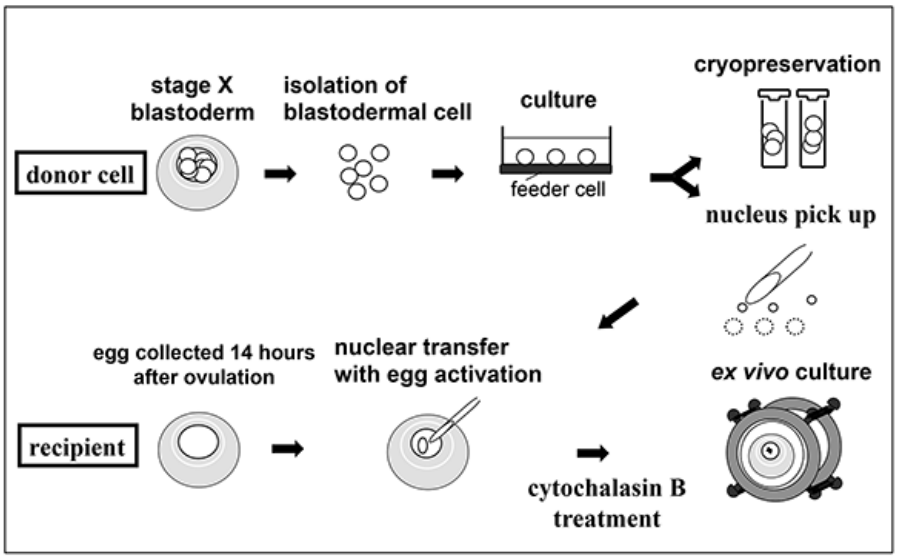

Figure 1. Schematic diagram of the somatic cell nuclear transfer (SCNT) system using blastodermal cell in aves.

References

Eyal-Giladi H, Kochav S. 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. *Developmental Biology*, 49:321–337.

Hamburger V, Hamilton HL. 1951. A Series of Normal Stages in the Development of the Chick Embryo. *Journal of morphology*, 88:49–92.

Han JY. 2009. Germ cells and transgenesis in chickens. *Comparative Immunology, Microbiology and Infectious Diseases*, 32: 61–80.

Kato A, Mizushima S, Shimada K, Kagami H, Ono T. 2014. Simple culture system for bobwhite quail and Japanese quail embryos from the blastoderm stage to hatching using a single surrogate eggshell. *Journal of Poultry Science*, 51: 203–206.

Kuhholzer B, Hawley RJ, Lai L, Kolber-Simonds D, Prather RS. 2001. Clonal lines of transgenic fibroblast cells derived from the same fetus result in different development when used for nuclear transfer in pigs. *Biology of Reproduction*, 64:1695–1698.

Miao, YL, Kikuchi K, Sun QY, Schatten H. 2009. Oocyte aging: cellular and molecular changes, developmental potential and reversal possibility. *Human Reproduction Updates*, 15: 573–585.

Mizushima S, Hiyama G, Shiba K, Inaba K, Dohra H, Ono T, Shimada K, Sasanami T. 2014. The birth of quail chicks after intracytoplasmic sperm injection. *Development*, 141:3799–3806.

Olszanska B, Stepinska U, Perry MM. 2002. Development of embryos from in vitro ovulated and fertilized oocytes of the quail (Coturnix coturnix japonica). *Journal of Experimental Zoology*, 292:580–586.

Ono T, Murakami T, Mochii M, Agata K, Kino K, Otsuka K, Ohta M, Mizutani M, Yoshida M, Eguchi G. 1994. A complete culture system for avian trasngenesis, supporting quail embryos from the single-cell stage to hatching. *Developmental Biology*, 161:126–130.

Stepinska U, Olszanska B. 2003. DNase I and II present in avian oocytes: a possible involvement in sperm degradation at polyspermic fertilization. *Zygote*, 11:35-42.

Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*, 394: 369–374.

PO-04-11

Seasonal variation of dog semen quality and freezability

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Objective

Artificial insemination (AI) with cryopreserved semen is widely applied in bovine reproduction, and it has resulted in a progressive improvement in the quality of cattle breeds. Development of an effective cryopreservation technique for canine semen might likewise offer the benefits of enabling maintenance of endangered canine breeds and species and of improving the utilization of specific genetic traits in male dogs of particular breeds such as guide dogs for the blind. The first report of successful AI using frozen-thawed dog semen was published in 1969 (Seager. 1969). However, the rates of successful pregnancy with cryopreserved sperm are still highly variable and generally lower than those with fresh semen (Thomassen et al., 2006). This indicates that the quality of dog sperm after thawing must be relatively poor and likely determines the low pregnancy rate (Thomassen et al., 2006). Thus, there is still considerable scope for improvement in dog sperm cryopreservation techniques, and such improvements will clearly have a positive effect on practical dog breeding. It is well known that the freezability of frozen semen is highly affected by the fresh semen quality (Pena et al., 2006; Flores et al., 2009). Therefore, the stable collection of fresh semen with good quality is important for acquiring the cryopreserved sperm which is expected to maintain its fertility. Seasonal variations of semen quality and fertility were reported in many species. In bovines, it was reported that ejaculate collected during the summer was more sensitive to freezing than collected in the winter (Orgal et al., 2012). In dogs, there were some reports that evaluate the seasonal effects on semen quality or hormonal changes of mongrel dogs in a mediterranean climate (Albrizio et al., 2013) and a sub-humid tropical climate (Ortega-Pacheco et al., 2006). Also, Kuroda and Hirose (1972) studied in Japan with spitz breeds that semen quality such as sperm concentration or viability and metabolism such as respiratory volume were fluctuated with the seasons. There are some differences between these reports such as climate, rearing environment and dog breeds, and they are expected to be important factor for spermatogenesis of the dog. Considering practical use for AI, understanding seasonal changes with the quality of cryopreserved sperm is important. Therefore, the aim of this study was to investigate the influence of seasonal variation upon semen quality and the freezability of sperm from Japanese guide dogs in order to confirm that such semen can be used throughout the year.

Methodology

Animals

Fresh semen was collected from three male dogs (Labrador retrievers and crossbreds Labrador retriever x Golden retriever, 1 to 5 years of age) raised in Tochigi, Japan, as breeding sires for guide dogs. Dogs were managed according to instructions from the East Japan Guide Dog Association. All experiments in this study were performed in accordance with the Utsunomiya University Guide for Experimental Animals.

Semen collection and processing

The year was divided into four seasons: spring (March to May); summer (June to August); fall (September to November) and winter (December to February). Experiments were conducted over three years. Semen samples were obtained after digital manipulation in the presence of a bitch in heat as a dummy. The sperm-rich fraction was isolated, although inevitably it contained small amounts of the first (pre-sperm) and third (prostatic) fractions. Ejaculates were kept at room temperature for transport to the laboratory (a total period of about 1.5 h). The quality of the fresh semen samples was assessed within 3 h of ejaculation. The original Tris-egg yolk-citrate extender (Ogata et al., 2015) was used to dilute semen samples. The samples were allowed to equilibrate for 3 h in a refrigerator at 4°C. An equal volume of extender containing 13% glycerol was then added to obtain a final concentration of 6.5% glycerol and 100×10^6 spermatozoa/ml.

Freezing and thawing

The sperm samples were loaded into 0.25 ml straws (IMV, L'Aigle, France) and frozen by placing them horizontally in a rack 6 cm above the surface of liquid nitrogen (LN_2) in a closed styrene foam box (16 cm x 24 cm x 16 cm) for 15 min; the straws were frozen in LN_2 vapor and plunged into the LN_2 (Okano et al., 2004). The straws were stored in LN_2 for at least one week before being thawed for evaluation of the sperm. Thawing was carried out in a water bath at 70 ℃ for 5 sec (Nothling and Shuttleworth. 2005), and the thawed sperm samples were placed into sealed 1.5 ml polypropylene tubes at room temperature (approximately 24°C).

Assessment of semen quality parameters

Sperm concentration: For each sample, fresh semen concentrations were recorded. Sperm concentrations were determined using a Hemacytometer (Reichert, Buffalo, NY, USA) and samples diluted 1:200 with surfactant-added saline, as previously described (Bane. 1952).

Sperm motility and longevity: Sperm progressive motility (SPM), sperm viability index (SVI), sperm viability, and acrosomal integrity were evaluated before and after freezing in order to determine sperm quality and freezability. SPM was estimated by phase-contrast microscopy (Olympus Optical Co., Ltd, Tokyo, Japan) at a magnification of 200 × on a warmed slide (38 ℃). Motility patterns were classified using the WHO grades with some modifications (World Health Organization. 1999): + + + , progressively motile at a high speed; + + , progressively motile at a moderate or low speed; + , motile without progression; -, immotile. The proportions (%) of sperm in each grade were assessed independently by two observers, and the average value per sample was recorded as the final motility. SPM scores were obtained from the sperm samples classified as having a + + + motility pattern. The effects of the different treatments were also compared using the SVI. This index is based on the four patterns of sperm motility and their relative proportions in each group. SVI was calculated using a previously described formula (Fukui et al., 2004) with some modifications: (% + + + sperm) + (% + + sperm × 0.75) + (% + sperm × 0.5).

Sperm viability and acrosomal integrity: Sperm viability and acrosomal integrity were evaluated by using combined Hoechst 33258/chlortetracycline staining (H258/CTC), which was performed with a modified version of the protocol described by Fraser et al. (1995). Briefly, the suspended samples were stained with 0.1 μl/ml of Hoechst bis-benzimide 33258 in Bracket and Oliphant (BO) medium for 3 min at room temperature. Excess dye was removed by centrifugation at 600 × g for 10 min. The precipitated spermatozoa were resuspended in 45 μl of BO medium, mixed with the same volume of CTC solution (750 mM CTC, 5 mM cysteine, 130 mM NaCl and 20 mM Tris, pH 7.8) and then fixed with 8 μl of 12.5% (w/v) paraformaldehyde. The cells were analyzed using a fluorescence microscope (Olympus) at a magnification of 400 ×; a minimum of 100 spermatozoa per slide were screened. After H258/CTC staining, the spermatozoa were first categorized as live or dead according to their Hoechst staining using a 330 to 385 nm filter and a DM400 dichroic mirror (U-MWU2; Olympus). Only sperm judged as living (Hoechst negative) were examined for CTC staining using a 400 to 440 nm filter and a DM455 dichroic mirror (U-MWBV2; Olympus). Live spermatozoa were then further categorized according to the CTC fluorescence patterns as described by Hewitt et al (1998). Three patterns of fluorescence were present in live spermatozoa: fluorescence over the whole head (F: non-capacitated), fluorescence-free band in the post-acrosomal region (B: capacitated); and almost no fluorescence over the whole head except for a thin band in the equatorial segment (AR: acrosome reacted). Viable spermatozoa with both F and B patterns were assumed to represent acrosomally intact spermatozoa.

Statistical analysis

Results were expressed as means ± SEM and statistically analyzed using the StatView v. 5.0 software (Abacus Concepts Inc., Berkeley, CA, USA). Factorial ANOVA with the Tukey-Kramer method was used to compare semen parameters. Differences with values of $P < 0.05$ were considered to be statistically significant.

Result and Discussion

Seasonal effects on the means ± SEM values of semen parameters of fresh and frozen-thawed sperm are presented in Table1. There were no significant differences in fresh semen concentrations across the four seasons. SPM (Fig. 1), SVI and viability were significantly reduced after freezing ($P < 0.05$), and this reduction was evident across all seasons. No significant differences were detected in SPM, SVI, viability or acrosomal status in terms of seasonality (Table 1). In this study, we focused on the seasonal variations in quality and freezability of canine sperm. There

were no significant differences in the sperm concentrations of fresh semen and SPM, SVI, viability and acrosomal integrity of fresh and frozen-thawed sperm among the four seasons. Environmental thermal stress is well known as the main cause of seasonal variation in mammal semen quality. In bulls, alteration of seminal plasma composition and decreasing of post-thaw sperm motility and acrosomal integrity were observed in summer (Sharma et al., 2014). Breeding sires for the guide dogs are required to be reared in the indoor. Therefore, their reproductive conditions for spermatogenesis were not so affected by the environmental heat stress and could maintain the semen quality in summer season. Testosterone is a key factor in male reproductive activity and semen quality. Although boar is non-seasonal breeders, seasonal variations in serum testosterone concentration and semen quality have been reported (Borg et al., 1993). Inaba (1985) and Taha (1981) reported that there was no seasonal variation in serum testosterone concentration of male dogs. For these reasons, it could be inferred that semen quality and freezability of male dogs were not fluctuated throughout the year.

Conclusion

Our present data acquired from Japanese male breeding dogs, indicate that the quality and freezability of dog semen remains in a stable condition throughout the year, and that semen samples can be used throughout the year.

Table1. Seasonal effect on characteristics of fresh and frozen-thawed canine semen

Season	Concentration (10^8sperm/ml)	SPM (%)		SVI (%)		Viability (%)		Acrosomal integrity (%)	
	fresh	fresh	frozen-thawed	fresh	frozen-thawed	fresh	frozen-thawed	fresh	frozen-thawed
Spring	9.3±2.1	82.5±1.5	53.8±2.5	85.1±1.3	61.8±3.3	87.5±1.7	69.5±4.1	94.4±1.3	92.7±1.6
Summer	6.8±1.5	83.0±2.5	50.8±6.1	84.7±1.8	59.1±4.5	85.5±2.1	65.4±1.7	92.2±1.1	94.3±1.1
Autumn	8.1±1.1	74.5±6.8	50.0±4.2	84.1±2.8	64.5±2.8	84.9±1.8	71.1±3.0	91.5±1.0	94.6±1.0
Winter	6.9±2.2	72.2±7.0	44.2±5.6	82.6±3.1	51.4±3.1	79.6±2.6	67.2±3.6	92.3±2.0	94.5±1.3

Data are presented as means ± SEM for ten ejaculates (n=10) per season (Spring, Summer, Autumn, Winter), per status (fresh, frozen-thawed).

Acrosomal integrity is expressed as the sum of pattern F (non-capacitated) and pattern B (capacitated).

Abbreviations: SPM, sperm progressive motility: SVI, sperm viability index.

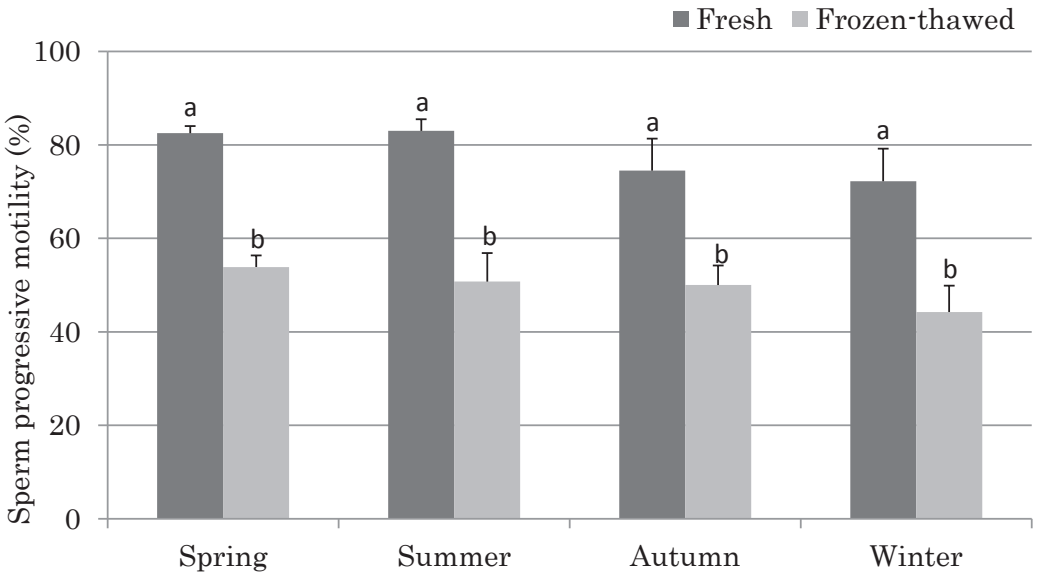

Fig.1. Seasonal variations in sperm progressive motility of fresh and frozen-thawed canine sperm.
Means ± SEM. Ten replicate experiments were performed.
Different letters (a, b) indicate significant differences between fresh and frozen-thawed samples ($P < 0.05$).

Reference

Albrizio M, Siniscalchi M, Sasso R, Quaranta A. Effects of the environment on dog semen parameters and testosterone concentration. Theriogenology 2013; 80: 800-804.

Bane A. A study on the technique of hemocytometric determination of sperm motility and sperm concentration in bull semen. Cornell Vet 1952; 42: 518-531.

Borg KE, Lunstra DD, Christenson RK. Semen characteristics, testicular size, and reproductive hormone concentrations in mature duroc, meishan, fengjing, and minzhu boars. Biol Reprod 1993; 49: 515-521.

Flores E, Fernandez-Novell JM, Pena A, Rodriguez-Gil JE. The degree of resistance to freezing-thawing is related to specific changes in the structures of motile sperm subpopulations and mitochondrial activity in boar spermatozoa. Theriogenology 2009; 72: 784-797.

Fraser LR, Abeydeera LR, Niwa K. Ca2+-regulating mechanisms that modulate bull sperm capacitation and acrosomal exocytosis as determined by chlortetracycline analysis. Mol Reprod Dev 1995; 40: 233-241.

Fukui Y, Togawa M, Abe N, Takano Y, Asada M, Okada A, Iida K, Ishikawa H, Ohsumi S. Validation of the sperm quality analyzer and the hypo-osmotic swelling test for frozen-thawed ram and minke whale (Balaenoptera bonarensis) spermatozoa. J Reprod Dev 2004; 50: 147-154.

Hewitt DA, England GC. An investigation of capacitation and the acrosome reaction in dog spermatozoa using a dual fluorescent staining technique. Anim Reprod Sci 1998; 51: 321-332.

Inaba T, Matsui N, Shimizu R, Takahashi S. Serum concentrations of free testosterone in beagle dogs. Jpn J Anim Reprod 1985; 31: 43-47 (in Japanese).

Kuroda H, Hiroe K. Studies on the metabolism of dog spermatozoa. I.Seasonal variation on the semen quality and of the aerobic metabolism of spermatozoa. Jpn J Anim Reprod 1972; 17: 89-98 (in Japanese).

Nothling JO, Shuttleworth R. The effect of straw size, freezing rate and thawing rate upon post-thaw quality of dog semen. Theriogenology 2005; 63: 1469-1480.

Ogata K, Sasaki A, Kato Y, Takeda A, Wakabayashi M, Sarentonglaga B, Yamaguchi M, Hara A, Fukumori R, Nagao Y. Glutathione supplementation to semen extender improves the quality of frozen-thawed canine spermatozoa for transcervical insemination. J Reprod Dev 2015; 61: 116-122.

Okano T, Murase T, Asano M, Tsubota T. Effects of final dilution rate, sperm concentration and times for cooling and glycerol equilibration on post-thaw characteristics of canine spermatozoa. J Vet Med Sci 2004; 66: 1359-1364.

Orgal S, Zeron Y, Elior N, Biran D, Friedman E, Druker S, Roth Z. Season-induced changes in bovine sperm

motility following a freeze-thaw procedure. J Reprod Dev 2012; 58: 212-218.

Ortega-Pacheco A, Segura-Correa JC, Bolio-Gonzalez ME, Jimenez-Coello M, Linde Forsberg C. Reproductive patterns of stray male dogs in the tropics. Theriogenology 2006; 66: 2084-2090.

Pena FJ, Nunez-Martinez I, Moran JM. Semen technologies in dog breeding: an update. Reprod Domest Anim 2006; 41 Suppl 2: 21-29.

Seager SWJ. Successful pregnancies utilizing dog semen. Artif Insem Dig 1969; 17: 6-16.

Sharma L, Pandey V, Nigam R, Singh P, Saxena A, Swain DK. Seasonal variations in seminal plasma proteins of buffalo. Reprod Domest Anim 2014; 49: 387-391.

Taha MB, Noakes DE, Allen WE. The effect of season of the year on the characteristics and composition of dog semen. J Small Anim Pract 1981; 22: 177-184.

Thomassen R, Sanson G, Krogenaes A, Fougner JA, Berg KA, Farstad W. Artificial insemination with frozen semen in dogs: a retrospective study of 10 years using a non-surgical approach. Theriogenology 2006; 66: 1645-1650.

World Health Organization. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge University Press 1999; 4th edition.

PO-04-18 Preliminary Study of Ovulation Induction by Low Dose of eCG and Timed Artificial Insemination with hCG in Swamp Buffalo

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Introduction

The swamp buffaloes (*Bubalus bubalis*) are belong to water buffalo and predominantly in Southeast Asia. Although the total world buffalo population has increased from 120 to 160 million during the last two decades, the number of swamp buffaloes has rapidly declined, especially in Thailand. The buffalo population in Thailand has decreased from 1.24 million heads in 2012 to 864,064 heads in 2014 and most of them are kept by small holder (DLD, 2015). Several major problems in buffalo reproduction are inherent late maturity, lack of obvious estrus behavior and prolonged inter-calving interval (Nanda et al., 2003). Anoestrus after calving in swamp buffalo longer than dairy buffaloes (EI-Wishy, 2007). Modern reproductive technology, such as artificial insemination, in vitro fertilization and embryo transfer, which are routinely applied in the dairy cattle industry, have to be improved and adapted to the buffalo (Scherf, 2000). A prolonged anoestrous period after parturition is one of the major problems occurring in beef cattle worldwide as well as in Thailand (Jarassaeng et al., 2013). A variety of hormonal injection programs have been introduced to solve the problem and resulted in a wide range of responses in beef cattle despite improve pregnancy rate in anoestrus cows (Jarassaeng et al., 2014). Kajaysri et al. (2015) introduced the CIDR insertion with eCG and hCG in anoestrus swamp buffaloes and found results of 40 % ovulation rate. The objective of this study was to investigate the pregnancy rate after ovulation induction and TAI program in swamp buffalo.

Materials and methods

Four heifer buffaloes at age of 2 years and four primiparous swamp buffaloes at more than 150 days postpartum were introduced into this study. The *Controlled internal drug release*(*CIDR*)*devices* (Eazi-Breed CIDR Cattle Insert, Zoetis Animal Health) device was inserted intravaginally for 14 days and injected with 500 microgram of cloprostenol (Estrumate®, MSD Animal Health) and 300 IU of equine chorionic gonadotropin (eCG) (Folligon®, *Intervet Ltd*., Boxmeer, The*Netherlands*) was injected on the CIDR removal day. All buffaloes were kept in the pen during insertion of CIDR device. All buffaloes were inseminated with Timed-AI at 72 hours after CIDR removal and injected with 1500 IU of human chorionic gonadotropin (hCG) (*Chorulon*®, *Intervet Ltd*., Boxmeer, The*Netherlands*). The pregnancy diagnosis was done by rectal palpation at 60 days after Timed-AI.

Results and discussion

The results showed that ovulation induction was successfully in all buffaloes. The CIDR was lost in one heifer buffalo. The overall pregnancy rate was 71% (5/7) after timed-AI. However, this hormonal program and TAI increased the number of buffaloes submitted to AI within a short period and resulted in an acceptable pregnancy rate. The lost of CIDR from vagina which is a drawback of the device was also found in other reports (Zaabel et al., 2009). Several ovulation induction with timed-AI have been introduced to anoestrus animals with more program from researcher, however, the potential in pregnancy rate not satisfaction, GnRH with PGF could be used in the same of dairy cattle (Rastegarnia et al., 2004). Our result was comparable to that of another recent in which CIDR was inserted for 14 days followed by estus detection and AI (Zaabel et al., 2009). In one study, the Ovsynch protocol with timed-AI was applied to buffalo and resulted in lower pregnancy rate compared to this study (Chaikhun et al., 2010). However, researcher in Indonesia achieved a higher pregnancy rate after using a similar Ovsynch program with timed-AI (Sianturi et al., 2011).

Conclusion

In conclusion, the results suggested that an ovulation induction program using CIDR and low dose eCG followed by Timed-AI can be used efficiently in swamp buffaloes.

Acknowledgements

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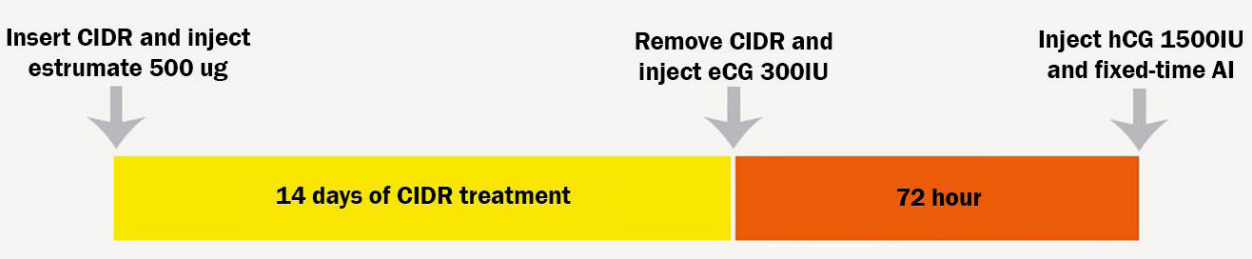

References

Chaikhun, T., Tharasanit, T., Rattanatep, J., De Rensis, F. and Techakumphu, M. 2010. Fertility of swamp buffalo following the synchronization of ovulation by the sequential administration of GnRH and PGF_2alpha combined with fixed-timed artificial insemination. Theriogenology 74(8):1371-6.

Department of Livestock Development (DLD). 2014. Statistical Yearbook, Bangkok,Thailand. http://www.dld.go.th/th/images/stories/procure/2558/25571 1/25571110_2.pdf. Accessed in 2 July 2016.

Jarassaeng, C., SIrisarhien, S., Sangkaew, A., Sri-ngam, S., Suwannathada, P., Aiumlamai, S., Prongcharoen, M., and Suebkom. C. 2013. Ovulation induction and fixed-time artificial insemination during anoestrus in postpartum beef cattle.

The 14th KVAC, 6-7 June 2013.

Jarassaeng, C., Sirisathien, S., Sangkaew, A., Sri-ngam, S., Suwannathada, P., Aiumlamai, S., Seesupa, S., Suebkom, C. and Sadee, P 2014. Progesterone profiles in ovulation induction by long time insertion of CIDR®-B with low dose eCG and TAI with hCG in anoestrus in beef cattle. The 15th KVAC, 24-25 April, 2014.

Kajaysri, J., Chumchoung, C. and Photikanit, G. 2015. Estrus and ovulation responses in anestrus postpartum swamp buffaloes following synchronization with a controlled internal drug release device and prostaglandin f2α based protocols. Buffalo Bulletin 34 (3): 357-368.

Nanda A.S., Brar, P.S., and Prabhakar, S. 2003. Enhancing reproductive performance in dairy buffalo: major constraints and achievements. Reprod Suppl 61: 27-36.

Rastegarnia, A., Niasari-Naslaji, A., Hovareshti, P., Sarhaddi, F., and Safaei, M. 2004. The effect of different doses of gonadorelin on ovarian follicle dynamics in river buffalo (*Bubalus bubalis*). Theriogenology 62(7):1283-91.

Scherf B, 2000: World Watch List for Domestic Animal Diversity. Food and Agriculture Organization of the United Nations. Rome, Italy. http://www.fao.org/docrep/009/x8750e/x8750e00.HTM

Sianturi, R.G., Purwantara, B., Supriatna, I., Amrozi and Situmorang, P. 2012. Optimizing artificial insemination on swamp buffalo (bubalus bubalis) through synchronization of estrus and ovulation. JITV 17(2): 92-99.

Zaabel, S.M., Hegab, A.O., Montasser, A.E., and El-Sheikh, H. 2009. Reproductive performance of anestrous buffaloes treated with CIDR. Anim Reprod 6(3):460-464.

PO-04-19

Effects of Sericin Supplementation on Cryopreserved sperm survival of Thai Native Chicken

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INTRODUCTION

The importance of AI in poultry reproduction, both for commercial production and genetic conservation, has stimulated research interest in developing the proper diluents and technique for liquid and cryopreserved semen storage (Donoghue and Wishart. 2000; Blesbois, 2007). The challenges are due in part to some special physiological properties of avian semen, which result in susceptibility to damage during the freeze-thaw process (Cerolini et al., 1997) leading to membrane rupture, and thus affecting semen quality and fertilizing ability (Long, 2006). The important factor to decrease the quality of frozen semen is free radical and engender to lipid peroxidation. These processes decrease the motility and fertility of sperm. Therefore, addition a suitable antioxidants is crucial for post-thaw spermatozoa fertilizing ability.

Sericin derived from thecocoon of silkworms (*Bombyx mori*). It is a natural macromolecular and water-soluble globular protein. Sericin has 18 kinds of amino acid such as hydroxyl, carboxyl and amino group (Wai et al., 2015). Kato et al. (1998) reported that sericin has role antioxidant and inhibits lipid peroxidation.

Sericin supplementation in extender of buffalo bull semen could improve semen quality which protected sperm from oxidative stress (Kumar et al., 2015). This result was similar to another report which conducted in Thai native bull by Dorji et al. (2015). Untile recently, there is no study about sericin supplementation on frozen-thawed semen quality of chicken. Therefore, the present study was to investigate the effect of sericin supplementation on motility and viability of frozen-thawed semen in Thai native chicken.

MATERIALS AND METHODS

Semen collection

Twenty-four Thai native cocks (Pradu Hang Dam) 12 months of age were used in this study. Animals were kept in individual cages. Cockerels were fed 130g/head/day, and water was provided *ad libitum*. The animals were reared under natural environmental conditions throughout the experiment. The study was conducted at the research farm of the Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand.

Semen from each individual was collected by the dorso-abdominal massage method (Burrows, and Quinn, 1937). The collection of spermatozoa from the domestic fowl and turkey, and placed in a 1.5 mL microtube. Semen samples were evaluated under a microscope within 15 min following collection, and were selected on the basis of meeting the following criteria: mass motility score ≥ 4 (score range 0-5); sperm concentration ≥ 3 × 109 sperm/mL (hemocytometer counting method); and sperm viability (nigrosin-eosin staining) ≥ 90%. To maximize semen quality and quantity, thecollection was always performed by the same people, under the same conditions, time, and massage method. Special care was taken to avoid contamination of semen with feces, urate, and transparent fluid, which lower semen quality.

Dilution and Cryopreservation Procedure

Pooled semen samples were divided into four aliquots and diluted 1:3 (v/v) with Schramm diluents (Schramm 1991) with different concentrations of sericin (0, 0.025, 0.05, 0.1%), The diluted semen samples were cooled down from 25 ºC to 5 ºC for 1 h, and N,N-dimethylformamide (DMF) was added to a final concentration of 6% (v/v). Semen was immediately loaded into 0.5 mL plastic straws, sealed with polyvinylpyrrolidone (PVP) powder and equilibrated at 5 ºC for 15 min. After equilibration, the filled straws were laid horizontally on a rack 11 cm above the surface of liquid nitrogen (-35ºC) for 12 min, then, placed 3 cm above liquid nitrogen vapor (-135ºC) for 5 min, and subsequently immersed in liquid nitrogen (Vongpralub et al. (2011). Semen straws were transferred to a liquid nitrogen container for storage. After storage for at least three days, the straws were thawed individually in an ice-water bath at 5 ºC for 5 min and then evaluated for various sperm functions.

Assessment of Frozen/Thawed Sperm Quality

For each assessment of motility, the frozen sperm was diluted in each extender with a ratio of 1:15 and dropped onto 2X-CEL slide sperm analysis chamber and covered with coverslip and motility analysis started thereafter. Sperm motility was evaluated using computer-assisted semen analysis (CASA). An IVOS Model 10 spermatozoa analyzer was used with Olympus software to process video material recorded in '.'avi' format.

Sperm membrane integrity was assessed with dual fluorescent probes, SYBR-14 and propidium iodide, according to the method described by Partyka et al. (2010). Briefly, each sample was diluted to a concentration of 50 × 106 spz/mL. Portions (250 µL) of the diluted samples were dropped into a cytometric tube and 5 µL of SYBR-14 working solution was added. The working solution was obtained by diluting a commercial solution of SYBR-14 in distilled water at a ratio of 1:49. Samples were mixed and incubated at room temperature for 10 min. The cells were counterstained with 5 µL PI for 5 min and then fixed with 30 mL 20% formaldehyde. The sperm was then evaluated under a fluorescent microscope IX71. The PI - negative and SYBR-14 - positive population showing green fluorescence was considered to be live, with sperm plasma membrane intact (PMI).Assessment of plasma membrane integrity

Statistical analysis

Experimental data were analyzed using a program SAS 9.0 statistical software package. Duncan's New Multiple Range Test (Steel and Torrie, 1980) was used to test the difference in the mean of group experiment. A probability level of $P \leq 0.05$ was considered as significant. Six replications were conducted for all of the parameters.

RESULTS AND DISCUSSION

The results of sperm motility revealed sericin supplemented in extenders with 0.05 and 0.1% increased sperm motility and progressive motility ($P<0.01$) (Table 1) but not the percentage of live sperm compared to control (Table 2).

It is well known that sericin has an important role as an antioxidant for decreasing ROS reaction. ROS are common products of normal cellular metabolism but an excessive production of ROS resulted in an oxidative stress and lipid peroxidation (Mazur et al., 2000). The lipid peroxidation decreased motility and fertility of spermatozoa. Kato et al. (1998) reported sericin could suppress lipid peroxidation and tyrosinase activity. Sericin supplementation with 0.05% resulted in greater sperm motility. These results are supported by Pushpa et al. (2013) and the finding of Kumar el al. (2015) where sericin at theconcentration of 0.5% was reported to increase the survival of cryopreserved buffalo semen by protecting sperm against oxidative stress. The addition of sericin to bovine *in vitro* culture medium has also improved embryo development by preventing H_2O oxidative stress (Santiago-Moreno, 2012). Moreover, this silkworm protein has antimicrobial property (Push et al., 2013; Sarovant et al., 2003).

In conclusions, the addition of sericin enhanced the motility of frozen storage of chicken semen. This protein from silkworm might improve the semen quality by its antioxidant property.

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Table 1 Effect of sericin adding into semen extender on motility (mean±SE) characteristics of post-thawed Thai native chicken semen.

Treatment	MOT	PMOT	VAP	VSL	VCL
T1	51.80±5.26[b]	19.40±2.54[b]	72.82±8.64	57.52±4.71	129.72±10.39[b]
T2	52.60±2.88[b]	19.80±2.48[b]	72.74±4.16	55.98±3.08	135.40±4.68[ab]
T3	58.40±2.96[a]	25.60±2.07[a]	78.90±5.60	58.98±5.16	140.72±5.95[a]
T4	54.60±2.70[ab]	24.80±3.27[a]	75.78±8.08	56.24±4.96	134.32±4.90[ab]
CV	6.25	10.08	8.86	8.49	3.78
MSE	3.39	2.25	6.65	4.85	5.11
P-value	0.04	0.001	0.44	0.75	0.03

[a-c] superscripts within columns indicate significant differences.
* mean ± standard error.
MOT = motile sperm.
PMOT = progressive motile sperm.
VAP = average path velocity.
VSL = straight-line velocity.
VCL = curvilinear velocity

Table 2 Effect of sericin adding into semen extender on viability (mean±SE) of post-thawed Thai native chicken sperm.

Treatment	Live (SYBR-14 +) (%)	Dead (PI+) (%)
T1	49.01±5.55	50.99±5.55
T2	49.57±7.53	50.43±7.53
T3	50.84±4.95	49.16±4.95
T4	51.07±4.31	48.93±4.31
P-value	0.97	0.97
CV	11.80	11.86

REFERENCES

Burrows, W.H., and J.P. Quinn. 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry. Sci.16:19–24.

Blesbois, E. 2007. Current status in avian semen cryopreservation.World Poult. Sci. J. 63:213–222.

Cerolini, S., K.A. Kelso, R.C. Noble, B.K. Speake, F.Pizzi and L.G. Cavalchini. 1997. Relationship betweenspermatozoan lipid composition andfertility during aging of chickens. Biol. Reprod. 57:976–980.

Donoghoue, A.M. and Wishart. G.J. ,2000. Storage of poultry semen. Anim. Reprod. Sci. 62:213– 232.

Dorji, P., T. Vongprolub, V. Pattarajinda, V. Chankitisakul and P. Chuawongboon. 2015. Effect of sericin supplement in Tris-egg Yolk Citrate extender on quality of ryopreserved semen from thai native bulls. BJNRD, 2(1): 11-18.Donoghue, A.M., and

Guthrie, H.D. and G.R. Welch. Determination of intracellular reactive oxygen species and High mitochondrial membrane potential in percoll- treated viable boar sperm using fluorescence-activate flow cytometry. J. Anim. Sci. 2006; 84: 2089-2100.

Kato, N., S. Sato, A. Yamanaka, H. Yamada, N. Fuwa and M. Nomura. 1998. Silk protein sericin inhibits lipid peroxidation and tyrosinase activity. Biosci. Biotechnol. Biochem. 62: 145-147.

Kumar, P., D. Kumar, P. Sikka and P. Singh. 2015. Sericin supplementation improves semen freez ability of buffalo bulls by minimizing oxidative stress during cryopreservation. Animal Reproduction Science. 152: 26–31.

Long, J.A. 2006. Avian semen cryopreservation: What are the biological challenges ?.Poult. Sci. 85:232–236.

Mazur, P., I. Katkov, N. Katkova and J. Cristar. 2000. The enhancement of the ability of mouse sperm to survive freezing and thawing by the use of high concentrations of glycerol and the presence of an Escherichia coli membrane preparation (oxyrase) to lower the oxygenconcentration. Cryobiology, 40: 187-209.

Partyka, A., W. Nizanski and E. Lukaszewicz. 2010. Evaluation of fresh and frozen-thawed fowl semen by flowcytometry. Theriogenology 74:1019–1027.

Pushpa, A., B.V.G. Vishnu and T. Reddy K.S. Preparation of nano silk Sericin based hydrogels from silk industry wast. J. Environ. Res. Develop. 2013; 8: 243-253.

Santiago-Moreno, J., C. Castaño, A. T. oledano-Díaz, M. A. Coloma, A. López-Sebastián, M. T. Prieto, and J. L. Campo. 2012. Cryoprotective and contraceptive properties of egg yolk as an additive in rooster sperm diluents. Cryobiology 65:230–234.

Sarovant, S., B. Sudatis, P. Meesilpa, B.P. Grady and R. Magaraphan. The use of sericin as an antioxidant and antimicrobial for polluted air treatment. Rev. Adv. Mater. Sci. 2003; 5:193-198.

Schramm, G.P. 1991. Suitability of different antifreeze agents for cryoprotection of cock sperm. Monatsh. Veterinarmed. 46:438–440.

Steel, R. G. D. and J. H. Torrie. 1980. Principle and procedures of statistics: A Biomaterial Approach (2ndEd). McGraw Hill Book Co., New York, U.S.A.Vongpralub, T., A.

Utha, J. Pongpeng, P. Sonseeda and W. Yindee. 2011. A comparison of Simple vapour method and programmable freezer on motility and fertility of frozen Thai native chicken semen. Pages 649–652 in Proceedings of the 3rdInternational Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2011), Nakhon Ratchasima, Thailand.

Wei, T., M. Z. Li and R. J. Xie, 2005. Preparation and structure of porous silk sericin materials. En. 290: 188–194. gMacromol. MaterBiochem. 62: 145-147.

PO-04-22

Regulation of sperm capacitation by molecules present in the female oviduct

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After ejaculation, mammalian sperm have to be capacitated in oviduct (Yanagimachi, 1994). Generally, only capacitated sperm are able to be fertilized to egg. In many mammals, capacitated sperm exhibit "acrosome reaction" and "hyperactivation" as an obvious phenotype. The acrosome reaction is a specialized exocytosis occurring at the sperm head, and is required for penetration of the zona pellucida and binding to egg (Yanagimachi, 1994). During capacitation, mammalian sperm show motility change, such as from "activation" to "hyperactivation" (Yanagimachi, 1994; Mohri et al., 2012; Fujinoki et al., 2016). At first, mammalian sperm are activated. Activated sperm show a small bend amplitude in flagellar movement and swim linearly. After incubation for some hours (4 hours in many rodent sperm), most of motile spermatozoa show hyperactivated motility. However, movement pattern of hyperactivated sperm basically depends on mammals (Yanagimachi, 1994; Fujinoki et al., 2016). In hamster and mouse, hyperactivated sperm show a large amplitude and a large asymmetric beating pattern in flagellar movement and sometimes writhe and swim in the form of eight characters (Yanagimachi, 1994; Fujinoki et al., 2016). In rat, hyperactivated sperm show the large amplitude of head, the arched movement of middle peace of flagellum and the decreasing of progressive movement (Yanagimachi, 1994; Fujinoki et al., 2016).

In the oviduct, there are many molecules that have physiological functions, for example progesterone, estradiol, melatonin, serotonin and gamma-aminobutyric acid (GABA) and so on (Coy et al., 2012). It has been known well that those molecules induce or suppress acrosome reaction of mammalian sperm (Baldi et al., 2009; Fujinoki 2009; Coy et al., 2012). Although recent studies suggested that some oviductal molecules affected mammalian sperm hyperactivation, it was not known well whether they affect hyperactivation of mammalian sperm (Fujinoki 2009; Coy et al., 2012; Fujinoki et al., 2016). So, I examined whether these molecules affected sperm hyperactivation in my recent studies (Noguchi et al., 2008; Fujinoki 2008; Fujinoki 2010; Fujinoki 2011; Kon et al., 2014; Fujinoki and Takei 2015). In the present study, I discuss whether they competitively or cooperatively regulate sperm capacitation based on results obtained from my recent studies (Noguchi et al., 2008; Fujinoki 2008; Fujinoki 2010; Fujinoki 2011; Kon et al., 2014; Fujinoki and Takei 2015).

I used syrian hamster (*Mesocricetus auratus*) as model animal because hyperactivation of syrian hamster sperm is recognized clearly (Noguchi et al., 2008; Fujinoki 2008; Fujinoki 2010; Fujinoki 2011; Kon et al., 2014; Fujinoki and Takei 2015). Sperm were obtained from caudal epididymis of matured male hamster, and were suspended in the mTALP medium to swim up. After swim up at room temperature, they were incubated for 4 hour at 37ºC under 5% (v/v) CO_2 in air to be capacitated. Motile sperm were observed by a video-microscope with phase contrast illumination unit. After observation, sperm capacitation were analyzed. I used hyperactivation as capacitation index because hyperactivation is analyzed by observation of intact sperm.

In syrian hamster, progesterone, melatonin and serotonin enhanced sperm hyperactivation in a dose dependent manner (Noguchi et al., 2008; Fujinoki 2008; Fujinoki 2011). Progesterone and melatonin enhanced sperm hyperactivation through membrane progesterone receptor and melatonin receptor type 1, respectively (Noguchi et al., 2008; Fujinoki 2008). Effective concentrations of progesterone in sperm hyperactivation were 10 ng/ml to 40 ng/ml, and most effective concentration of it was 20 ng/ml (Noguchi et al., 2008). Effective concentrations of melatonin were 1 pM to 1 μM (Fujinoki 2008). In contrast, serotonin dose-dependently affected sperm hyperactivation through two types of serotonin receptor (5HT receptor) (Fujinoki 2011). Low concentration (fM to pM) of serotonin enhanced sperm hyperactivation through $5HT_2$ receptor. While high concentration (pM to nM) of serotonin enhanced sperm hyperactivation through $5HT_4$ receptor. On the other hand, estradiol and GABA suppressed enhancement of sperm hyperactivation in a dose dependent manner (Fujinoki 2010; Kon et al., 2014; Fujinoki and Takei 2015). Estradiol at 2 ng/ml and 20 ng/ml suppressed enhancements of sperm hyperactivation by progesterone and melatonin (Fujinoki 2010; Fujinoki and Takei 2015). Negative effects of estradiol on enhancement of sperm hyperactivation by progesterone and melatonin were associated with membrane estradiol receptor. GABA at 5 nM and 5 μM suppressed enhancements of sperm hyperactivation by progesterone although it did not suppress enhancement of sperm hyperactivation by melatonin (Kon et al., 2014; Fujinoki and Takei 2015). Negative effect of GABA on enhancement of sperm hyperactivation by progesterone was associated with $GABA_A$ receptor/Cl^- channel. As shown in Fig. 1, I investigated interactions among progesterone, melatonin,

serotonin, estradiol and GABA on sperm hyperactivation. Progesterone, melatonin and serotonin act as enhancer of sperm hyperactivation. And estradiol and GABA act as suppressor of progesterone-enhanced and melatonin-enhanced hyperactivation. However, in present study, I did not show interactions among serotonin, estradiol and GABA. In future studies, I will examine interactions among them.

Generally, concentration of progesterone increases after ovulation (Schillo, 2009). While concentration of estradiol increases before ovulation and decreases after ovulation (Schillo, 2009). Because progesterone acts as enhancer of sperm hyperactivation and estradiol acts as suppressor of progesterone-enhanced hyperactivation, it is assume that enhancement of sperm hyperactivation is suppressed by suppressor before ovulation and sperm hyperactivation is enhanced by enhancer after ovulation (Fujinoki et al., 2016). In addition, GABA concentration in oviduct is high before ovulation and is low after ovulation (Louzan et al., 1986). Melatonin is released from ovary with follicular fluid at ovulation (Brzezinski et al., 1987; Rönnberg et al., 1990). Moreover, serotonin products in cumulus cells (Dubé and Amireault 2007). Because GABA was suppressor and melatonin and serotonin were enhancer, their effects also support above assumption (Fujinoki et al., 2016). As shown in Fig. 2, therefore, pre-ovulation during sexual receptivity is suppressor dominant oviductal condition whereas post-ovulation is enhancer dominant oviductal condition. When females meet males during sexual receptivity, females copulate with males (Schillo, 2009). However, females do not always copulate with males at ovulation period. Thus, I speculate that sperm need to be capacitated to be fertilized according to ovulation and maturation of eggs.

In conclusion, I showed that progesterone, melatonin and serotonin were an enhancer of sperm hyperactivation and estradiol and GABA were a suppressor of above enhancers in Syrian hamster. Generally, those molecules are secreted from the ovary and/or the cumulus cell-oocyte-complex (Schillo, 2009). Because their secretion are associated with the estrous cycle, it is assumed that sperm are capacitated monitoring concentration of these molecules.

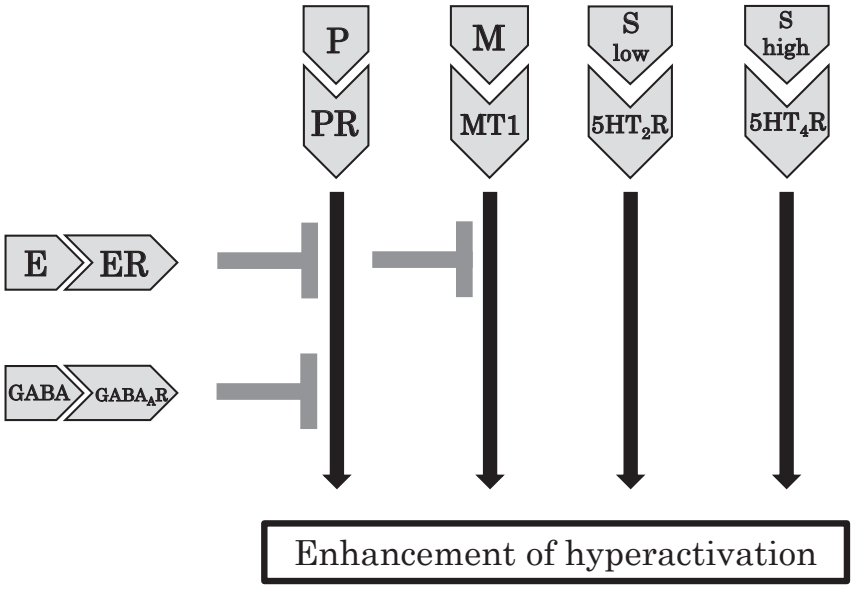

Figure 1 Scheme of interactions among progesterone, estradiol, melatonin, serotonin and GABA on enhancement of sperm hyperactivation. P: progesterone, PR: progesterone receptor, M: melatonin, MT1: melatonin receptor type 1, S low: low concentration of serotonin, S high: high concentration of serotonin, $5HT_2R$: $5HT_2$ receptor, $5HT_4R$: $5HT_4$ receptor, E: estradiol, ER: estrogen receptor, GABA: gamma-aminobutyric acid: $GABA_AR$: $GABA_A$ receptor/Cl^- channel.

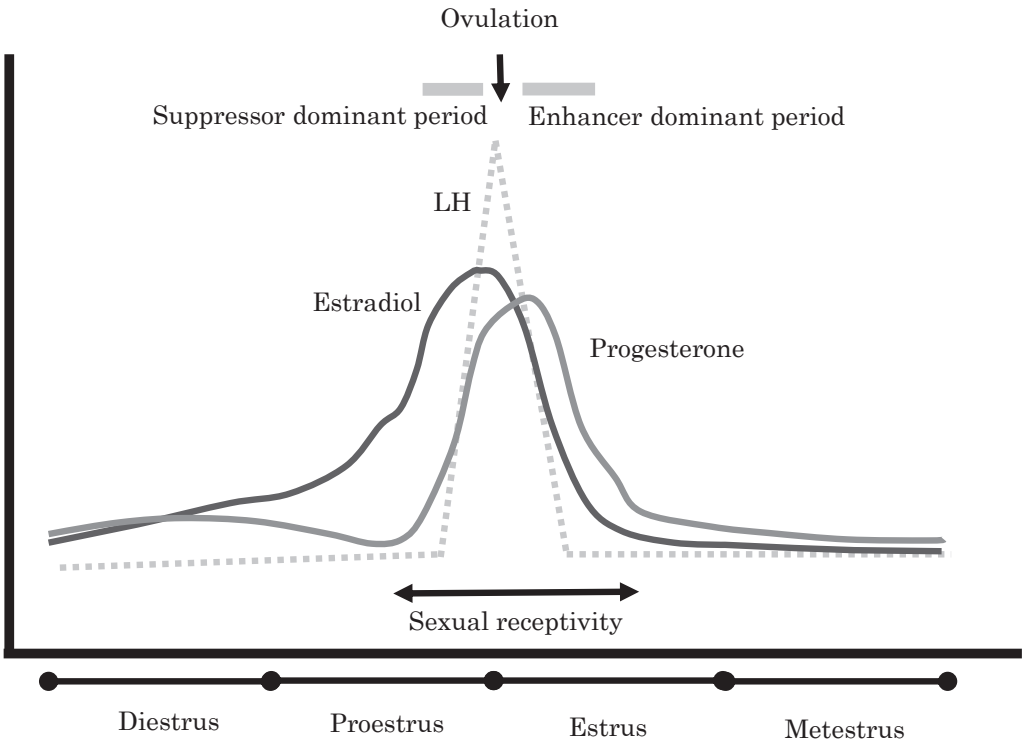

Figure 2 Scheme of relationships between estrous cycle and suppression and enhancement of sperm hyperactivation.

Reference

Baldi E et al. 2009 Nongenomic activation of spermatozoa by steroid hormones: facts and fictions. *Mol Cell Endocrinol* 308: 39–46.

Brzezinski A et al. 1987 Melatonin in human preovulatory follicular fluid. *J Clin Endocrinol Metabol* 64: 865-867.

Coy P et al. 2012 Roles of the oviduct in mammalian fertilization. *Reproduction* 144: 649-660.

Dubé F and Amireault P 2007 Local serotonergic signaling in mammalian follicles, oocytes and early embryos. *Life Sci* 81 1627-1637.

Fujinoki M 2008 Melatonin-enhanced hyperactivation of hamster sperm. *Reproduction* 136: 533-541.

Fujinoki M 2009 Non-genomic regulation of mammalian sperm hyperactivation. *Reprod Med Biol* 8: 47-52.

Fujinoki M 2010 Suppression of progesterone enhanced hyperactivation in hamster spermatozoa by estrogen. *Reproduction* 140: 453-464.

Fujinoki M 2011 Serotonin-enhanced hyperactivation of hamster sperm. *Reproduction* 142: 255-266.

Fujinoki M and Takei GL 2015 Estrogen suppresses melatonin-enhanced hyperactivation of hamster spermatozoa. *J Reprd Dev* 61: 287-295.

Fujinoki M et al. 2016 Non-genomic regulation and disruption of spermatozoal in vitro hyperactivation by oviductal hormones. *J Physiol Sci* 66 207–212.

Kon H et al. 2014 Suppression of progesterone-enhanced hyperactivation in hamster spermatozoa by γ-aminobutyric acid. *J Reprod Dev* 60: 202-209.

Louzan P et al. 1986 Gamma-aminobutyric acid in the genital tract of the rat during the oestrous cycle. *J Reprod Fertil* 77: 499-524.

Mohri H et al. 2012 Tubulin-dynein system in flagellar and ciliary movement. *Proc Jpn Acad*, Ser B 88: 397-415.

Noguchi T et al. 2008 Regulation of hyperactivation of hamster spermatozoa by progesterone. *Reprod Med Biol* 7: 63-74.

Rönnberg L et al. 1990 Circadian and seasonal variation in human preovulatory follicular fluid melatonin concentration. *J Clin Endocrinol Metabol* 71: 493-496.

Schillo KK 2009 Reproductive Physiology of Mammals: From Farm to Field and Beyond. Delmar, New York.

Yanagimachi R. 1994 Mammalian fertilization. *In*: Knobil E, Neill JD (ed.), The Physiology of Reproduction Vol. 2, 2nd ed. Raven Press, New York: pp189-317.

PO-04-23 Both estrone and estriol non-genomically suppress luteinizing hormone secretion from the bovine anterior pituitary cells via the estradiol receptor GPR30

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Introduction

During the luteal phase of the estrous cycle, nanomolar level of estradiol (E2) binds to nuclear-localized estrogen receptors α or β (ER α or ER β), and alters gene transcription in the hypothalamus and the anterior pituitary (AP). This induces important feedback effects that control gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) levels. However, E2 also binds with a plasma membrane receptor in the AP, thereby suppressing LH, but not follicle stimulating hormone (FSH), in a rapid, non-genomic manner (Arreguin-Arevalo and Nett, 2005; Iqbal et al., 2007).

G protein-coupled receptor 30 (GPR30; or G protein-coupled estrogen receptor 1) is a plasma membrane E2 receptor (Maggiolini and Picard, 2010). GPR30 of cattle gonadotropes contributes to rapid negative E2 feedback regulation of GnRH-induced LH secretion (Rudolf and Kadokawa, 2013; Nakamura et al. 2015). Physiological concentrations of selective agonists of ER α and ER β (Arreguin-Arevalo and Nett, 2005), and both physiological and pharmacological concentrations of selective agonists of another putative membrane receptor, the STX receptor, have no rapid effect on GnRH-stimulated LH release from AP cells (Rudolf and Kadokawa, 2014). Therefore, GPR30 is the only clearly identified gonadotrope membrane E2 receptor and its study is important for understanding reproduction in animals.

We recently reported that GPR30 in bovine AP cells mediates the production of picomolar concentrations of E2. In addition, we found that the nonsteroidal mycoestrogen, zearalenone (ZEN), and ZEN's five metabolites, suppressed GnRH-induced LH secretion non-genomically (Nakamura et al., 2015; Nakamura and Kadokawa, 2015). The relative strength of the non-genomic inhibiting effects caused by ZEN or its five metabolites differed from the relative strength of their genomic effects. Therefore, it is possible that any estrogen-like chemical, even if it exhibits only a weak genomic effect via ER α or ER β, would suppress LH secretion strongly via GPR30.

Estrone (E1) and estriol (E3) are the weaker endogenous agonist for ER α and ER β than E2. Therefore, this study was designed to evaluate the hypothesis that GPR30 bound to E1 or E3 suppresses, non-genomically, the GnRH-induced release of LH from bovine AP cells in vitro.

Materials and Methods

Effects of E2, E1, and E3 on GnRH-induced LH secretion from bovine AP cells

All experiments were approved by the Committee on Animal Experiments of the School of Veterinary Medicine, Yamaguchi University.

APs were obtained from post-pubertal Japanese Black heifers (n = 8, 26 months of age). Each experiment began with enzymatic dispersal of AP cells using a method described previously (Suzuki et al., 2008). The dispersed cells were suspended in phenol red-free Dulbecco's modified Eagle's medium (DMEM) containing 1% nonessential amino acids, 100 IU/mL penicillin, 50 μg/mL streptomycin, 10% horse serum, and 2.5% fetal bovine serum. Horse serum and fetal bovine serum had been treated previously with dextran-coated charcoal to remove steroid hormones. Cells were plated in 24-well culture plates and maintained at 37°C in a humidified atmosphere of 5% CO_2 for 82 h. The wells were washed twice with PBS and then incubated with 490 μL of DMEM containing 0.1% BSA for 2 h. Cell monolayers were then pre-treated with 5 μL of DMEM alone, 5 μL of DMEM containing 0.1–1000 nM of E2, E1, or E3 for 5 min with gentle shaking. Cells were then treated for 2 h with 5 μL of 100 nM GnRH dissolved in DMEM to stimulate LH secretion. Pre-treatment with 0.1, 1, 10, 100, or 1000 nM of E2, E1, or E3 resulted in final concentrations, after GnRH treatment, of 0.001, 0.01, 0.1, 1, or 10 nM, respectively. The final concentration of GnRH was 1 nM in all treatments (Kadokawa et al., 2014), except in the control wells that were pre-treated with 5 μL of DMEM for 5 min and then with 5 μL of DMEM without GnRH for 2 h. The GnRH wells were pre-treated with 5 μL of DMEM for 5 min and then incubated with GnRH for 2 h. After incubation with or without GnRH for 2 h, all media were collected for LH assay.

Analysis of the effects of G36 on E2-, E1-, or E3-induced suppression of GnRH-induced LH secretion from bovine AP cells

APs were obtained from post-pubertal Japanese Black heifers (n = 11, 26 months of age). AP cells were dispersed, plated, and incubated at 37°C in 5% CO_2 for 82 h. After washing with PBS, the cell monolayers were incubated with 485 μL of DMEM containing 0.1% BSA for 2 h. Cells were pre-treated with 5 μL of DMEM with or without the GPR30 antagonist, G36 (final concentration, 0.01 nM), for 5 min with gentle shaking. Subsequently, 5 μL of DMEM with or without E2, E1, or E3 (final concentration of each, 0.01 nM) was added to each well for 5 min with gentle shaking. This was followed by incubation with 5 μL of 100 nM GnRH in DMEM (final concentration, 1 nM) for 2 h to stimulate LH secretion. After 2 h of incubation, media were collected for analysis of LH.

Effects of E2, E1, and E3 on the cAMP increment in cultured AP cells

cAMP is the key molecule in the cytoplasmic pathway to increase LH secretion from ovine gonadotrophic cells, by modulating Ca2 + -activated K + channels (Adams et al., 1979; Sikdar et al., 1989). Our previous study reported the suppressive effects of E2 pre-treatment on the cAMP increment in cultured AP cells when the E2 pre-treatment suppressed LH secretion non-genomically (Nakamura et al., 2015). Therefore, cAMP was measured in cultured AP cells in the presence and absence of pre-treatment with E2, E1, or E3.

APs were obtained from post-pubertal Japanese Black heifers (n = 6, 26 months of age). After enzymatic dispersal, AP cells were cultured for 82 h. After washing with PBS, the cells were incubated with 490 μL of DMEM containing 0.1% BSA for 2 h. Cells were pre-treated with 5 μL of DMEM alone or with 5 μL of DMEM containing either E2, E1, or E3 (final concentration of each, 0.01 nM). After gently shaking for 5 min, cells were treated for 2 h with 5 μL of 100 nM GnRH (final concentration, 1 nM, except the "control") dissolved in DMEM containing dopamine (0.5 μM) and phosphodiesterase inhibitor [0.5 mM 3-isobutyl-1-methyl-xanthine (MIX)]. Dopamine and MIX are required to measure cAMP in cultured gonadotrophic cells from heterogeneous AP cells because the amount of cAMP in lactotrophs fluctuates; moreover, phosphodiesterase decreases the amount of cAMP to below the detection limit (Adams et al., 1979). The concentrations of dopamine and MIX used in the present study were identical to those used in a previous investigation of cAMP in cultured ovine or bovine AP cells (Adams et al., 1979; Nakamura et al., 2015). After 2 h of treatment, the wells were washed twice with PBS and then used for cAMP extraction with a cAMP Select EIA Kit (Cayman Chemical, USA) according to the manufacturer's protocol and following our previous study (Nakamura et al., 2015).

Effects of E2, E1, and E3 on mRNA levels of LHα, LHβ, and FSHβ

Our previous study reported no significant effect of E2 pre-treatment on the expression of LHα, LHβ, and FSHβ mRNA in cultured AP cells when the suppression of LH secretion occurred non-genomically (Nakamura et al., 2015). Therefore, gene expression was measured in cultured AP cells in the presence and absence of pre-treatment with E2, E1, and E3. APs were obtained from post-pubertal Japanese Black heifers (n = 6, 26 months of age). After enzymatic dispersal, AP cells were cultured for 82 h. After washing with PBS, the cells were incubated with 490 μL of DMEM containing 0.1% BSA for 2 h. Cells were pre-treated with 5 μL of DMEM alone or 5 μL of DMEM containing either E2, E1, and E3 (final concentration of each, 0.01 nM). After gently shaking for 5 min, cells were treated for 2 h with 5 μL of 100 nM GnRH (final concentration, 1 nM, except the "control") dissolved in DMEM. After 2 h of incubation, total RNA was extracted from the wells. Potentially contaminating genomic DNA was digested with DNase I. cDNA was synthesized from 2 μg of RNA from each sample in 20 μL reactions with random hexamer primers. cDNA samples were used for qRT-PCRs for LHα, LHβ, and FSHβ, and 2 housekeeping genes, chromosome 2 open reading frame 29 (C2orf29) and suppressor of zeste 12 (SUZ12). Details of the qRT-PCR assay were described in our previous paper (Nakamura et al., 2015). The amounts of LHα, LHβ, and FSHβ mRNA were normalized to the geometric means of C2orf29 and SUZ12 expression.

Data analysis

For each pituitary gland, the mean concentration of LH in the control samples was set at 100%. For samples obtained from each pituitary gland, the mean LH concentration after each treatment was expressed as a percentage of the control value. In order to evaluate the effects of treatment with various concentrations of E2, E1, or E3, the statistical significance of differences in LH concentrations was determined using ANOVA with concentrations of LH as the dependent variable and the different concentrations of E2, E1, or E3 as the

independent variable. Post-hoc comparisons were done using Fisher's protected least significant difference (PLSD) test. In order to evaluate the effects of G36 on E2-, E1-, or E3 -induced suppression of GnRH-induced LH secretion from bovine AP cells, differences in LH concentrations between treatments were analyzed using ANOVA with LH concentration as the dependent variable and treatment as the independent variable, followed by Fisher's PLSD test. The mean concentration of cAMP and also the amounts of LHα, LHβ, and FSHβ mRNA in the treated samples for each of the pituitary glands were averaged, and the means were expressed as percentages of the control value. The statistical significance of differences in gene expression was analyzed by using one-factor ANOVA, followed by Fisher's PLSD test. The level of significance was set at $P < 0.05$. Data are expressed as means ± SEM.

Results

As shown in Figure 1, GnRH-stimulated LH secretion from AP cells was inhibited by 1 to 100 pM of E2, 1 to 10 pM of E1, 1 pM to 1 nM of E3.

Pre-treatment for 5 min with the GPR30-specific antagonist, G36, inhibited the E2, E1 or E3 suppression of LH secretion from cultured AP cells (Figure 2). G36 alone had no significant effect on LH secretion.

E2, E1 and E3 decreased cytoplasmic cAMP (Figure 3), but not gene expression of LHα, LHβ and FSHβ subunits (Figure 4).

Discussion

In the present study, both E1 and E3, as well as E2, suppressed LH secretion from cultured bovine AP cells rapidly. In addition, these chemicals decreased cytoplasmic cAMP, but not the expression of LHα, LHβ, and FSHβ mRNA. Therefore, these results suggest that both E1 and E3, as well as E2, non-genomically suppress LH secretion from bovine AP cells.

We used 0.01 nM of G36 in this study because of our previous work showing that this concentration inhibited the suppression of LH secretion from bovine AP cells induced by both 0.01 nM E2 and 0.01 nM ZEN (Nakamura and Kadokawa, 2015). We also reported that 0.01 nM G36 alone had no significant effect on LH secretion (Nakamura and Kadokawa, 2015). In the present study, treatment with 0.01 nM E1 or E3 suppressed LH secretion, which was inhibited by 0.01 nM G36. G36 alone again had no significant effect on LH secretion. E2 affects various pathways in GnRH neurons and induces cross-talk between cell surface receptors, GPR30, and the nuclear receptors ERα and ERβ (Terasawa and Kenealy, 2012). When viewed in the context of this recent study, data from the present study suggest that GPR30 plays an important role in suppressing LH secretion, but that ERα and ERβ are also involved in reducing its secretion.

Conclusion

Therefore, these results suggested that both E1 and E3 non-genomically suppress LH secretion from the bovine AP cells via GPR30.

Figure legends

Figure 1. The effects of various concentrations of (A) E2, (B) E1, or (C) E3 in media containing 1 nM GnRH on LH secretion from cultured bovine AP cells. + P < 0.05 and + + P < 0.01 compared to control. *P < 0.05 and **P < 0.01 compared to GnRH alone.

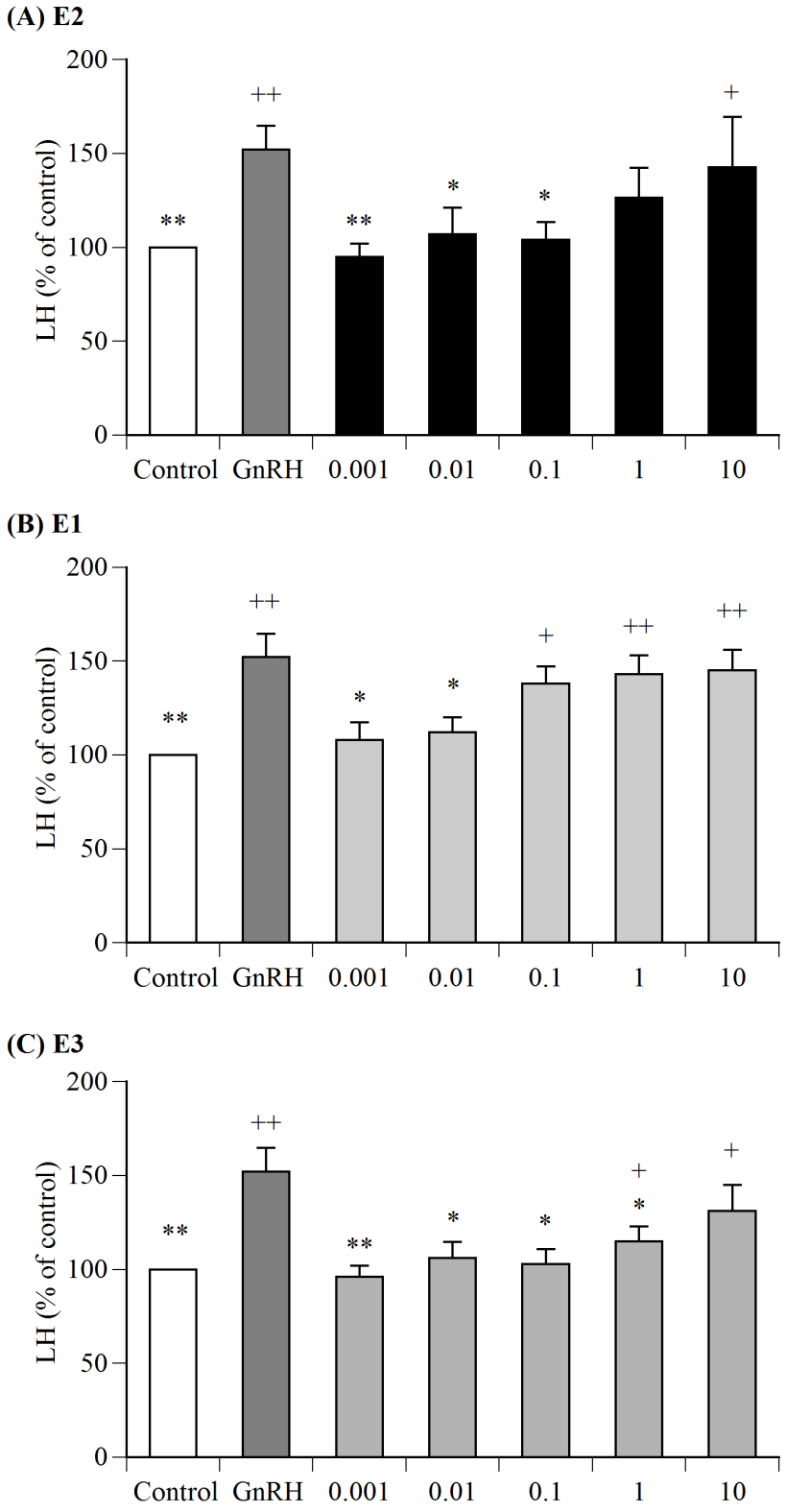


Figure 2. The effects of the G protein-coupled receptor 30 antagonist, G36, on E2, E1, or E3 suppression of GnRH-induced LH secretion from cultured bovine AP cells. + P < 0.05 and + + P < 0.01 compared to control. *P < 0.05 and **P < 0.01 compared to GnRH alone.

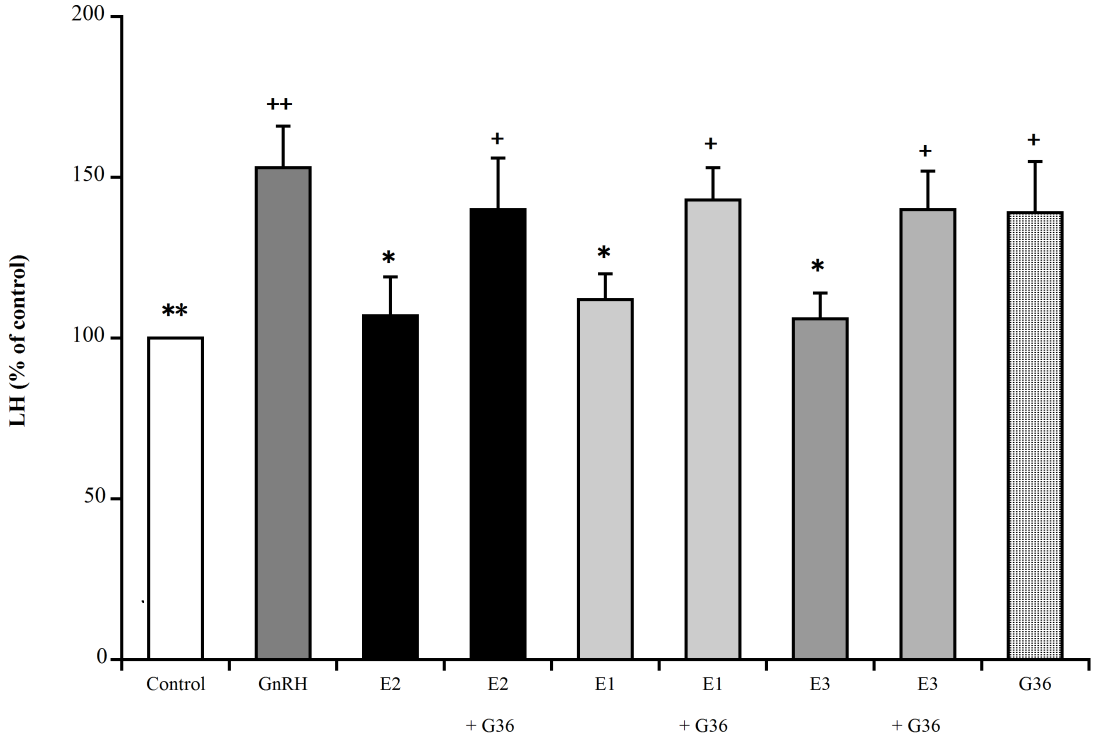


Figure 3. Comparison of the effects of 0.01 nM of E2, E1, or E3 on the cAMP increment in cultured bovine AP cells treated with 1 nM GnRH. + P < 0.05 and + + P < 0.01 compared to control. *P < 0.05 and **P < 0.01 compared to GnRH alone

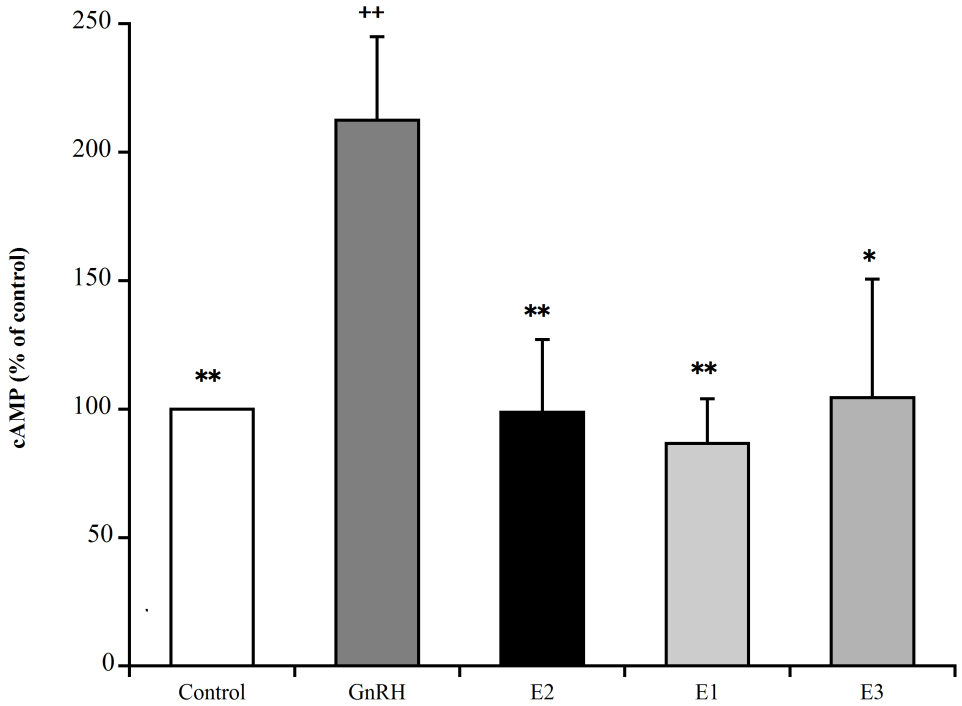
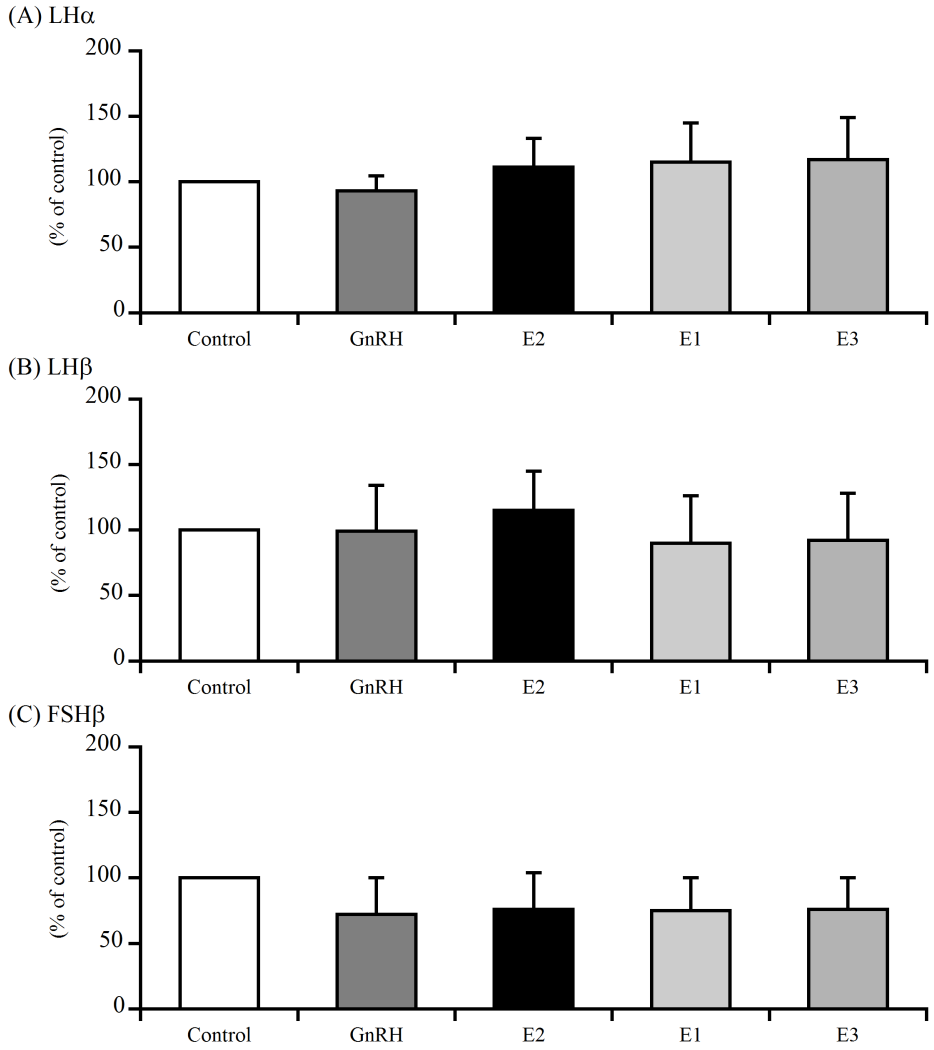


Figure 4. Comparison of the effects of 0.01 nM of E2, E1, or E3 on mRNA expression levels of (A) *LHα* (B) *LHβ*, and (C) *FSHβ* in cultured bovine AP cells treated with 1 nM GnRH. No significant changes were evident.



References

Adams TE, Wagner TO, Sawyer HR, Nett TM. GnRH interaction with anterior pituitary. II. Cyclic AMP as an intracellular mediator in the GnRH activated gonadotroph. Biol. Reprod. 1979;21:735-747.

Arreguin-Arevalo JA, Nett TM. A nongenomic action of 17beta-estradiol as the mechanism underlying the acute suppression of secretion of luteinizing hormone. Biol Reprod 2005;73:115-122.

Iqbal J, Latchoumanin O, Clarke IJ. Rapid in vivo effects of estradiol-17beta in ovine pituitary gonadotropes are displayed by phosphorylation of extracellularly regulated kinase, serine/threonine kinase, and 3′ ,5′ -cyclic adenosine 5′ -monophosphate-responsive element-binding protein. Endocrinology 2007;148:5794-5802.

Kadokawa H, Pandey K, Nahar A, Nakamura U, Rudolf FO. Gonadotropin-releasing hormone (GnRH) receptors of cattle aggregate on the surface of gonadotrophs and are increased by elevated GnRH concentrations. Anim Reprod Sci 2014;150:84-95.

Maggiolini M, Picard D. The unfolding stories of GPR30, a new membrane-bound estrogen receptor. J Endocrinol 2010;204:105-114.

Nakamura U, Rudolf FO, Pandey K, Kadokawa H. The non-steroidal mycoestrogen zeranol suppresses luteinizing hormone secretion from the anterior pituitary of cattle via the estradiol receptor GPR30 in a rapid, non-genomic manner. Anim Reprod Sci 2015;156:118-127

Nakamura U, Kadokawa H. The nonsteroidal mycoestrogen zearalenone and its five metabolites suppress LH secretion from the bovine anterior pituitary cells via the estradiol receptor GPR30 in vitro. Theriogenology 2015;84:1342-1349.

Rudolf FO, Kadokawa H. Expression of estradiol receptor, GPR30, in bovine anterior pituitary and effects of GPR30 agonist on GnRH-induced LH secretion. Anim Reprod Sci 2013;139:9-17.

Rudolf FO, Kadokawa H. Effects of STX, a novel estrogen membrane receptor agonist, on GnRH-induced luteinizing hormone secretion from cultured bovine anterior pituitary cells. J Vet Med Sci 2014;76:1623-1625.

Sikdar SK, McIntosh RP, Mason WT. Differential modulation of Ca2+-activated K+ channels in ovine pituitary gonadotrophs by GnRH, Ca2+ and cyclic AMP. Brain Res. 1989;496:113-123

Suzuki S, Kadokawa H, Hashizume T. Direct kisspeptin-10 stimulation on luteinizing hormone secretion from bovine and porcine anterior pituitary cells. Anim Reprod Sci 2008;103:360-365.

Terasawa E, Kenealy BP. Neuroestrogen, rapid action of estradiol, and GnRH neurons. Front Neuroendocrinol 2012;33:364-375.

PO-04-24 Melamine impairs female fertility via suppressing protein level of Juno in mouse eggs

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Introduction

Melamine (1,3,5-triazine–2,4,6-triamine, or C3H6N6) is a nitrogen heterocyclic triazine compound (Cook et al. 2005; Ingelfinger 2008) which has been widely used as an industrial chemical in many plastics, adhesives, glues, and laminated products such as plywood, cement, cleansers, fireretardant paint, and more (Hau et al. 2009; Ingelfinger 2008). Melamine developed as a chemical in the 1830s, and had varied and widespread legitimate uses. A food safety incident outbroke in China in 2008 which was involved milk and infant formula along with other food materials and components being adulterated with melamine had attracted much attention to the limited usage of melamine (Ingelfinger 2008). Accumulating evidence has revealed that long-term exposure to melamine could damage the reproductive systems in mammals, and lead to male infertility and fetal toxicity in the rat (Bock & Jackson 1957). Also, previous report by us has shown that melamine feeding renders female mice subfertile (Duan et al. 2015).

Fertilization is a culminating event in mammals, involved in two haploid cells, the egg and the sperm. They meet in the female reproductive tract, interact, and finally fuse to become a new, genetically distinct, diploid cell (Bianchi et al. 2014; Bianchi & Wright 2014). Accomplishment of fertilization needs several sequential steps of gamete interaction: Capacitated sperm bind to the zona pellucida surrounding eggs, and then release acrosomal contents by exocytosis and penetrate the ZP. After that, acrosome-reacted sperm reach, bind to and fuse with the egg membrane to form fertilized egg (Wassarman et al. 2004). ZP is a glycoproteinaceous translucent matrix that surrounds the mammalian eggs and embryos, and plays important roles during oogenesis, sperm-egg binding, fertilization and implantation (Avella et al. 2013; Wassarman 1999; Wassarman et al. 2001). This matrix of human is composed of four glycoproteins ZP1, ZP2, ZP3, and ZP4, whereas mouse ZP is composed of ZP1, ZP2 and ZP3 (ZP4 being a pseudogene) (Avella et al. 2013; Gupta et al. 2012). In the supramolecular structure model, sperm-binding site is an N-terminal domain of ZP2 that depends on the cleavage status of ZP2 (Avella et al. 2013; Clark 2011). Subsequent to sperm membrane fusion with oolema, cleavage of ZP2 helps in prevention of polyspermy (Burkart et al. 2012).

Ovastacin is a pioneer component of mammalian cortical granules which belongs to a member of the large astacin family of metalloendoproteases (Bond & Beynon 1995; Dumermuth et al. 1991). This oocyte-specific protein is exocytosed from cortical granules triggered by fertilization and is responsible for post-fertilization ZP2 cleavage to block sperm binding and polyspermy. Thus, in ovastacin-deficient mice, albeit fertilization, sperm are still able to bind to zona pellucida surrounding embryos because ZP2 remains intact, which accordingly, renders ovastacin-deficient females subfertile due to the polyspermy (Burkart et al. 2012). In addition, Fetuin-B has been recently identified as the inhibitor of ovastacin, and genetic ablation of Fetuin-B causes premature ZP hardening and, consequently, female infertility (Dietzel et al. 2013; Stocker et al. 2014). This result shows that premature cleavage of ZP2 can result in infertility in mice.

Glycophosphatidylinositol (GPI)-anchored receptors on the egg surface are essential for fertilization because sperm lacking them render eggs infertile (Alfieri et al. 2003; Coonrod et al. 1999). A breakthrough was made in 2014 when Gavin J. Wright's group identified folate receptor 4 (Folr4) as the Izumo1 receptor, displayed on the surface of egg they named this protein "Juno" after the Roman goddess of fertility and marriage (Bianchi et al. 2014). Juno is expressed on the surface of oocyte, a GPI-anchored protein that is essential for female fertility. Juno-deficient mice are infertile because eggs lacking Juno cannot fuse with normal acrosome reacted sperm (Bianchi et al. 2014; Bianchi & Wright 2014).

Our most recent report indicates that melamine negatively affects female fertility in mice (Duan et al. 2015). Here, we further explore the possible molecular basis at several levels during fertilization, and our data provide the evidence showing that melamine compromises female fertility via regulating the protein level of Juno, rendering eggs non-fusible with sperm.

Materials and Methods

Ethic statement

Our study was approved by the Animal Research Institute Committee guidelines of Nanjing Agricultural University, China. Mice were housed in a temperature-controlled room with proper darkness-light cycles, fed with a regular diet, and maintained under the care of the Laboratory Animal Unit, Nanjing Agricultural University, China. The mice were killed by cervical dislocation.

Animals and feeding treatment

The female 4-week-old ICR mice were housed in separate cages at controlled condition of temperature (20–23°C) and illumination (12h light-dark cycle), and had free access to food and water throughout the period of the study. After 1 week acclimation to the laboratory environment, mice were randomly assigned to 3 groups (n = 40), with an average body weight of 18 g and were each orally given 0, 10 or 50mg/kg/d of melamine dissolved in water for 8 weeks. The animals were observed each three days, and there are no one died during administration.

In vitro fertility

Cauda epididymides were lanced in a dish of human tubal fluid (HTF) medium (EMD Millipore, Billerica, MA) to release sperm, followed by being capacitated for 1 h (37°C, 5% CO2) and added to ovulated eggs at a concentration of 4 x 10^5/ml sperm in 100μl HTF for 5 hr at 37°C, 5% CO2. The presence of two pronuclei was scored as successful fertilization.

Zygote Harvest and Culture

ICR mice (6- to 8-weeks-old) were injected with pregnant mare serum gonadotrophin (PMSG). After 48 hours they were injected with human chorionic gonadotrophin (hCG) and immediately mated with male mice. Zygotes were harvested 18 h later and cultured in K modified simplex optimized medium (KSOM; Chemicon, Billerica, MA, USA) under paraffin oil at 37°Cand 5% CO_2.

Immunofluorescent and confocal microscopy

Ovulated eggs were fixed in 4% paraformaldehyde in PBS (pH 7.4) for 30 minutes and permeabilized in 0.5% Triton-X-100 for 20 min at room temperature. Then, oocytes were blocked with 1% BSA-supplemented PBS for 1 h and incubated at 4°C overnight or at room temperature for 4 h with rat monoclonal anti-mouse folr4 antibody (1:100, BioLegend, CA) or rabbit polyclonal anti-mouse ovastacin antibody (1:100, obtained from Dr. Jurrien Dean). After washing four times (5 min each) in PBS containing 1% Tween 20 and 0.01% Triton-X 100, eggs were incubated with an appropriate secondary antibody for 1 h at room temperature. Alexa Fluor 555 donkey anti-rabbit IgG (H + L) was obtained from Invitrogen (Carlsbad, CA). After washing three times, eggs were stained with PI or Hoechst 33342 (10 μg/ml) for 10 min. Finally, eggs were mounted on glass slides and viewed under a confocal laser scanning microscope (Carl Zeiss 700).

Western blot analysis

Ovulated eggs or two-cell embryos were lysed in 4× LDS sample buffer with 10× reducing reagent (Life Technologies-Invitrogen) and heated at 100°C for 5 min. Proteins were separated on 12% Bis-Tris precast gels, transferred to PVDF membranes, blocked in 5% nonfat milk in TBS (Tris buffered saline, pH 7.4) with 0.1% Tween 20 (TBST) for 1 h at room temperature, and then probed with 1:500 dilution of M2c.2 antibody (obtained from Dr. Jurrien Dean) at 4°C overnight. After washing three times in TBST (10 min each), blots were incubated 1 h with a 1:10,000 dilution of HRP (Horse Radish Peroxidase) conjugated goat anti-rabbit IgG (Santa cruz, Texas). Chemiluminescence was performed with ECL Plus (Piercenet) and signals were acquired by Tanon-3900.

Sperm binding assay

Caudal epididymal sperm were isolated from wild-type ICR mice and placed under oil (Sigma-Aldrich, MO) in HTF medium previously equilibrated with 5% CO2 and capacitated by an additional 1 hr of incubation at 37°C. Sperm binding to ovulated eggs or two-cell embryos isolated from control and melamine-treated mice was observed using capacitated sperm and control two-cell embryos as a negative wash control. Samples were fixed in 4% PFA for 30 min, stained with Hoechst 33342. Bound sperm were quantified from z projections acquired by confocal microscopy, and results reflect the mean ± S.E.M. from at least three independently obtained samples, each containing 10-12 mouse eggs/embryos.

Statistical analysis

The data were expressed as mean ± SEM and analyzed by one-way ANOVA, followed by LSD's post hoc test, which was provided by SPSS16.0 statistical software. The level of significance was accepted as $p<0.05$.

Results

Melamine exposure compromises the *in vitro* fertilization

Melamine feeding model was set up by eight-week diet, and was classified into control (0mg/kg/d), low-dose (10mg/kg/d) and high-dose (50mg/kg/d) groups. To confirm the *in vivo* fertility result reported previously, eggs from three groups were collected and used for *in vitro* fertilization, respectively. As shown in Fig. 1, the fertilization rate of low-dose group is comparable to that of control group (85.0 ± 2.2% VS 81.9 ± 1.4%), but the rate of how-dose group is significantly lower than and low dose (50mg/kg/d) group, the high dose (50mg/kg/d) group was significantly decreased ($P < 0.01$). Because there was no obvious defect on the fertilization in low-dose group, we only compared control and high-dose groups in below experiments.

Melamine does not result in mislocalization and premature exocytosis of ovastacin in eggs

To determine the possible reason causing the failure of fertilization, we first examined the localization and protein level of ovastacin, an oocyte-specific metalloprotease in the cortical granules which is responsible for post-fertilization cleavage of N-terminus of ZP2, because mislocalization and premature release of ovastacin before fertilization in unfertilized eggs would lead to zona hardening so that compromise the fertilization. To validate this, we performed the immunostaining of ovastacin under the same condition in control and melamine fed groups, and measured the immunofluorescent intensity. As shown in Fig. 2, in 0h group ovastacin was localized under the oocyte subcortical region and excluded in cortical granule free domain, however, in 12h and 24h groups both localization and signal intensity of ovastacin were comparable between control and high-dose groups (11.1 ± 0.6% VS 9.9 ± 0.4%), indicating that melamine would not lead to the mislocalization and premature exocytosis of ovastacin.

Melamine does not affect sperm binding ability on eggs

Next, we tested if melamine has effect on the sperm–zona pellucida binding *in vitro*. Based on the fact that sperm bind to the N-terminus of ZP2 in unfertilized eggs but not 2-cell embryos in which has been cleaved by ovastacin released by cortical granules, we set up 2-cell embryos as the negative control for the sperm binding assay. The immunofluorescent analysis showed that the number of sperm binding to the surface of zona pellucida surrounding eggs from both control and high-dose groups is comparable (94 ± 3.45 VS 95.75 ± 4.347) (Fig. 3A, B,C), suggesting that impairment of the fertilization capability of eggs by melamine does not result from the zona binding defect. Because sperm binding to zona is determined by the cleavage status of ZP2, we also performed the western blot using the antibody M2c.2 which recognizes the C-terminus of mouse ZP2. In the control group, ZP2 remained intact in unfertilized eggs and cleaved in 2-cell embryos as expected (Fig. 3D).

Melamine exposure reduces Juno protein level on the egg membrane

Since we have already ruled out the defect on the zona pellucida, we further explored the possible candidates on the egg membrane. Juno is a recently-found receptor on the egg membrane which binds to Izumo1 in the sperm head to mediate the sperm-egg fusion. We performed the immunostaining of Juno, and found that it was evenly distributed on the egg membranes (Fig. 4A). Furthermore, we measured the immunofluorescent intensity of egg membranes in control and high-dose groups, and the result showed that the protein level of Juno in the high-dose group was remarkably lower than that in control group (11.6 ± 0.3 VS 24.2 ± 0.6, $P < 0.01$, Fig. 4B). Thus, the subfertility phenotype induced by melamine is probably caused by the decreased level of Juno on the egg membrane.

Discussion

Melamine (2, 4, 6-triamino-1, 3, 5-triazine), a chemical material, is a widely used industrial chemical that is not considered acutely toxic with a high LD_{50} in animals, and the oral LD_{50} ranges from 3.2 g/kg to 7.0 g/kg in mice (Skinner et al. 2010). However, long-term exposure to melamine could lead to infertility in rat males (Bock & Jackson 1957). Also, our most recent report indicates that melamine negatively affects female fertility in mice (Duan et al. 2015).

In the present study, we further investigated the possible molecular mechanism regarding the effect of melamine

on the fertility of female mice. We found that feeding mice with the melamine-contained diet had no effects on the ovastacin localization and exocytosis, as well as the sperm-zona pellucida binding, but indeed compromised the Juno protein level on the egg membrane, which might be the major cause leading to the female subfertility.

Fertilization is a unique and multi-step event that initiates the onset of development. During mammalian fertilization, capacitated sperm must bind to and penetrate the specialized extracellular matrix of the egg, known as zona pellucida, and then fuse with the oolemma to become the fertilized eggs (Wassarman et al. 2004). The mouse genetic studies have defined the N-terminal domain of ZP2 as the sperm binding site in the zona pellucida, and the sperm binding is determined by the ZP2 cleavage status independent of fertilization (Avella et al. 2013). Following fertilization, ZP2 undergoes proteolytic cleavage by an oocyte-specific astacin-like metalloendoprotease, first reported as ovastacin (citation), released from the cortical granules, and sperm no longer bind to mouse embryos (Bleil et al. 1981). However, if ovastacin is exocytosed during oogenesis or oocyte maturation before fertilization, it will prematurely cleave the N-terminus of ZP2 and result in less or no sperm binding, leading to the fertilization failure. Based on these understandings, we examined the possible reasons that would cause the melamine-induced female subfertility in mice one after another. Normal localization and protein level of ovastacin in melamine exposed eggs indicated that impairment of fertility is not due to the defect of ovastacin. Next, we tested the sperm binding to zona pellucida. Both sperm binding assay and western blot analysis of ZP2 cleavage revealed that melamine does not result in the zona defect.

Juno is an essential cell-surface protein as the receptor for Izumo1 on the plasma membrane of mouse eggs (Bianchi et al. 2014). Juno and Izumo1 play crucial role in sperm-egg fusion in mice (Bianchi et al. 2014; Inoue et al. 2005; Ohnami et al. 2012). In other words, both Juno-deficient females and Izumo1-deficient males are infertile because their gametes cannot fuse to their wild-type partner's cells. Therefore, we aimed Juno as our next candidate. Our immunostaining and signal measurement results showed that high-dose (50mg/kg/d) feeding of melamine to female mice led to significant decrease of Juno protein level on the plasma membrane of unfertilized eggs. This finding consistently interprets the subfertility phenotype, because the small amount of remaining Juno renders some melamine-exposed eggs still fertilizable.

Taken together, we present data here to demonstrate that melamine negatively affects female fertility through suppressing Juno protein level in mice. As for how melamine regulates its protein expression or degradation, it needs the more in-depth investigation in the future.

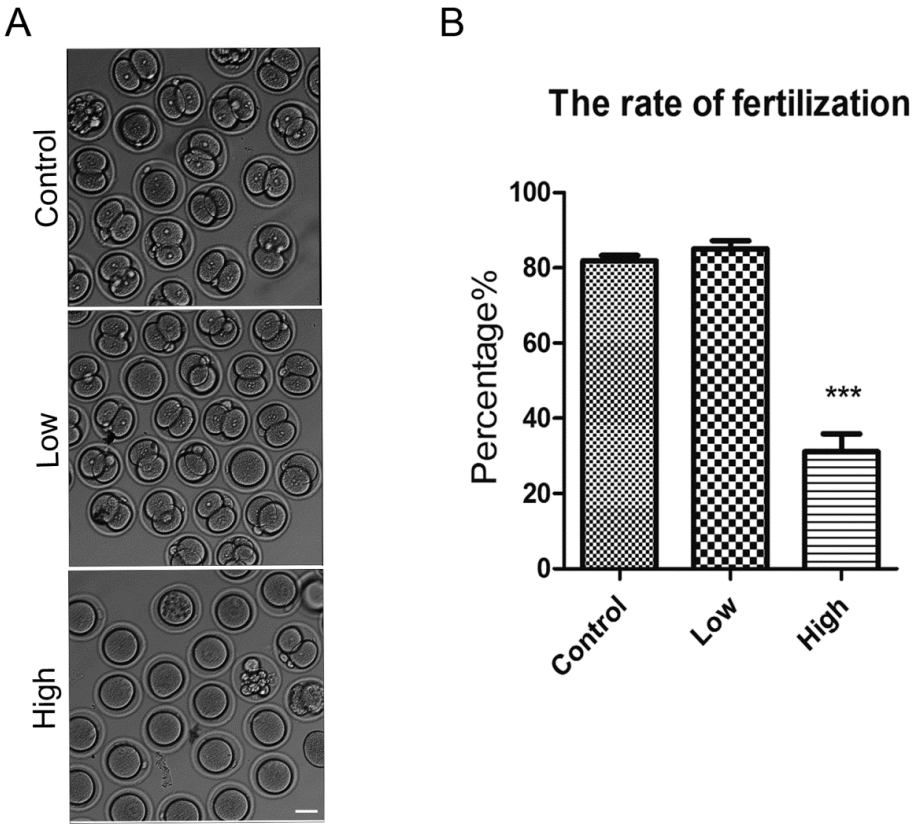

Fig. 1. *In vitro* fertilization of ovulated eggs from melamine fed mice. (A) Representative images of fertilized eggs in control and melamine-treated mice. Most of eggs were not fertilized in high-dose treatment group. Scale bar, 40μm. (B) *In vitro* fertilization rates, control oocytes (n=91), low group (n=112), and high group (n=124). Fertilization was determined by the presence of 2 pronuclei 12 hr after insemination. Data were presented as mean percentage (mean ± SEM) of at least three independent experiments. Asterisk denotes statistical difference at a $P < 0.05$ level of significance.

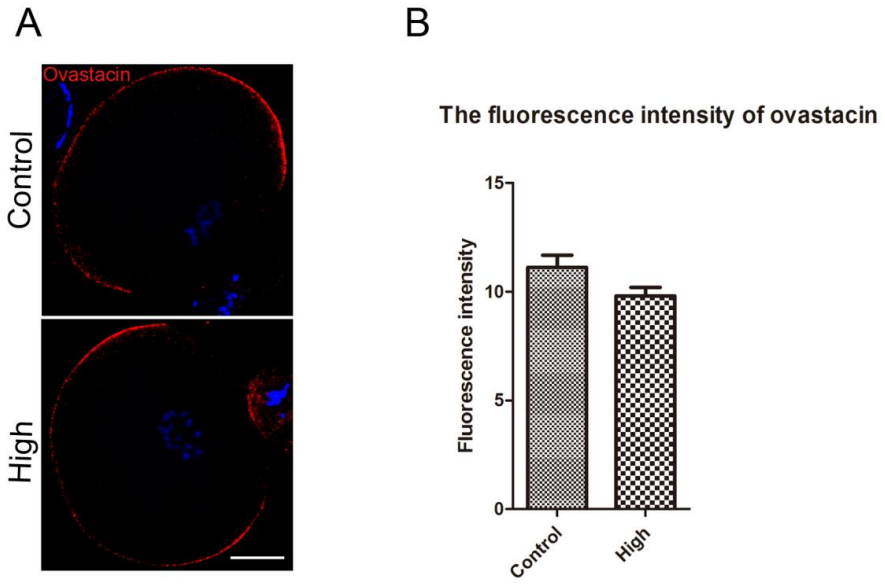

Fig. 2. Effect of melamine feeding on the ovastacin localization and protein level in ovulated eggs. (A) Ovastacin were stained with rabbit polyclonal anti-mouse ovastacin antibody and examined by confocal microscopy. Scale bar, 20μm. (B) Measurement of fluorescent intensity of ovastacin signals. There was no significant difference of ovastacin signals between control (n=15) and treatment (n=15) groups. Data were presented as mean percentage (mean ± SEM) of at least three independent experiments. Asterisk denotes statistical difference at a $P < 0.05$ level of significance.

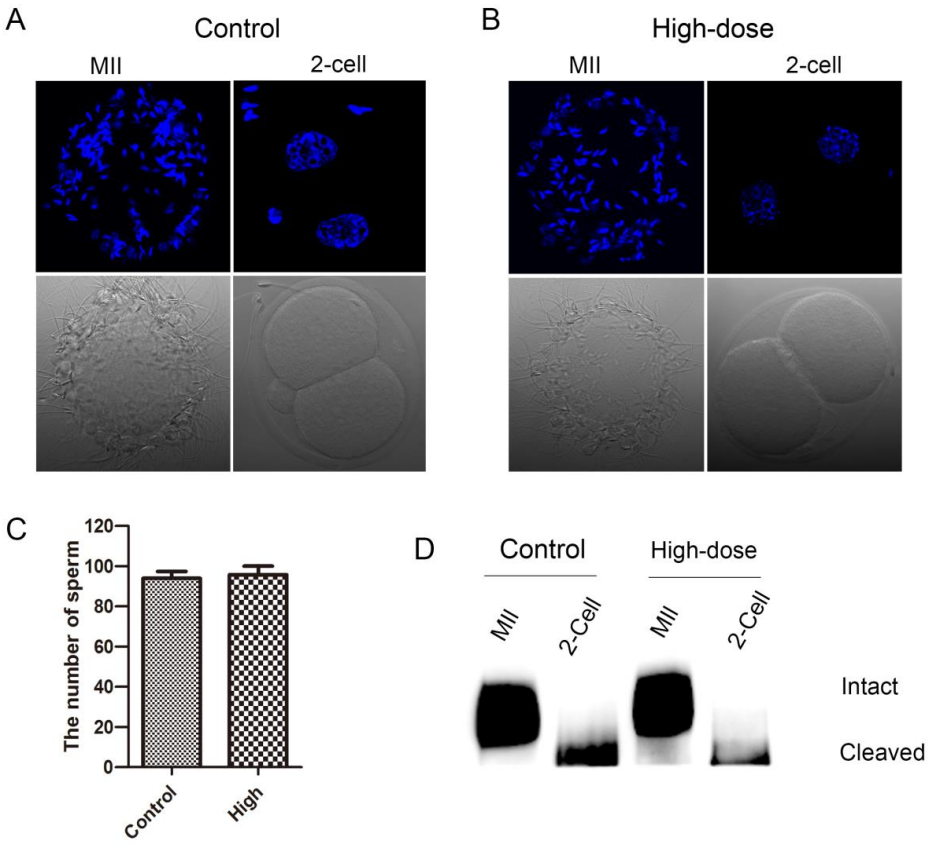

Fig. 3. Sperm binding capability and ZP2 cleavage status. (A, B) Eggs and two-cell embryos from control and melamine fed mice were incubated with capacitated sperm for 1 hr. After washing with a wide-bore pipette to remove all but two to six sperm on normal two-cell embryos (negative control), eggs and embryos with sperm were fixed and stained with Hoechst 33342. Scale bar, 20μm. (C) The number of sperm binding the surface of zone pellucida surrounding eggs. There was no significant difference between control (n=10) and treatment (n=8) groups. (D) Western blot analysis of ZP2 cleavage status in eggs and two-cell embryos using M2c.2 antibody that recognizes the C-terminal domain of ZP2. The size of intact ZP2 is 120 kD, the size of cleaved C-terminal fragment of ZP2 is 90 KD.

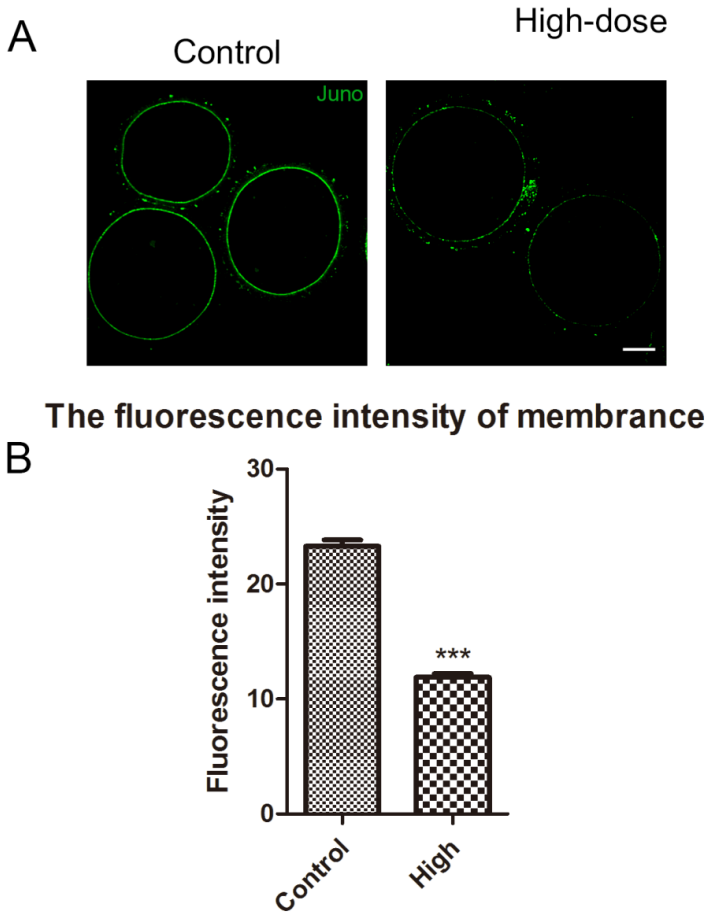

Fig. 4. Effect of melamine feeding on the protein level of Juno in ovulated eggs. (A) Juno was stained with rat monoclonal anti-mouse folr4 antibody and examined by confocal microscopy. Scale bar, 20μm. (B) Measurement of fluorescent intensity of Juno signals. The protein level of Juno in egg membrane was significantly reduced in high-dose group (n=15) compared to control group (n=25). Data were presented as mean percentage (mean ± SEM) of at least three independent experiments. Asterisk denotes statistical difference at a $P < 0.05$ level of significance.

References

Alfieri JA, Martin AD, Takeda J, Kondoh G, Myles DG, and Primakoff P. 2003. Infertility in female mice with an oocyte-specific knockout of GPI-anchored proteins. *J Cell Sci* 116:2149-2155.

Avella MA, Xiong B, and Dean J. 2013. The molecular basis of gamete recognition in mice and humans. *Mol Hum Reprod* 19:279-289.

Bianchi E, Doe B, Goulding D, and Wright GJ. 2014. Juno is the egg Izumo receptor and is essential for mammalian fertilization. *Nature* 508:483-487.

Bianchi E, and Wright GJ. 2014. Izumo meets Juno: preventing polyspermy in fertilization. *Cell Cycle* 13:2019-2020.

Bleil JD, Beall CF, and Wassarman PM. 1981. Mammalian sperm-egg interaction: fertilization of mouse eggs triggers modification of the major zona pellucida glycoprotein, ZP2. *Dev Biol* 86:189-197.

Bock M, and Jackson H. 1957. The action of triethylenemelamine on the fertility of male rats. *Br J Pharmacol Chemother* 12:1-7.

Bond JS, and Beynon RJ. 1995. The astacin family of metalloendopeptidases. *Protein Sci* 4:1247-1261.

Burkart AD, Xiong B, Baibakov B, Jimenez-Movilla M, and Dean J. 2012. Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *J Cell Biol* 197:37-44.

Clark GF. 2011. The molecular basis of mouse sperm-zona pellucida binding: a still unresolved issue in developmental biology. *Reproduction* 142:377-381.

Cook HA, Klampfl CW, and Buchberger W. 2005. Analysis of melamine resins by capillary zone electrophoresis with electrospray ionization-mass spectrometric detection. *Electrophoresis* 26:1576-1583.

Coonrod SA, Naaby-Hansen S, Shetty J, Shibahara H, Chen M, White JM, and Herr JC. 1999. Treatment of mouse oocytes with PI-PLC releases 70-kDa (pI 5) and 35- to 45-kDa (pI 5.5) protein clusters from the egg surface and inhibits sperm-oolemma binding and fusion. *Dev Biol* 207:334-349.

Dietzel E, Wessling J, Floehr J, Schafer C, Ensslen S, Denecke B, Rosing B, Neulen J, Veitinger T, Spehr M, Tropartz T, Tolba R, Renne T, Egert A, Schorle H, Gottenbusch Y, Hildebrand A, Yiallouros I, Stocker W, Weiskirchen R, and Jahnen-Dechent W. 2013. Fetuin-B, a liver-derived plasma protein is essential for fertilization. *Dev Cell* 25:106-112.

Duan X, Dai XX, Wang T, Liu HL, and Sun SC. 2015. Melamine negatively affects oocyte architecture, oocyte development and fertility in mice. *Hum Reprod* 30:1643-1652.

Dumermuth E, Sterchi EE, Jiang WP, Wolz RL, Bond JS, Flannery AV, and Beynon RJ. 1991. The astacin family of metalloendopeptidases. *J Biol Chem* 266:21381-21385.

Gupta SK, Bhandari B, Shrestha A, Biswal BK, Palaniappan C, Malhotra SS, and Gupta N. 2012. Mammalian zona pellucida glycoproteins: structure and function during fertilization. *Cell Tissue Res* 349:665-678.

Hau AK, Kwan TH, and Li PK. 2009. Melamine toxicity and the kidney. *J Am Soc Nephrol* 20:245-250.

Ingelfinger JR. 2008. Melamine and the global implications of food contamination. *N Engl J Med* 359:2745-2748.

Inoue N, Ikawa M, Isotani A, and Okabe M. 2005. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. *Nature* 434:234-238.

Ohnami N, Nakamura A, Miyado M, Sato M, Kawano N, Yoshida K, Harada Y, Takezawa Y, Kanai S, Ono

C, Takahashi Y, Kimura K, Shida T, Miyado K, and Umezawa A. 2012. CD81 and CD9 work independently as extracellular components upon fusion of sperm and oocyte. *Biol Open* 1:640-647.

Skinner CG, Thomas JD, and Osterloh JD. 2010. Melamine toxicity. *J Med Toxicol* 6:50-55.

Stocker W, Karmilin K, Hildebrand A, Westphal H, Yiallouros I, Weiskirchen R, Dietzel E, Floehr J, and Jahnen-Dechent W. 2014. Mammalian gamete fusion depends on the inhibition of ovastacin by fetuin-B. *Biol Chem* 395:1195-1199.

Wassarman PM. 1999. Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* 96:175-183.

Wassarman PM, Jovine L, and Litscher ES. 2001. A profile of fertilization in mammals. *Nat Cell Biol* 3:E59-64.

Wassarman PM, Jovine L, Litscher ES, Qi H, and Williams Z. 2004. Egg-sperm interactions at fertilization in mammals. *Eur J Obstet Gynecol Reprod Biol* 115 Suppl 1:S57-60.

Diagram

PO-04-30 The effect of the grazing grass species on the meat quality grade of the beef carcass in Japan

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Introduction

Beef production systems that feed large amounts of imported grain to cattle in barns are common in Japan. This system produces marbling-rich beef. Recently, Japanese customers have come to demand not only marbling-rich beef, but also lean beef (Japan Meat Information Service Center, 2016), which has increased the need for technical development of high-quality domestic lean beef production in Japan. The authors conducted a research project for lean beef production system development using year-round grazing with domestic roughage such as corn silage and eco-feed (feed made of recycled food waste), similar to condensed barley distillers' product (Nakamura et al., 2010, 2011; Kaneko et al., 2012). In southeastern Japan, especially in low altitude areas of Kyushu, both winter grass (e.g. Italian ryegrass) and summer grass (e.g. Palisadegrass) are necessary for year-round grazing. The intake value and feed components of winter grass and summer grass differ. Additionally, weather conditions differ among seasons. In this area, high temperatures in summer suppress weight gain. Therefore, the meat quality of this beef production system incorporating year-round grazing can be expected to change according to the season when steers are slaughtered.

This study was conducted to clarify the effects of grazing grass species on the meat quality grade of beef carcasses in Japan.

Material and Method

Grazing was conducted at Kyushu Okinawa Agricultural Research Center, Japan (32°53', 130°44', 78 m a.s.l.) during 2012–2015. Six Japanese Brown steers, all around 10 months old, were introduced from a livestock market in October and December of 2012. After one week of acclimation, the steers began grazing on a Bahiagrass (*Paspalum notatum Fluegge*) pasture with supplemental feeding of concentrate. At 5 March 2013, concentrate feeding was stopped. From 29 May 2013, corn silage and Italian ryegrass silage were given as supplemental feed when grass was insufficient. Details of pasture grass and supplemental feeding are presented in Table 1. Three steers were slaughtered on 4 June 2015 after half a year of Italian ryegrass grazing (shown as "IR" below: average live weight, 753 kg; average age, 40 months). The remaining three steers were grazed half a year more at the Bahiagrass pasture and were then slaughtered on 12 November 2015 (shown as "Ba" below: average live weight, 710 kg; average age, 45 months). The meat quality grade of the respective beef carcasses was rated by certified graders in accordance with the standard devised by the Japan Meat Grading Association. We analysed differences between carcasses of the two groups using one-way ANOVA.

Results and Discussion

Results of the yield grade show that the carcass weights of IR were greater than those of Ba, but no significant difference was found. The rib-eye area and the rib thickness of IR were greater than those of Ba. One of the causes of these differences is the difference of body weight. Of the six steers, the three largest were the IR steers, although the grazing period of Ba steers was a half-year longer than those of IR steers. The body weights of the IR steers when the Italian ryegrass grazing finished were 70 kg larger than those of Ba. Although we had expected similar body weights before finishing Bahiagrass grazing during half a year, the body weight of Ba steers had increased only 30 kg. The nutritional value of Bahiagrass appears to be insufficient. Moreover, during Bahiagrass grazing, body weight gain was suppressed because of the summer heat. On the other hand, Schmidt et al. (2014) reported effects on average daily gain, hot carcass weight, and fat thickness according to differences of summer forage species grazing during finishing. Results of this study suggest that these factors, grass species, and weather conditions cause a shortage of the carcass weight and the fat thickness of the Ba carcass.

No significant difference in meat quality grades was found. Results show almost no impact on the meat quality grade according to grass species. Schmidt et al. (2014) reported differences according to summer forage species grazed during finishing: effects were found for the marbling score and lightness of lean meat, but no effect was

found on the colour of lean meat or subcutaneous fat. This study found that the lean meat brightness of IR tended to be higher than that of Ba (P=0.12). The possibility exists that the grass species alters the lean meat brightness. Worldwide, reports of the relevant literature describe differences in chemical components of meat related to taste and health, such as compositions of amino acids and fatty acids, flavour components, and customer sensory evaluation (e.g., French et al., 2000; Schmidt et al., 2014). Further study is necessary to clarify differences of meat quality for Wagyu because of different grass species, and seasons.

Conclusion

Results show that differences of nutritional values of grass and weather condition potentially affected the beef yield grade. Nevertheless, almost no effect on meat quality grade was found according to grass species differences.

Keywords

Japanese Brown Cattle, Meat quality grade, Grass species, Grazing

Table 1 The summery of grazing grass and supplemental feeding.

Date	Grass	Supplemental feeding
From autumn 2012	Bahiagrass with overseeded Italian ryegrass	concentrate
From 3rd Mar 2013	Italian ryegrass	non
From 29th May 2013	Bahiagrass	corn silage (adlibitum)
From 27th Jun 2013	Palisadegrass	non
From 8th Nov 2013	Bahiagrass	corn silage and itarian ryegrass silage (adlibitum)
From 17th Mar 2014	Italian ryegrass	non
From 4th Jun 2014	Palisadegrass, Bahiagrass or Sudengrass	non
From 26th Nov 2014	Italian ryegrass	non
From 20th Feb 2015	Italian ryegrass	corn silage (adlibitum)
From 9th Mar 2015	Italian ryegrass	non
From 2nd Jun 2015 until 11th Nov 2015	Bahiagrass	non

Table 2 Carcass characteristics.

	Grass before Slaughtering		*P*-value
	Italian ryegrass	Bahiagrass	
Components of the yield grade.			
Carcass weight(kg)	434.3 ± 13.7	389.5 ± 38.0	0.13
At the 6-7th rib section			
Rib-eye area (cm^2)	48.7 ± 2.3	41.3 ± 1.2	0.008
Rib thickness (cm)	6.6 ± 0.3	5.4 ± 0.5	0.02
Subcutaneous fat thickness (cm)	2.1 ± 0.5	1.6 ± 0.7	0.32
Components of the quality grade.			
Beef marbling score1	2.7 ± 0.6	3.0 ± 0.0	0.37
BMS No.2	3.0 ± 1.0	3.0 ± 0.0	1.00
Beef color score1	2.7 ± 0.6	2.0 ± 0.0	0.12
BCS No.3	4.7 ± 0.6	5.3 ± 0.6	0.23
Brightness1	2.7 ± 0.6	2.0 ± 0.0	0.12
Maet firmness and texture score1	2.3 ± 0.6	2.0 ± 0.0	0.37
Firmness1	2.3 ± 0.6	2.0 ± 0.0	0.37
Texture4	3.0 ± 0.0	2.7 ± 0.6	0.37
Beef fat score1	3.7 ± 0.6	3.0 ± 0.0	0.12
BFS No.5	5.3 ± 0.6	5.7 ± 0.6	0.51
Luster, and quality1	3.7 ± 0.6	3.0 ± 0.0	0.12

1 Scores, brightness, firmness and luster, and quality: 1 = poor, 5 = very fine.

2 BMS (beef marbling standard) No.: 1 = nothing, 12 = very abundant.

3 BCS (beef color standard) No.: 1 = light, 7 = deep.

4 Texture: 1 = rough, 5 = fine.

5 BFS (beef fat standard) No.: 1 = white, 5 = yellow.

References

French P., Stanton C., Lawless F., O'Riordan EG., Monahan FJ., Caffery PJ. & Maloney AP. 2000. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J. Anim. Sci. 78:2849–2855.

Japan Meat Information Service Center. 2016. Awareness survey report on meat at 2015. http://www.jmi.or.jp/publication/publication_detail.php?id=251Accessed July 13, 2015 (in Japanese only)

Kaneko M., Nakamura Y. & Yamada A. 2012. Effect of establishment methods on growth of *Brachiaria brizantha* (A. Rich.) Stapf as annual summer grass in temperate zone. Journal of Warm Regional Society of Animal Science, Japan 55:41–47. (in Japanese with English abstract)

Nakamura Y., Hirano K., Nakanishi Y., Kamiya M., Tsuneishi E., Kato N., Hayashi Y. & Yamada A. 2010. A beef production system and meat quality using grazing and self-sufficient feed. *J*ournal of Warm Regional Society of Animal Science, Japan 53:41–49. (in Japanese with English abstract)

Nakamura Y., Morita S., Kaneko M., Kato N., Hayashi Y., Tsuneishi E. & Yamada A. 2011. Comparison of meat quality of *M. Semitendinosus* of Japanese Brown (Kumamoto) Steer Produced by conventional and pasture feeding. Journal of Warm Regional Society of Animal Science, Japan 54:49–60. (in Japanese with English abstract)

Schmidt JR., Miller MC., Andrae JG., Ellis SE. & Duckett SK. 2014. Effect of summer forage species grazed during finishing on animal performance, carcass quality, and meat quality. J. Anim. Sci. 2013.91:4451–4461.

PO-04-33

Utilisation of Sunnhemp (Crotalaria juncea) as Roughage Source in beef cattle

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Abstract

Sunnhemp *(Crotalaria juncea)* has been proposed for improving soil fertility and combating weeds in Thailand. However, the previous studies have found that the cutting intervals of Sunnhemp at 50 days can be achieved through the greater DM and nutrient yields. Sunnhemp should contain a large amount of nutrients for an animal. The objective of this study was to determine the effects of the utilisation of Sunnhemp as roughage source in beef cattle. The experiment was conducted to determine the effect of Sunnhemp supplementing with urea treated rice straw (UTS) on Brahman × Thai-Native cattle performance. Twelve cattle, averaging 218 ± 14 kg body weight (BW) and approximately the 14-17 month. All cattle were stratified randomly and assigned in a randomized complete block design (RCBD) into 4 treatments (0, 25, 50 and 75% respectively) of 3 cattle each. The treatments were the level of Sunnhemp supplementing with UTS at 0, 25 and 50% respectively there were no significantly differences in BW, ADG and DMI. However, BW, ADG were significantly decreased at 75% of Sunnhemp supplementing with UTS. Sunnhemp can be readily grown and also offers the benefits to Thai farmers who could use Sunnhemp for feeding beef cattle.

Introduction

The leguminous shrub sunnhemp (*Crotalaria juncea*) has been proposed for improving soil fertility and combating weeds. in Thailand, although no quantified data is available on the cutting height and cutting age on production of sunnhemp (*Crotalaria juncea*) to livestock, the performance of the animals is reported to be good. The both work that gives some detail is that of Balaraman and Vankaterkrishman(1974) who determined the nutritive value of sunnhemp (*Crotalaria juncea*) of 24.95%, CF 31.62%, EE 2.81%, NEF 27.62%, ash 13% Ca 1.47% and P 0.36% respectively, Balaraman and Vankaterkrishman (1974). Reddy (1969) also recorded CP yields 18.1%, CF 38.1%, EE 1.1%, NEF 34.1%, ash 7.8% Ca 1.4% and P 0.25% respectively. Stage of maturity at harvest significantly affects the yield, crude protein and crude fiber content of sunnhemp. In Tanzania, Sarwatt et al. (1990) reported yields of *Crotalaria ochroleuca* 450, 1,800 and 3,364 kg DM/ha at each cut when harvested at 4, 6 and 8 weeks respectively. These crude protein yields reflected a percentage of 33.7% crude protein from cutting at 4 week intervals 30.2% crude protein from cutting at 6 week intervals and 28.6% crude protein from cutting at 8 week intervals. Sunnhemp therefore, offers the Thai cattle farmer a useful alternative protein source for cattle diets rather than relying on the more expensive. Sunnhemp, legume for improving soil fertility, can be readily grown and also offers the benefits to Thai farmers who could use sunnhemp for feeding cattle. The objective of this experiment was to determine the effects of the utilisation of Sunnhemp as roughage source in beef cattle**.**

Materials and methods

Animals and Treatments

The experiment was conducted to determine the effect of Sunnhemp supplementing with urea treated rice straw (UTS) on Brahman × Thai-Native cattle performance. Twelve cattle, averaging 218 ± 14 kg body weight (BW) and approximately the 14-17 month. All cattle were stratified randomly and assigned in a randomized complete block design (RCBD) into 4 treatments (0, 25, 50 and 75% respectively) of 3 cattle each. All cattle were fed approximately 3 kg/d of 14% CP concentrate and free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 70 days, with the first 10 days was the adjustment period, followed by 60 days of measurement period.

Laboratory analyses

Feed offered and left after eating of individual cattle were collected on 2 consecutive days of each week and dried at 60 °C for 48 h. At the end of the experiment feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through 1 mm screen and analyzed for chemical composition. Dry matter (DM) was determined by hot air oven at 60°C for 48 h. The crude protein (CP) was

determined by Kjeldahl analysis (AOAC, 1990). Ether extract (EE) was determined using petroleum ether in a Soxtec System (AOAC, 1990). Fiber fraction, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. Ash content was determined by ashing in a muffle furnace at 600°C for 3 h. The chemical analysis was expressed on the basis of the final DM.

Statistical Analysis

Measured data of intake, ADG and BW were analyzed by ANOVA for randomized complete block design (RCBD) using the Statistical Analysis System (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of p

Result and Discussion

Feed chemical composition

The chemical composition of the concentrate, Sunnhemp and Urea-treated rice straw (UTS) in the experiment is show in Table 1. As result, the proximate composition of Sunnhemp high in protein and crude fiber, NDF, ADF and cellulose. Sunnhemp was found to contain appreciable content of fiber fractions as NDF and ADF (53.04% and 47.12% on DM basis, respectively) as well as cellulose and hemicellulose (32.59% and 11.70% on DM basis, respectively) levels. Moreover, the Sunnhemp crude protein was on overall equal to 19.61% on DM basis. Our findings on Sunnhemp nutritional composition were similar to those reported by Balaraman and Vankaterkrishman (1974) who determined the nutritive value of sunnhemp (*Crotalaria juncea*) of 24.95%, CF 31.62%, EE 2.81%, NEF 27.62%, ash 13% Ca 1.47% and P 0.36% respectively, Balaraman and Vankaterkrishman (1974). Reddy (1969) also recorded CP yields 18.1%, CF 38.1%, EE 1.1%, NEF 34.1% and ash 7.8% respectively. The CP content of Sunnhemp was higher than the concentrate diet and UTS, and the fiber levels of Sunnhemp (CF, NDF, ADF and ADL) were lower than UTS respectively.

Intake and live weight

The average values for on feed intake, live weight, and live weight change of beef cattle are presented in Table 2. Dry matter intake tended to decrease as the proportion of Sunnhemp in diets increased but concentrate intake was not affected by dietary.

The effects of Sunnhemp on growth performance of beef cattle showed that the initial and final body weights (kg) did not differ significantly between groups ($P>0.05$). However, BWC, ADG were significantly decreased at 75% of Sunnhemp supplementing with UTS. ($P<0.05$) From our findings, it is suggested that the Sunnhemp supplementing with UTS (50:50) could be used to roughage for beef cattle. However, further studies will be needed to evaluate an availability of the Sunnhemp also for other ruminant species under different growth and physiological stages.

Keywords: Sunnhemp, Beef cattle, Performance, Utilisation

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Table 1. Chemical compositions of the experimental diets

Item	Concentrate	Sunnhemp	Urea-treated rice straw
DM	90.04 ± 0.12	90.62 ± 0.69	66.14 ± 0.30
CP	14.62 ± 1.99	19.61 ± 0.65	7.77 ± 0.10
EE	3.43 ± 0.08	2.43 ± 0.32	0.66 ± 0.64
Ash	5.45 ± 0.57	6.01 ± 1.06	4.29 ± 0.14
CF	12.48 ± 1.05	26.80 ± 2.39	35.80 ± 0.14
NDF	31.67 ± 1.78	53.04 ± 0.30	75.50 ± 0.14
ADF	18.57 ± 0.48	41.34 ± 0.12	51.40 ± 0.18
ADL	5.27 ± 0.53	8.75 ± 0.12	11.18 ± 0.94

Table 2. Effect of treatment on DMI, ADG, BW and BWC of beef cattle.

	Sunnhemp level				SEM	P value
	0%	25%	50%	75%		
DMI (Kg/d)	8.79^{a}	8.51^{a}	8.31^{ab}	8.01^{b}	0.15	0.04
Concentrate	2.70	2.70	2.70	2.70	-	-
Roughage	6.09^{a}	5.81^{a}	5.60^{ab}	5.31^{b}	0.16	0.05
Initial Weight (Kg)	225.4	217.6	221.1	219.2	3.2	0.72
Final Weight (Kg)	254.7	250.1	246.5	239.9	4.8	0.13
BWC (Kg)	29.3^{ab}	32.5^{a}	25.4^{bc}	20.7^{c}	3.1	0.05
ADG (g/d)	488^{ab}	542^{a}	423^{bc}	345^{c}	41.2	0.04

References

AOAC, (1990). Official Methods of Analysis 15th Ed. Association of Official Analytical Chemists. Washington. DC.

Balaraman, N. and R. Vankaterkrishman. (1974). Nutritive value of sunnhemp (Crotalaria juncea Linn) hay for sheep. Indian Vet. J. 51(5):337-341.

Reddy, M. R. (1969). Sunnhemp hay can cut down concentrate needs of cattle. Indian Farming 18:45-46.

Van Soest, P.J., J.B., Robertson and B.A. Lewis. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597.

Sarwatt, S. V., F. E. J. Mkiwa, A.B. Lwoga and B.H. Dzowela. (1990) Nutritive value of *Crotalaria ochroleuca*: the effect of supplementation on feed utilisation and performance of growing sheep. Utilisation of Research Results on Forage and Agricultural By-product Materials as Animal Feed Resources in Africa. Ethiopia. International Livestock Centre for Africa. 330 -340.

SAS, (1996). SAS Procedures Guide Release 6.3 Edition. SAS Institute Inc. Cary. NC: 441. Skerman P J (1977). Lablab purpureus (L) Sweet. In: Tropical forage legumes. FAO. Plant Production and Protection Series No.2. FAO. Rome.

Steel, R. D. G. and Torrie, J. H., (1986). Principles and Procedures of Statistics. A Biometrical Approach. 5th Ed. McGraw-Hill International Book Company New York.

PO-04-34

Evaluation of incorporating an improved Leucaena forage for grass-fed beef production in a tropical ecosystem

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Abstract

In the U.S., there is an increasing demand by consumers to purchase locally grown agricultural food products. Within this food movement, demand for grass-fed beef is increasing and providing an opportunity for beef cattle producers to enter into this niche market. However, the high fiber and low protein content of the forages in tropical pastoral systems often limit the production of quality grass-fed beef due to slow gain, extended finishing time, and consequent compromise in carcass quality characteristics. The objective of this study was to evaluate the incorporation of an improved variety of a high protein tree legume, Leucaena (*Leucaena leucocephala cv. Wondergraze),* on animal growth performance, days to market and carcass and meat quality traits in a tropical ecosystem. Two treatment pastures, identical in size, paddock numbers and grazing management, were developed: the control pasture (CP) was predominantly guinea grass (*Panicum maximum*), and the treatment Leucaena pasture (LP) was a mix of 60% guinea and 40% Leucaena. Twenty-five crossbred weaned steers were randomly assigned to each pasture group of 20 ha, and animals in both group were managed in the same way. Results show a significant improvement in average daily gain (0.74 vs 0.53 kg/day) and lower days to market (432-d vs 609-d) for the LP compared to the CP. In comparison to CP, hot carcass weights were heavier (335.8 vs. 321.4 kg), USDA marbling scores (421.2 vs. 381.1) and backfat thickness (0.58 vs. 0.38 cm) were greater, rib eye area was larger (87.7 vs. 81.3 cm^2) and age by dentition lower (2.1 vs. 3.0 yr) for the LP. Results show that Leucaena incorporated pasture produced 0.041 kg more body weight gain per ha per day and improved carcass quality traits.

Introduction

The demand by consumers for locally-grown, agricultural food products are growing (Martinez et al., 2010). Similar trend is happening in Hawaii and provides an opportunity for beef cattle producers to increase beef production. However, highly productive pastoral lands are limited by rainfall and temperature parameters (Fukumoto et al., 2015) in island ecosystems. Improving the pastoral production capacity for sustained grass-finish production is a goal for producers targeting the local market.

Leucaena leucocephala is leguminous tree or shrub widely naturalized throughout the Tropics (Cook et al, 2005), producing highly nutritious forage for ruminant production. Its deep tap root system provides for drought-tolerance. Through collaborative research efforts in Australia (Dalzell, et al., 2005), a team of scientists, private industry partners and government agencies, developed a unique forage production model for integrating Leucaena into pastoral systems for enhanced beef production. The Australian Leucaena model increased beef production up to 4-fold (Shelton, 2007).

The objective of the study was to evaluate the beef cattle performance, carcass characteristics, meat tenderness, and forage quality of beef cattle grown and finished on improved Leucaena pastures for sustainable beef production in a tropical ecosystem.

Materials and Methods

Two treatment pastures, identical in size (20-ha), number of paddocks (10 equal size paddocks) and management were developed. The *Leucaena leucocephala* pasture (LP) was planted with the cultivar *Wondergraze* (Leucseeds, Pty, Ltd. Queensland, Australia). Seeds were directly sowed into 4.3 m wide prepared seedbeds, arranged in double rows spaced 61 cm apart, spacing of double rows were 7.3 m apart and established within existing stands of guinea grass (*Panicum maximum*). The control pasture (CP) consisted of existing stands of guinea grass. Twenty-five weaned crossbred steer calves were randomly assigned to each treatment pasture and were provided water and mineral supplementation *ad. libitum* and were managed on a 70-day pasture rotation. Forage samples were collected prior to cattle rotation into a new paddock to measure the forage value at 70-days of re-growth. Forage samples were dried in a force draft oven at 50°C, ground and sent for nutrient analyses by NIR (Dairy One Lab,

Ithaca, NY, USA). Individual animal weights were recorded at the start of the evaluation period and recorded after each rotation at approximately every 70-days. Animal health was monitored and appropriate treatments were provided, as needed. A visual assessment of market readiness or finish was made and a final weight was recorded within a week of slaughter.

Due to the limited processing capacity at the local abattoir, market steers were processed in 5 separate harvest periods. Carcass data collected include hot carcass weight, marbling score, backfat thickness, rib eye area, maturity score, age by dentition and USDA grade equivalent. Following slaughter, 2.5 cm thick boneless rib eye samples were collected from the 12th rib, individually vacuum-sealed and shipped to the meat laboratory at the University of Hawaii at Manoa, USA. Upon arrival, the steak samples were repacked and aged in the refrigerator for 2 weeks from the slaughter date and then stored at -20° C for later shear force measurement. Once all rib eye steaks were collected, shear force measurements were carried out. Sealed samples were thawed overnight in a refrigerator. The thawed samples were cooked in a water bath at 70°C for one hour, cooled to room temperature for one hour and chilled overnight in a refrigerator (Wheeler, et al. 2005). The steaks were unwrapped, gently dried with paper towels and 6 core samples (1.3 cm diameter) were taken parallel to the longitudinal orientation of the muscle fiber for each steak. The force required to cut the cores were measured by a Warner-Bratzler machine (G-R Manufacturing, Manhattan, KS, USA). The shear force value was the mean of the maximum force required to cut each set of core samples.

The effects of live weight gains, carcass characteristics and shear force values between treatment groups were determined by ANOVA procedure using Prism6 program (Graphpad, San Diego, CA, USA).

Results and Discussion

Livestock performance results are summarized in Table 1. Average daily gains were significantly higher (0.74 vs 0.53 kg/day) and days to market (432-d vs 609-d) was significantly shorter for steers in the LP as compared to the CP group. ADG for steers in the LP group was 39.6% higher and took 29% fewer days to reach market size as compared to the CP group.

Table 2 summarizes the carcass characteristics and shear force measurement. Hot carcass weight was heavier (335.8 vs. 321.4 kg), marbling score (421.2 vs. 381.1) and backfat thickness (0.58 vs. 0.38 cm) was higher, rib eye area was larger (87.7 vs. 81.3 cm^2) and age at slaughter was lower (2.1 vs. 3.0 yr) for the LP group as compared to the CP group . The USDA-equivalent quality grade was higher for LP carcasses, averaging low Choice compared to Select for CP. However, shear force values of rib eye steak samples were lower (more tender) for the CP compared to the LP group (4.1 vs 5.0 kg). This anomaly may have resulted from the how the animals were handled at slaughter or sampling errors in the steak samples received from each treatment, 10 for the LP and 25 for the CP.

Table 3 summarizes selected chemical content of the individual forages and an estimate of the quality in the combination of guinea grass and Leucaena at a 60:40 ratio (Shelton and Dalzell). Leucaena forage had higher crude protein, lower fiber levels (higher digestibility), higher non-fibrous carbohydrate and, higher relative feed value than guinea grass. The minimum crude protein requirement for growing and finishing beef cattle is 16.4% and 12.3%, respectively (NRC, 1976).

Conclusion

In conclusion, results of the study show that incorporation of an improved *Leucaena leucocephala*, cv. *Wondergraze*, into a tropical pastoral rotational grazing system significantly enhanced average daily gains, shortened days to harvest and improved carcass traits as compared to guinea grass pastures. The improvement in animal growth and carcass traits is likely due to the enhancement of nutritional quality of grass:legume forage mixture.

Keywords: Tropical beef production, Leucaena, Forage, Meat quality

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Table 1. Comparison of the animal performance on guinea grass compared to guinea grass and *Leucaena* pastures, Hamakua, Hawaii Island.

	Guinea Grass Control (CP)	**GG + Leucaena Treatment (LP)**
Ave. Starting Weight, kg	294.6 ± 4.54	644.0 ± 6.08
Ave. Ending Weight, kg	628.6 ± 8.94	630.5 ± 8.53
On-Test Gain/head, kg	308.3 ± 5.85	316.9 ± 8.21
On-Test ADG, kg/day	0.53 ± 0.01	0.74 ± 0.02 **
On-Test Days to Harvest, days	608.9 ± 0.41	432.2 ± 12.4 **

Average ± Standard Error
** means within a row are significantly different, $p < 0.01$

Table 2. Comparison of carcass weight, marbling score, rib eye area, back fat thickness, age by dentition and shear force of steers on guinea grass and guinea grass and Leucaena pastures.

	Guinea Grass Control (CP)	**GG + Leucaena Treatment (LP)**
Carcass weight, lb.	321.4 ± 5.31	335.8 ± 4.76 *
Marbling score	381.2 ± 12.8	421.2 ± 11.0 *
USDA Grade Equivalent	Select	Low Choice
Rib eye area, in^2	81.3 ± 1.42	87.7 ± 1.48**
Back fat thickness, in	0.38 ± 0.02	0.58 ± 0.05 **
Age by dentition, yr	3.0 ± 0.06	2.1 ± 0.19 **
Shear force, kg	4.1 ± 0.17	5.0 ± 0.22 **

Average ± Standard Error; Marbling Score: Slight=300, Small=400
* means within a row are significantly different, $p < 0.05$
** means within a row are significantly different, $p < 0.01$

Table 3. Nutritional content of selected quality components of guinea grass and Leucaena and the estimated content of the planted forage ratio of 60% guinea and 40% Leucaena.

	CP	ADF	NDF	NFC	Ash	TDN	RFV
	---------- % Dry Matter Basis ----------						
Guinea Grass	18.8	326	643	8.2	11.4	60	92
Leucaena	29.4	23.3	32.2	32.3	9.3	71	205
60% GG:40% GG+L	23.0	28.9	51.5	17.8	10.6	64.4	137

n=4, 67-day regrowth.
CP=crude protein, ADF=Acid detergent fiber, NDF=neutral detergent fiber, NFC=non-fibrous carbohydrates, TDN=total digestible nutrients, RFV=relative feed value

References

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. and Schultze-Kraft, R. 2005. Tropical Forages: an interactive selection tool., CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia. *Leucaena leucocephala* forage fact sheet. http://www.tropicalforages.info/key/Forages/Media/Html/Leucaena_leucocephala.htm

Dalzell, S.A., H.M. Shelton, B.F. Mullen, P.H. Larsen, and K.G. McLaughlin. 2006. Leucaena: A guide to establishment and management. Meat and Livestock Australia, Ltd., Sydney, Australia.

Fukumoto, G.F., Thorne, M.S., Silva, J.H. and Deenik, J.L 2015. Suitability map for forage-finished beef production using GIS technology: Hawai`i Island. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii, USA, PRM-7. http://www.ctahr.hawaii.edu/oc/freepubs/pdf/PRM-7.pdf

Martinez, Steve, et al. *Local Food Systems: Concepts, Impacts, and Issues*, ERR 97, U.S. Department of Agriculture, Economic Research Service, May 2010.

Shelton, M. and Dalzell, S. 2007. Production, economic and environmental benefits of leucaena pastures. Tropical Grasslands, v. 41, p 174-190.

Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. 2005. Shear force procedure for meat tenderness measurement. Roman L. Hruska U. S. Meat Animal Research Center. http://www.ars.usda.gov/SP2UserFiles/Place/30400510/protocols/ShearForceProcedure.pdf.

PO-04-35 Evaluation of Palatability for Crop Species and Conserving Methods in Korean Native Cattle

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Objective

About 5.6 million ton of forages were consumed for ruminant in South Korea. Self-sufficient of forage reached at 82%, but half of them were consisted of rice straw. To improve the forage supply situation, the government supports forage production in paddy field. There are various crop species for suitable in paddy field. The main crop species for paddy field in Korea are Barley, Rye and Italian ryegrass. Each crop has specific characteristics in growing habits, harvesting time, conservation methods and animal palatability. So, these experiments were conducted to evaluation of palatability for crop species and conservation methods in Korean native cattle.

Methodology

All three palatability evaluation tests, i) hay and haylage in rye, ii) hay and haylage in Italian ryegrass, and iii) Haylage between Italian ryegrass and barley, were conducted for 7 days with 3 kg/head/day of concentrate feed. Three Korean native cattle after four caving were fed on an ad libitum basis forage and weighting the residuals two times per day. Forage crops (Barley, Rye and Italian ryegrass) were planted in November, 2014 and harvested in May, 2015. All forage were conserved as hay and haylage. Crude protein (CP) was analyzed according to Official methods (AOAC, 1990) and total degestible nutrient (TDN) was obtained by formula of Holland et al. (1990) : TDN=88.9-(0.79xADF%). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analysed following the method of Goering and Van Soest (1970) and *in vitro* dry matter digestibility (IVDMD) was determined by Tilley and Terry (1990) method.

Results

The forage quality of rye hay and haylage were similar, total digestible nutrient (TDN) was slightly higher at rye hay as 56.04 and 55.69%, crude protein (CP) was higher at rye haylage as 7.04 and 6.77%. Forage quality of Italian ryegrass (IRG) was higher than those of rye. CP content of IRG hay and haylage was 9.84 and 10.90%, TDN was 57.36 and 58.81%. Barley haylage was higher in TDN but lower in CP content as 63.96 and 7.64%, compared to rye and IRG. The palatability of rye haylage was higher than that of rye hay by 1.68 and 1.14 DMkg/head/day. But, palatability of IRG showed different trend, hay was slightly higher than haylage by 2.29 and 2.27 DMkg/head/day, respectively. The palatability was not significantly different between hay and haylage($p<0.05$). In the evaluation between Italian ryegrass and barley haylage, Italian ryegrass was markedly higher than that of barley haylage($p<0.05$) by 4.91 and 1.96 DM kg/head/day, respectively. According to Kim et al.(2015), palatability of Italian ryegrass silage was higher than that of barley and rye silage. In this experiments, Italian ryegrass haylage was higher palatability than barley haylage.

Conclusion

According to these results, we suggest that the best palatable forage crop is Italian ryegrass and farmers must decide the conserving method (hay or haylage) to fit into their situation.

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Table 1. Fiber composition and forage quality of conserved forages.

Item		ADF (%)	NDF (%)	CP (%)	IVDMD (%)	TDN (%)	RFV
Rye	Hay	41.59	64.00	6.77	58.10	56.04	82
	Haylage	42.04	66.61	7.04	67.86	55.69	78
IRG	Hay	39.62	60.55	9.84	66.10	57.36	89
	Haylage	38.09	59.18	10.90	69.97	58.81	93
Barley	Haylage	34.63	54.10	7.64	69.92	63.96	106

* ADF : acid detergent fiber, NDF : neutral detergent fiber, CP : crude protein, IVDMD : *in vitro* dry matter digestibility, TDN : total digestible nutrient, RFV : relative feed value, IRG : Italian ryegrass

Table 2. Fresh and dry matter intakes of conserved forages.

Item		DM (%)	Intake (kg/head/day)	
			Fresh matter	Dry matter
Rye	Hay	81.33	2.03	1.64
	Haylage	67.06	3.04	2.04
Total			5.07	3.68
IRG	Hay	81.41	3.15	2.56
	Haylage	66.73	3.85	2.57
Total			6.95	5.13
IRG	Haylage	48.08	10.21	4.91
Barley	Haylage	48.92	2.20	1.08
Total			12.41	5.99

* Korean native cattle (BW 250-350 kg), Concentrate : 3 kg/head/day,

References

AOAC, 1990. Association of Official Analytical Chemists, Official Methods of Analysis. 15th Edition. Washington, DC.

Goering, H.K. and Van Soest, P.J. 1970. Forage fiber analysis. Ag. Handbook. No. 379. ARS. USDA. Washington D.C.

Holland, C., KEZAR, W., Kautz, W.P., Lazowski, E.J. Mahanna, W.C. and Reinhart, R. 1990. Pioneer Hi-Bred International, Inc., Des moines, IA.

Kim, J.G., Park, H.S., Ko, H.J., Jeong, J.S. and Kim, M.J. 2015. Assessment of animal palatability for domestic developed Italian ryegrass in South Korea. Proceeding of XXIII International Grassland Congress. India.

Tilley, J.A.M. and Terry, R.A. 1963. A two stage technique for *in vitro* digestibility of forage crops. Journal of Britannica Grassland Science 18:104-111.

PO-04-36

Forage selection of tropical forage legume species by Japanese Black cattle

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Introduction

Legumes play a significant role in many farming systems of the tropics and subtropics. They contribute to the nutritive value of animal diets, biological nitrogen (N) fixation, and landscape stability (Humphreys, 1995). In addition, legumes help with soil improvement, soil conservation, establishing wildlife food plots, and provide N for other crops; they are also used in the production of honey (Zemenchik et al., 1996; Sheaffer and Evers, 2007). Furthermore, forage legumes are key components to livestock rations regardless of whether or not they are grazed or harvested as hay or silage (Sheaffer and Evers, 2007). Little is known, however, about the cultivation and utilization of tropical forage legumes when compared to temperate forage legumes in Japan.

Diet selection by grazing animals is a function of preference modified by opportunity (Allen et al., 2011). "Preference" is a measure of the relative intake of alternative forages or forage constituents, especially where access to forage is unrestricted (Allen et al., 2011). It is well known that dry matter digestibility (DMD) and crude protein (CP) concentration of forage exert a positive influence on patch choice of sward and forage selection by animals (Smit et al., 2006; Van Dorland et al., 2007; Hirata et al., 2012). Moreover, the tannin content of forage has a negative influence on forage selection (Kumar and D'Mello 1995). Few studies have, however, investigated the chemical composition of tropical forage legumes and how it influences selection and preferences in cattle. In addition, the exact chemical component that influences preferences, and the type of tropical forage legumes are preferred are not clear.

The purpose of this study was to determine which tropical forage legume species were preferred by Japanese Black cattle and the chemical components that influenced those preferences.

Materials and Methods

The selection of six different tropical forage legume species by Japanese Black breeding cattle was investigated. These species included greenleaf desmodium (*Desmodium intortum*, Gd), *Aeschynomene americana* 'Glenn' (Ag), *A. americana* strain 93556 (A9), *A. villosa* strain 93621 (Av), phasey bean (*Macroptilium lathyroides*, Pb), and siratro (*Macroptilium atropurpureum*, Si). These were seeded on June 1, 2006, at the experiment field in the Sumiyoshi Field attached to the University of Miyazaki's Department of Agriculture. They were harvested on September 1 (test 1; Gd, Av, A9), September 8 (test 2; Pb, Si, Ag, Gd), September 19 (test 3; Gd, Av, A9), October 13 (test 4; Si, Ag, Gd), and October 20 (test 5; Gd, Av, A9) of 2006. These tropical forage legumes were cut to lengths of approximately 5 cm, with two kinds of legumes being presented in 1-kg lots to each cattle. Three Japanese Black breeding cattle (4-8 age) were used in the study. The cattle had feeding experience of the test legumes. Constant temporal intake (5 min) was measured using an alternate response item. The intake ratio of each species to the whole intake was then calculated. Each test was performed prior to the evening feed (17:00-18:00 h) in the stable.

All samples were dried in an oven set at 85°C for 72 h. The dried material was then crushed until it passed through a 1-mm screen. The CP concentration was determined according to methods described in AOAC (1990). The DMD was determined using a pepsin-cellulase technique, and corrected with a calibration equation to produce predicted *in vivo* values (Goto and Minson 1977). The soluble tannin concentration was determined according to Iwasa and Torii (1962). The relationship between forage CP concentration and DMD, as well as the intake ratio, was also determined.

All data obtained as ratios were converted to arcsine values. Differences in the intake ratio for abundance in each test were determined using *t*-tests. Differences between the species in DMD, CP concentration, and soluble tannin concentration in each test were compared using an ANOVA. The least significant difference between mean values was determined using Tukey's HSD test. Pearson's correlation was used to determine the associations between the intake ratio and the CP concentration, DMD, and tannin concentration ratios, and between forage selections by cattle that were exposed to each combination. The CP concentration and DMD ratios of the species included in the combination were also evaluated using the Pearson's correlation. All statistical analyses were performed using STATISTICA (version 10, StatSoft, Tulsa, OK, USA) software.

Results and Discussion

In test 1, the intake ratio of Av and A9 tended to be higher than Gd, but was not significantly different (Fig. 1a). In test 2, the intake ratio of Ag was higher than that of Si and Pb ($P < 0.05$), whereas the intake ratio of Pb was lower than that of Gd and Si ($P < 0.05$, Fig. 1b). Forage selection was expected to be highest in the order: Ag, Si, Gd, and Pb. In test 3, no significant difference was observed in intake ratio between Gd, Av, and A9 (Fig. 1c). In test 4, the intake ratio of Ag was significantly higher than that of Gd and Si ($P < 0.05$, Fig. 1d). There was no difference between Gd and Si. The intake ratio of Av in test 5 was higher than that of Gd ($P < 0.05$, Fig. 1e). There was no difference between A9 and Gd. Throughout the test period, Gd intake ratio increased with the growth of the plants, and was either the same as Av, A9, and Si, or lower.

Except in test 4, the Gd was lower in CP concentration and DMD. Furthermore, it had a higher tannin concentration than the other species (Table 1). The Av exhibited a higher CP concentration and DMD in test 1, but tended to decrease with plant growth. In contrast, the decrease of CP concentration and DMD in Si and Ag was small with the progression of growth.

A combination of Gd/Av, and Gd/A9, resulted in intake ratios of Gd increasing from 0.2 to approximately 0.5 during the growth season (Fig. 2). A positive correlation existed between the Gd intake ratio and the CP concentration ratios for Gd/Av and Gd/A9 ($r = 0.906$, $n = 6$, $P < 0.05$, Fig. 3). Of all the forage combinations tested in this study, a positive correlation also existed between forage selection by cattle exposed to each combination and the CP concentration ratios of the species included in the combination ($r = 0.698$, $n = 15$, $P < 0.05$, Fig. 4).

The Gd legume is not particularly palatable to animals because of its high tannin concentrations (Skerman et al., 1988; Cook et al., 2005). It is palatable enough, however, that it requires careful grazing management to persist (Cook et al., 2005). Generally, tannin in the forage has a negative influence on the forage selection by animals, especially high levels of tannin, which may depress the feed intake (Kumar and D'Mello 1995). Since Gd had a lower CP and DMD, and a higher tannin concentration than the other species, intake ratios differed between tests. The Gd legume was either the same as Av, A9, and Si, or was lower in intake ratio. The CP and DMD concentrations in the forage exerted a positive influence on patch choice on sward and forage selection by the cattle (Smit et al., 2006; Van Dorland et al., 2007; Hirata et al., 2012). However, in this study, only CP concentration ratios influenced forage selection by animal. The selective change for the animal reflected the change in CP concentration ratio. It was not able to clarify it about the influence of the DMD ratios. From these results, it became clear that CP was a factor that affected forage selection.

Conclusion

The results of this study suggested that the intake ratio of Gd was either the same or lower compared to Av, A9, and Si. The intake ratio of Av (test 1, 3, and 5) and Ag (test 2 and 4) tended to be higher than that of the other species. The Gd had a lower CP concentration and DMD, and higher tannin concentration than those of the other species. The results of the correlation analysis revealed that the forage selection of Japanese Black cattle reflected changes in the CP concentration ratio.

Acknowledgments

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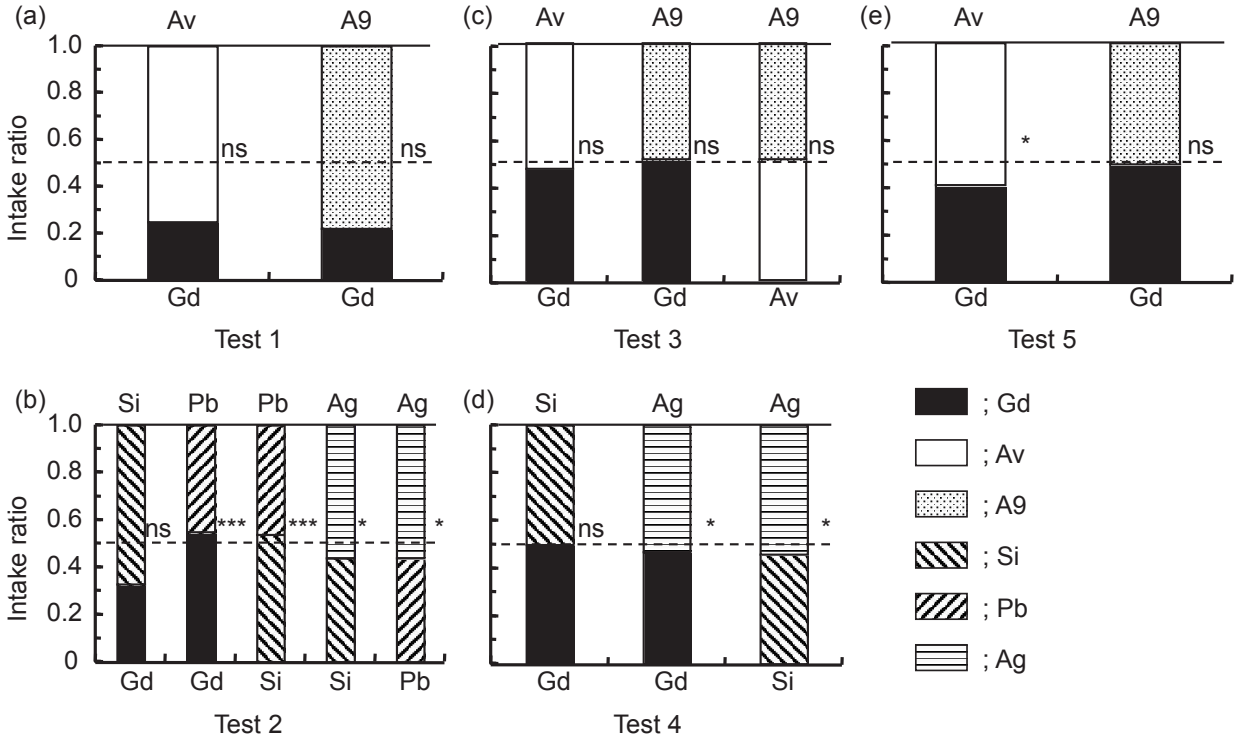

Figure 1. Intake ratio of each combination.
Values with ***, * and ns indicate significant differences (0.5), at $P < 0.001$, 0.05 and $P > 0.05$, respectively.

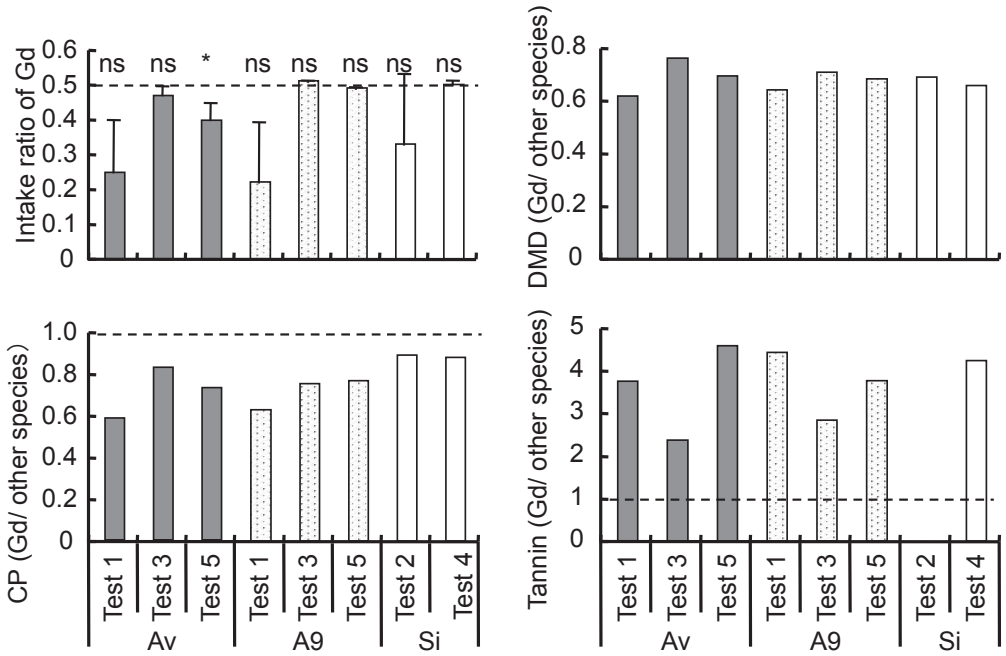

Figure 2. Intake ratio, crude protein (CP) concentration, dry matter digestibility (DMD) and tannin concentration ratio of Gd for Gd/Av, Gd/A9 and Gd/Si. Intake ratios indicated with * indicate significant differences (0.5) at $P < 0.05$, while ns indicate not significant differences. The random choice line was 0.5 (intake ratio), while equal line of the CP concentration, DMD and tannin concentration ratio was 1.0.

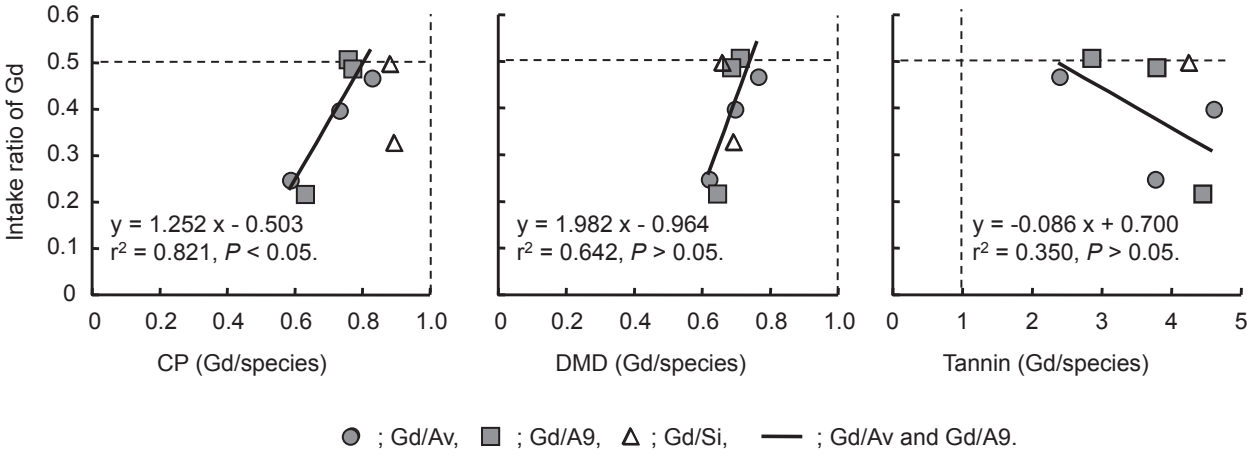

Figure 3. Relationship between the Gd intake ratio and the crude protein (CP) concentration, dry matter digestibility (DMD) and tannin concentration ratio for Gd/Av, Gd/A9 and Gd/Si. The random choice line was 0.5 (intake ratio), whereas the equal line of the CP concentration, DMD and tannin concentration ratio was 1.0.

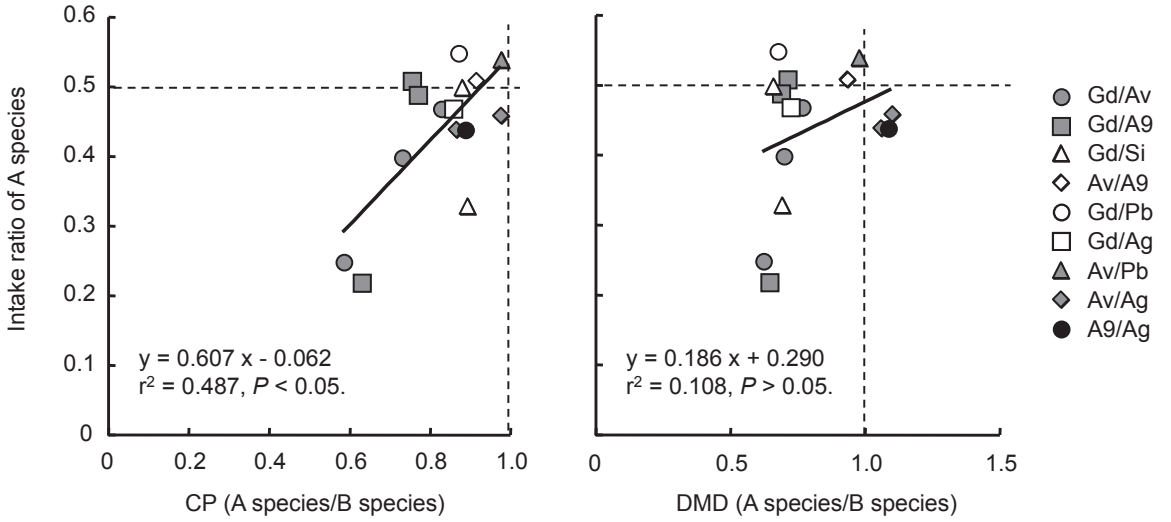

Figure 4. Relationships between intake ratio and crude protein (CP) concentration ratio, and dry matter digestibility (DMD) ratio of the other grass species (A species/B species). The random choice line was 0.5 (intake ratio), whereas the equal line of the CP concentration and DMD ratio was 1.0.

Table 1. Crude protein (CP) concentration, dry matter digestibility (DMD) and soluble tannin concentration.

Species	Test 1			Test 3			Test 5		
	CP (g/kg DM)	DMD	Tannin (g/kg DM)	CP (g/kg DM)	DMD	Tannin (g/kg DM)	CP (g/kg DM)	DMD	Tannin (g/kg DM)
Gd	163.7 b	0.432 c	20.5 a	188.0 c	0.452 c	11.6 a	142.7 b	0.456 b	24.3 a
Av	281.0 a	0.701 a	5.5 b	228.0 b	0.594 b	4.9 b	196.1 a	0.657 a	5.3 b
A9	261.0 a	0.675 b	4.6 c	250.0 a	0.639 a	4.1 c	186.0 a	0.668 a	6.5 b

Species	Test 2			Test 4		
	CP (g/kg DM)	DMD	Tannin (g/kg DM)	CP (g/kg DM)	DMD	Tannin (g/kg DM)
Gd	155.5 c	0.488 c	-	154.8 a	0.468 c	15.6 a
Si	174.6 bc	0.709 a	-	176.4 a	0.715 a	3.7 b
Pb	179.2 b	0.727 a	-	-	-	-
Ag	202.6 a	0.672 b	-	181.1 a	0.652 b	3.8 b

Means followed by different letters within the same variable differ ($P < 0.05$).

References

Allen VG, Batello C, Berretta EJ, Hodgson J, Kothmann M, Li X, McIvor J, Milne J, Morris C, Peeters A, Sanderson M (2011) An international terminology for grazing lands and grazing animals. Grass and Forage Science 66, 2-28.

AOAC (1990) *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Arlington, VA.

Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M, Schultze-Kraft R (2005) *Tropical Forages: An Interactive Selection Tool* is a collaborative effort between CSIRO Sustainable Ecosystems, Department of Primary Industries & Fisheries (Qld), Centro Internacional de Agricultura Tropical (CIAT) and the International Livestock Research Institute (ILRI). http://www.tropicalforages.info/index.htm 2016/07/11.

Goto I, Minson DJ (1977) Prediction of the dry matter digestibility of tropical grasses using a pepsin-cellulase assay. Animal Feed Science and Technology 2, 247-253.

Hirata M, Murakami K, Ikeda K, Oka K, Tobisa M (2012) Cattle use protein as a currency in patch choice on tropical grass swards. Livestock Science 150, 209-219.

Humphreys LR (1995) Diversity and productivity of tropical legumes. In *Tropical legumes in animal nutrition*. (D'Mello JPF, Devendra C Edt), p 1-21. CAB International, Wallingford, Oxon, UK.

Iwasa K, Torii H (1962) A colorimetric determination of tea tannin with ferrous tartrate. Japanese Society of Tea Science and Technology 19, 104-108.

Kumar R, D'Mello JPF (1995) Anti-nutritional factors in forage legumes. In *Tropical legumes in animal nutrition*. (D'Mello JPF, Devendra C Edt), p 95-133. CAB International, Wallingford, Oxon, UK.

Sheaffer CC, Evers GW (2007) Cool-season legumes for humid areas. In *Forages, Volume II, The Science of Grassland Agriculture, 6th edition* (R. F. Barnes, C. J. Nelson, K. J. Moore & M. Collins Edt), pp. 179–190. Iowa, USA: Blackwell Publishing.

Skerman PJ, Cameron DG, Riveros F (1988) *Tropical Forage Legumes*. FAO, Rome, Italy.

Smit HJ, Tamminga S, Elgersma A (2006) Dairy cattle grazing preference among six cultivars of perennial ryegrass. Agronomy Journal 98, 1213-1220.

Van Dorland HA, Wettstein H-R, Aeschlimann G, Leuenberger H, Kreuzer M (2007) Preference of dairy cows for ryegrass, white clover and red clover, and its effects on nutrient supply and milk quality. Archives of Animal Nutrition 61, 371-389.

Zemenchik RA, Wollenhaupt NC, Albrecht KS, Bosworth AH (1996) Runoff, erosion, and forage production from established alfalfa and smooth bromegrass. Agronomy Journal 88, 461-466.

PO-04-37 The application of chemical pretreatment on digestibility improvement of domestic alternative forage - an in vitro fermentation evaluation

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Objective

The alkali-treatment is an efficiency method for forage treatment, the combining of NaOH and H_2O_2 treatment resulted in higher dry matter intake and digestibility in feeding trail (Kerley et al., 1987; Cameron et al., 1988). The forage digestibility improvement is due to the alkali treatment broken the linkage between hemicellulose and lignin (Cameron et al., 1991; Kim and Lee, 2007). The sunn hemp and rice straw are common green manure crop and agriculture waste in Taiwan, respectively. A great quantity forage was imported for dairy industry in Taiwan every year, the increasing imported forage price resulted in the high feeding cost. Both sunn hemp (SH) and rice straw (RS) could be used as alternative feeding forages to replace the imported hay. However, the lower digestibility limited the utilization of both forage source in ruminant feeding.

This study attempted to investigate the application of chemical pretreatment on digestibility improvement of domestic alternative forage. The treatment group with better performance will be selected to combine with domestic protein feedstuff to formulate the testing diet. The testing diets were evaluated by in vitro digestion to select the appropriate diet for animal feeding trail.

Materials and methods

Chemical pretreatment and diet mixture

The sunn hemp (SH), and Taikeng 9 rice straw (RS) used in this study were harvested in fall. All raw materials were air dried at 60°C resulting in a dry matter content of 92-95%. The chopped materials (about 1 cm) were dried again at 65°C until dry matter content reach 95% than storage under dry condition in a plastic bag at room temperature. Five treatments (final concentration in dry matter) including (1) blank, (2) 5% NaOH, (3) 5% $Ca(OH)_2$, (4) 2.5% NaOH + 2.5% $Ca(OH)_2$ and (5) 5% NaOH + 2.5% H_2O_2 were used in this study. Samples (800 g/treatment) treated by (2), (3) and (4) were incubated at room temperature for 4 weeks. The treatment (5) was prepared according to Cameron et al. (1991) before digestion testing procedure.

The domestic protein source including sesame meal (SM) and sorghum distillery residue (SDR) were used in this study to formulated the similar rumen degradable/undegradable protein (RDP/RUP), forage neutral detergent fiber (FNDF) and crude protein (CP) diets (Table 1) for mixed diet evaluation test.

In vitro digestion test for pretreated forage and mixed diet

To evaluate the effect of rumen fluid source on pretreatment forage digestibility, rumen fluid collected from cannulated dairy cows fed Bermuda hay or alfalfa hay for 14 days were used as inoculum source of in vitro evaluation. Rumen fluid was strained through four layers of cheesecloth, and mixed in a 1:4 proportion with mineral solution (Tiller and Terry, 1963). The method used for in vitro digestion was based on the Rao et al. (2009), the Incubation was conducted in a 60-mL glass bottle containing 40 mL diluted rumen fluid and 400 mg substrate (particle size < 1 mm) under 39°C water bath. Incubations of mixed diets evaluation were conducted using rumen fluid from cannulated dairy cows fed diet contain 25% Bermuda hay, 25% alfalfa hay and 50% concentrate for 14 days.

During the incubation, the gas productions were recorded at 1, 2, 4, 8, 12, 24, 48 h, the rumen inoculum mixtures were sampled at the 48 h of incubation. At the end of incubation, the 10 mL mixed culture fluid was collected to assay the microbial protein synthesis according to Blummel et al. (2003) before separation. Residual culture fluid was separated by the nylon bag and the Liquid portion was collected to analyze VFA concentration according to Castillejos et al. (2006) with gas chromatography. The solid portion was lyophilized for DM and NDF assay.

The chemical composition of all raw material and treated samples were assayed by AOAC (2000) method and Van Soest Fiber Analysis system (Goering and Van Soest, 1970).

Data were analyzed using one-way ANOVA of SAS 8.02 for Windows (SAS Institute) and means were separated by the Duncan's multiple range tests.

Result and Discussion

The chemical composition of treated forage was showed in Table 2. The NaOH + H_2O_2 treatment resulted in the fewest physical appearance change among all treatments. However, treatments contain $Ca(OH)_2$ resulted in darker color after 4 wks. The composition assay result indicated that the non-structure carbohydrate (NSC) proportion was increased after NaOH treatment. The NaOH treatment also decreased the ADF and lignin in tested forage, especially in RS group. It suggested that alkali-pretreatment contributed to loss the structure of forage fiber.

The gas production kinetics result under different were showed in Figure 1. The legume feeding rumen fluid inoculum resulted in higher gas production in all treatments, the treatment with NaOH also increased the gas production. It suggested that the rumen fluid inoculation source had effect on fermentation performance of pretreatment forages. Cone et al. (2002) indicated that the different flora composition in inoculation source affected the digestibility of the same fermentation substrate under in vitro test. The feeding diet of inoculation source animal could be an important factor to effect the in vitro digestion performance (Reid et al., 1964; Milne, 1977)

The inoculation of legume feeding rumen fluid shown benefit on pretreatment forage digestion. All alkali-pretreatment increased the in vitro NDF digestibility (IVNDFD) and MCP synthesis after 48 h incubation (Table 3). Compared to SH group, the in vitro dry matter digestibility (IVDMD) result also indicated that pretreatment resulted in the higher IVDMD improvement on RS than on SH (Table 3).

The VFA composition had no significant difference among all pretreatments except RS with NaOH + $Ca(OH)_2$ treatment under the grass feeding rumen fluid was inoculated. The higher proportion of propionate was shown in RS with NaOH + $Ca(OH)_2$ treatment. The legume feeding rumen fluid inoculation test also indicated that pretreatments showed increasing of Ac/Pr on RS substrates (Table 3). However, the total VFA concentrate of SH fermentation was twice higher than RS fermentation. Cameron et al. (1991) indicated that NaOH + H_2O_2 treated wheat straw (AHP-WS) increased the NDF digestibility, but yields of milk and 4% fat-corrected milk (FCM) were decreased as the level of AHP-WS increased in the diet.

The effect of pretreatment forage on mixed diets were shown in Table 4. The $Ca(OH)_2$ pretreatment had no effect on gas production and IVDMD of SH. However, all pretreatment treatment improved the gas production and IVDMD on mixed diet contain RS. According to the IVDMD and gas production data from different pretreatments, it suggested that pretreatment with NaOH resulted in better fermentation performance. Compared to the control group, the IVDMD increased 8 to 10% after NaOH + H_2O_2 pretreatment forage were used in mixed diets. The gas production kinetic results indicated that the diet contain forages with NaOH + $Ca(OH)_2$ or NaOH + H_2O_2 pretreatment had the highest theory gas production. Previous study also indicated that alkali-treatment with H_2O_2 resulted in higher improvement of DM and NDF digestibility in sheep, total non ammonia-nitrogen flow at the abomasum was significantly higher on the NaOH-treated diet (Meeske et al., 1993).

According to the gas production rate constant data of mixed diets, both NaOH + $Ca(OH)_2$ and NaOH + H_2O_2 pretreated RS with SDR resulted in the highest fermentation rate (0.07-0.08 %/h). However, no positive effect was shown in mixed diet contain pretreated SH with SDR. It suggested that protein source should be included in diet formulation contained chemical pretreatment forage.

Conclusion

The results in this study indicated that chemical pretreatments could improve the fiber digestibility significantly. The inoculation source had effect on in vitro fermentation performance of forages. Concentrate and pretreated alternative forage combination study showed better gas production in treatments of NaOH + $Ca(OH)_2$ and NaOH + H_2O_2. Both RS and SH showed quality improvement after all pretreatments, and had promising gas production result when combined with proteinous feed ingredients.

Table 1. The composition of mixed diet substrate*

Treatment	Forage (g)	SM concentrate		SDR concentrate	
	SH	SM	Corn	SDR	Corn
Control	453.63	154.64	391.74	268.03	278.34
$Ca(OH)_2$	423.06	161.49	415.45	279.92	297.02
NaOH	412.70	163.82	423.49	283.95	303.35
NaOH+ $Ca(OH)_2$	418.60	162.49	418.91	281.66	299.75
NaOH+H_2O_2	468.47	151.31	380.23	262.26	269.27
	RS	SM	Corn	SDR	Corn
Control	340.74	302.28	356.98	523.95	135.31
$Ca(OH)_2$	341.42	302.37	356.20	524.11	134.47
NaOH	420.84	313.06	266.10	542.64	36.52
NaOH+ $Ca(OH)_2$	336.47	301.70	361.83	522.95	140.58
NaOH+H_2O_2	358.81	304.71	336.47	528.17	113.02

* SH = sunn hemp; RS = rice straw; SM = sesame meal; SDR = sorghum distillery residue.

Table 2. The effect of pretreatment on chemical composition of forages*

Pretreatment	Composition (% OM)**			
	NSC	NDF	ADF	ADL
SH				
Control	51.50	48.50	36.00	5.14
$Ca(OH)_2$	46.69	53.31	38.42	4.57
NaOH	48.00	52.00	38.35	5.39
NaOH+ $Ca(OH)_2$	47.44	52.56	36.85	4.86
NaOH+H_2O_2	53.04	46.96	36.22	4.93
RS				
Control	35.43	64.57	38.96	3.06
$Ca(OH)_2$	47.72	52.28	42.87	4.31
NaOH	35.56	64.44	42.37	3.61
NaOH+ $Ca(OH)_2$	34.61	65.39	42.61	4.22
NaOH+H_2O_2	38.69	61.31	37.23	2.67

* SH = sunn hemp; RS = rice straw.
**NSC = non-structure carbohydrate; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin.

Table 3. The effect of pretreatment on forage in vitro digestion, microbial protein synthesis (MCP, mg/g substrate) and fermentation products.

		Pretreatment**					
Forage*	Item	1	2	3	4	5	SEM
				grass			
SH	IVDMD%	54.28^{bc}	52.96^{c}	57.25^{ab}	60.74^{a}	57.37^{ab}	1.333
	IVNDFD%	42.82	71.65	71.75	58.17	62.26	
	MCP	23.97^{c}	21.81^{d}	20.88^{d}	29.94^{a}	26.52^{b}	0.516
	Ac (%)	40.70	42.66	43.91	42.59	43.24	1.799
	Pr (%)	21.46	21.23	21.43	21.33	20.90	0.296
	Bu (%)	23.91	23.90	22.58	23.86	23.77	0.715
	Ac/Pr	1.90	2.01	2.05	2.00	2.07	0.092
RS	IVDMD%	33.60^{b}	49.96^{a}	49.28^{a}	44.28^{a}	45.71^{a}	2.062
	IVNDFD%	54.19	70.92	67.48	69.61	67.49	
	MCP	23.06^{b}	23.21^{b}	28.32^{a}	25.50^{ab}	24.26^{b}	0.941
	Ac (%)	43.49	43.91	44.37	44.12	44.88	0.801
	Pr (%)	21.23^{b}	21.34^{ab}	21.88^{ab}	21.96^{a}	21.87^{ab}	0.226
	Bu (%)	23.37	23.16	22.44	23.11	22.43	0.601
	Ac/Pr	2.05	2.06	2.03	2.01	2.05	0.045
				legume			
SH	IVDMD%	55.79^{d}	58.92^{c}	65.29^{a}	60.99^{bc}	63.39^{ab}	0.912
	IVNDFD%	45.11	62.89	73.27	67.48	63.87	
	MCP	33.28	32.14	32.67	32.59	33.04	1.049
	Ac (%)	43.40	43.29	43.92	43.77	44.31	0.954
	Pr (%)	20.76	20.47	20.81	20.55	20.70	0.172
	Bu (%)	22.99	23.70	23.07	23.49	22.92	0.660
	Ac/Pr	2.09	2.11	2.11	2.13	2.14	0.036
RS	IVDMD%	36.24^{c}	48.78^{b}	53.14^{a}	47.97^{bc}	49.66^{ab}	1.133
	IVNDFD%	48.40	68.40	68.10	67.66	68.77	
	MCP	25.97^{b}	30.87^{a}	31.37^{a}	30.22^{a}	32.57^{a}	0.721
	Ac (%)	44.28^{b}	45.00^{ab}	44.60^{ab}	47.37^{a}	44.08^{b}	0.939
	Pr (%)	20.29^{b}	20.70^{ab}	20.70^{ab}	21.63^{a}	20.45^{ab}	0.391
	Bu (%)	23.44^{ab}	22.41^{b}	23.13^{ab}	21.93^{b}	23.97^{a}	0.478
	Ac/Pr	2.18	2.17	2.15	2.19	2.16	0.044

* SH = sunn hemp; RS= rice straw.

** Pretreatment: 1= control; 2 = $Ca(OH)_2$; 3 = NaOH; 4 = $Ca(OH)_2$+NaOH; 5 = NaOH+H_2O_2

Table 4. The effect of pretreatment forage on in vitro gas production (GP, mL/g OM) of mixed diet* after 48 h incubation.

	Pretreatment**					
Item	1	2	3	4	5	SEM
			SH-SM			
GP	59.36 ^{b}	64.26 ^{b}	70.99 ^{a}	71.43 ^{a}	73.16 ^{a}	1.694
IVDMD	61.73 ^{c}	65.79 ^{ab}	66.49 ^{ab}	63.88 ^{bc}	67.05 ^{a}	0.010
pH	5.41 ^{b}	5.47 ^{a}	5.45 ^{ab}	5.47 ^{a}	5.48 ^{a}	0.011
			SH-SDR			
GP	69.4 ^{a}	66.5 ^{a}	72.367 ^{a}	70.00 ^{a}	75.41 ^{a}	3.225
IVDMD	58.09 ^{b}	59.61 ^{b}	61.35 ^{b}	61.17 ^{b}	66.50 ^{a}	0.015
pH	5.49 ^{b}	5.62 ^{a}	5.57 ^{ab}	5.61 ^{a}	5.56 ^{ab}	0.027
			RS-SM			
GP	61.04 ^{b}	67.23 ^{a}	62.83 ^{a}	68.43 ^{a}	68.93 ^{a}	3.112
IVDMD	53.02 ^{a}	56.4 ^{a}	55.18 ^{a}	58.08 ^{a}	59.94 ^{a}	0.022
pH	5.35 ^{a}	5.30 ^{b}	5.34 ^{ab}	5.30 ^{b}	5.34 ^{ab}	0.014
			RS-SDR			
GP	52.80 ^{c}	65.56 ^{ab}	63.26 ^{bc}	75.07 ^{a}	72.93 ^{ab}	3.531
IVDMD	48.76 ^{c}	52.14 ^{bc}	53.33 ^{ab}	56.88 ^{a}	53.76 ^{ab}	0.012
pH	5.45 ^{ab}	5.40 ^{b}	5.50 ^{a}	5.44 ^{ab}	5.47 ^{a}	0.019

*SH = sunn hemp; RS= rice straw; SM = sesame meal; SDR = sorghum distillery residue

**Pretreatment: 1= control; 2 = $Ca(OH)_2$; 3 = NaOH; 4 = $Ca(OH)_2$+NaOH; 5 = NaOH+H_2O_2

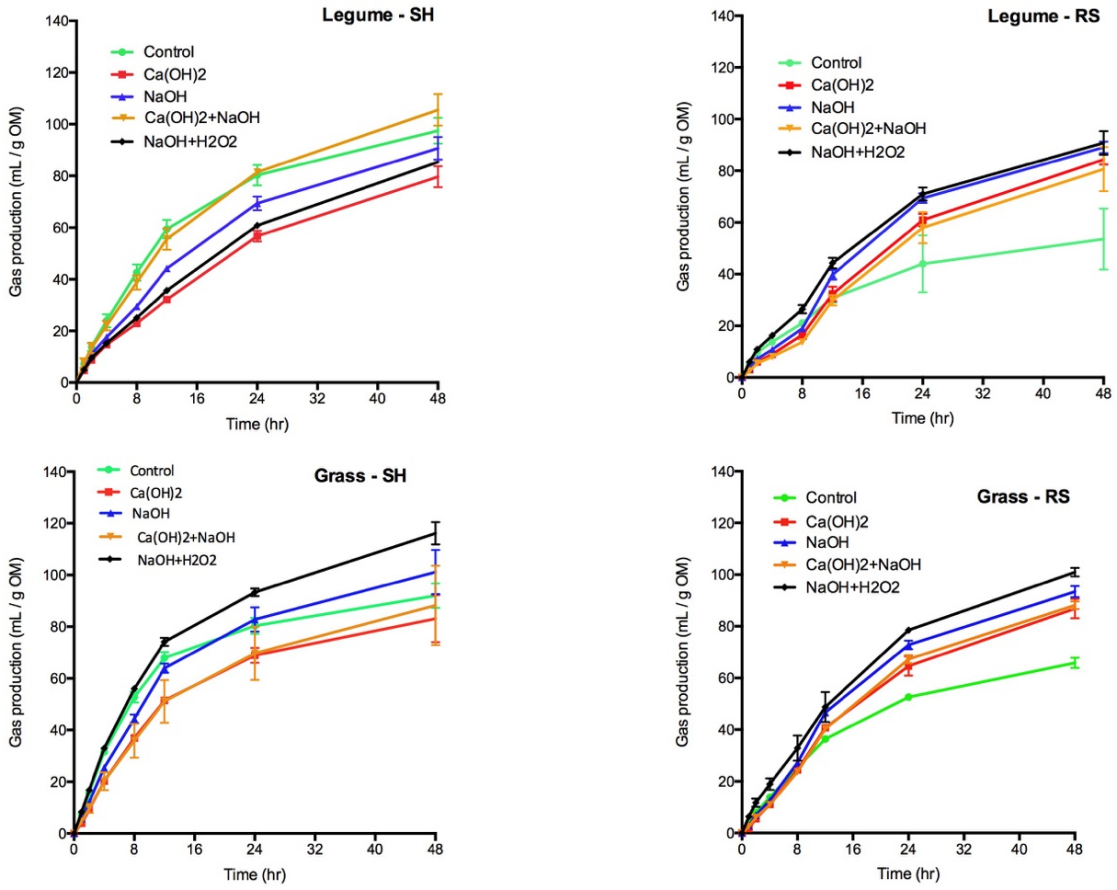

Figure 1. The effect of inoculation source on pretreatment forage gas production.

Grass = Bermuda hay feeding rumen fluid; Legume = alfalfa hay feeding rumen fluid; SH = sunn hemp; RS = rice straw.

Reference

AOAC. 2000. Official Methods of Analysis. Association of Official Analytical Chemist. EUA.

Blümmel, M., A. Karsli, and J. R. Russell. 2003. Influence of diet on growth yields of rumen micro-organisms *in vitro* and *in vivo*: influence on growth yield of variable carbon fluxes to fermentation products. Br. J. Nutr. 90, 625–634.

Cameron, M. G., G. C. Fahey, Jr., J. H. Clark, N. R. Merchen, and L. L. Berger. 1991. Effect of feeding alkaline hydrogen peroxide-treated wheat straw-based diets on intake, digestion, ruminal fermentation, and production responses by mid-lactation dairy cows. J. Anim. Sci. 69:1775-1787.

Cameron, M. G., G. C. Fahey, Jr., L. L. Berger, and N. R. Merchen. 1988. Comparison of sodium hydroxide-treated and alkaline hydrogen peroxide-treated wheat straw, manufactured using the horizontal mixer-sprayer method, on feed intake, growth performance, and nutrient digestibility by growing withers. *in*: Beef Cattle Res. Rep. *pp*.3-7. Univ. Illinois, Urbana-Champaign.

Castillejos, L., S. Calsamiglia, and A. Ferret. 2006. Effect of essential oils active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. J. Dairy Sci. 89:2649–2658.

Cone, J. W., A. H. Van Gelder, and H. Bachmann. 2000. Influence of inoculum source, dilution and storage of rumen fluid on gas production profiles. In: Gas Production: Fermentation Kinetics for Feed Evaluation and to Assess Microbial Activity. Proceedings of the EAAP Satellite Symposium on Gas Production, *pp.* 15-16. Proc. Br. Soc. Anim. Sci. Wageningen, Netherlands.

Goering, H.K., Van Soest, P.J., 1970. Forage fibre analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379, *pp.* 20. Agric. Res. Serv., USDA, Washington, DC, USA.

Kerley, M. S., G. C. Fahey, Jr., L. L. Berger, N. R. Merchen, and J. M. Gould. 1987. Effect of treating wheat straw with pH-regulated solution of alkaline hydrogen peroxide on nutrient digestion by sheep. J. Dairy Sci.70:2078-2084.

Kim, T. H. and Y. Y. Lee. 2007. Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. Appl. Biochem. Biotechnol. 136-140:81-92.

Meeske, R., H. H. Meissner, and J. P. Pienaar. 1993. The upgrading of wheat straw by alkaline peroxide treatment. The effect of NaOH and H_2O_2 on the site and extent of digestion in sheep. Anim. Feed Sci. Technol. 40:121–133.

Milne, J. A. 1977. A comparison of methods of predicting the *in vivo* digestibility of heather by sheep. J. Br. Grassland Soc. 32: 141–147.

Rao, S. C. and B. K. Northup. 2009. Capabilities of four novel warm-season legumes in the southern great plains: biomass and forage quality. Crop Sci. 49:1096-1102.

Reid, R. L., G. A. Jung, and S. Murray. 1964. Techniques *in vitro* and *in vivo* using the measurement of nutritive quality in a bluegrass pasture. J. Anim. Sci. 23: 700-710.

PO-04-39 Effects of providing fatty acid–calcium soap with high oleic acid content on fatty acid composition in Japanese Black steers

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INTRODUCTION

Demand has grown in recent years for animal products, with an emphasis on taste. Production methods to acquire beef with special characteristics are becoming the preferred way to fulfill this need. Fatty acid composition is one of the most important qualities of meat: of the fatty acids, oleic acid and unsaturated fatty acids (USFAs) are reportedly the ones most associated with flavor (Dryden & Marchello, 1970; Melton, Amiri, Davis, & Backus, 1982).

Fatty acid–calcium soap, a feed ingredient that bypasses the rumen, is commercially available. By avoiding hydrogen addition effects due to contact with rumen microbiota, fatty acid–calcium soaps effectively increase the absorption of USFAs in the lower gastrointestinal tract. However, some have reported that the addition of large amounts of fatty acid–calcium soap reduces feed palatability (Tamaki & Ishigaki, 1992; Naruse, Morita, & Hashiba, 1996). Based on these findings, feeding cattle with small amounts of fatty acid–calcium soap having high oleic acid content is expected to raise oleic acid content in beef without diminishing the palatability of feed.

We therefore decided to investigate the effects of providing Japanese Black steers with fatty acid–calcium soap (containing ~41% oleic acid by real weight), at the end of the fattening period, on the fatty acid composition of their intermuscular lipid.

MATERIALS AND METHODS

Animals, Experimental Period, and Feed

Eleven Japanese Black steers were used. The experimental period was set at four months prior to shipment; experiments began when steers were 27 months of age. From eight months of age, steers were provided with concentrated feed blended with bean curd lees at an original weight ratio of 50% (10–17 kg/d, Table 1), and rice-plant straw as roughage (1.0–1.5 kg/d). The breakdown of the ingredients in the concentrated feed by weight ratio was: 50% bean curd lees, 10% commercial concentrate (ICHIBAN, JA Higashi Nihon Kumiai Shiryou), 10% flaked barley, 10% flaked corn, 10% beet pulp, and 10% wheat bran. Concentrated feed was stirred to uniformity and then sealed for a week or more for lactic acid fermentation treatment.

Experimental Design

The fatty acid–calcium soap was derived from soybean and rapeseed oils (Bypass Mate L, Yuka Sangyo Co., Ltd., Table 2): the amount provided to steer was set at 135.5 g/d as described in an earlier study (Kobayashi & Ishizaki, 2011). All steers were provided identical feed until experiments started: the experimental group (5 animals) was then provided concentrated feed mixed with fatty acid–calcium soap daily from four months prior to shipment, while the control group (6 animals) was given feed lacking the fatty acid–calcium soap. During the experimental period, the amount of feed provided and the amount of feed left over were measured daily and feed intake calculated; body weight was measured monthly.

Blood Characteristics

Blood was sampled from the jugular vein at 27 months of age (immediately before experiments started), at 29 months of age, and at 31 months of age (immediately before shipment). After serum separation at 3,000 rpm for 30 min, general biochemical testing was carried out using a blood testing measurement device (IDEXX Vet Test, IDEXX Laboratories Co., Ltd.).

Dry matter andCrude Fat Content of Meat

Meat samples, 1 cm in thickness, were collected the day after slaughter from the longissimus thoracis muscle between the sixth and seventh thoracic vertebrae; meat was vacuum-sealed and cryopreserved at –40°C until analysis. Samples were thawed in a refrigerator for 24 h, starting the day before analysis. Samples were then minced, and their dry matter andcrude fat contents analyzed as described in a Technical Manual (Tanabe et al., 2000).

Fatty Acid Composition of Intermuscular lipid

Samples of fat were taken from the region between the longissimus thoracis and the trapezius muscle. After cryopreservation at –40°C, extraction and purification of the fat was carried out on the basis of the method of Bligh & Dyer (1959), and fatty acids were extracted by methyl esterification according to the method of Christopherson et al. (1969). The solution was analyzed by a gas chromatograph (GC-2010; Shimadzu Co., Ltd.; SP2560 column), and proportions of fatty acids calculated.

Statistical Analysis

Statistical analysis consisted of comparisons of mean observed values: for these, the Student's t-test was conducted using SPSS statistical processing software. Statistical significance for comparisons was set at $p<0.05$.

RESULTS AND DISCUSSION

Daily gain in body weight did not differ between the groups (experimental group, 0.56 kg/d; control group, 0.51 kg/d). No differences were observed in feed intake measures in terms of either concentrated feed intake (11.3 kg/d vs. 12.7 kg/d) or crude feed intake (1.0 kg/d vs. 1.1 kg/d). Some studies have reported fatty acid–calcium soap to worsen feed palatability (Tamaki & Ishigaki, 1992 ;Naruse et al., 1996). However, in this study, no significant differences in terms of body weight gain or feed intake measures were observed between groups.

Blood characteristics are shown in Table 3. Serum cholesterol at 31 months of age was significantly higher in the experimental group than in the control group. Our results seems similar to those reported by Takahashi et al. (2010), who found that feeding 180 g of fatty acid–calcium soap to Japanese Black cows significantly increased their serum cholesterol.

The fatty acid composition of intermuscular lipid is shown in Table 4. As a percentage of total fatty acids, C18:0 levels were significantly lower in the experimental group than in the control group (7.18% vs. 12.56%, respectively), while C18:1 levels were significantly higher (59.79% vs. 53.83%). In addition, total USFA levels, monounsaturated fatty acid (MUFA) levels, and unsaturated/saturated fatty acid ratio were significantly greater in the experimental group. Otawara et al. (2000) fed 300 g/d of fatty acid–calcium soap to Japanese Black steers for three months, and reported that compared with a control group, the experimental group had 8.9% higher oleic acid (47.4% vs. 38.5%) and 6.9% higher total USFAs (63.3% vs. 56.4%) as percentages of total fatty acids in subcutaneous fat. Miura et al. (1993) and Kobayashi & Ishizaki (2011) reported that feeding with fatty acid–calcium soap caused oleic acid levels, calculated as a percentage of total fatty acids, to increase in the longissimus thoracis muscle. Similar to these reports, in the present study, feeding with fatty acid–calcium soap, which has relatively high oleic acid content, is believed to have raised the proportions of USFAs and oleic acid within intermuscular lipid.

In conclusion, providing fatty acid–calcium soap with relatively high oleic acid content to Japanese Black steers during the end of their fattening period can elevate the proportions of USFAs and oleic acid in fat, without lowering production characteristics like feed intake, growth, and carcass grading.

Table 1. The composition of the feed [a]

Component	Lactic acid fermented feed, including bean card less 50%
Dry matter, %	52.6
Crude protein, %	17.6
Crude fat, %	4.3
Crude ash, %	4.1
Nitrogen free extract, %	63.1
Crude fiber, %	10.9

[a] Values except dry matter are expressed on a dry matter basis.

Table 2. Fatty acid profile of fatty acid-calcium soap

Item	Fatty acid-calcium soap
Total fatty acid, %	82.0
Fatty acids, % of total	
C14:0	0.7
C16:0	11.0
C16:1	0.8
C18:0	4.5
C18:1	50.0
C18:2	28.0
C18:3	5.0

Table 3. Blood Characteristics

Group	Age at which sampled (Mo)	Glu[a] (mg/dl)	BUN[a] (mg/dl)	T-Cho[a] (mg/dl)	AST[a] (U/L)	GGT[a] (U/L)
Experiment	27	76.4±3.7	15.0±1.2	217.4±10.1	62.8±3.0	40.8±4.1
	29	74.4±2.6	14.8±1.2	244.2± 9.3	59.4±3.0	38.6±3.7
	31	78.0±2.6	14.2±1.1	240.4±14.2*	69.0±5.6	41.2±4.3
Control	27	70.3±3.8	16.0±2.5	265.5±40.2	95.3±35.8	80.0±42.4
	29	71.5±5.4	14.8±2.0	229.8±11.9	66.0±9.5	37.0±3.2
	31	70.5±2.3	14.3±1.1	188.3± 9.5	68.8±3.0	30.0±2.5

Means±SE

* indicates a significant difference compared with the control group (P<0.05)

[a] Glu=Glucose, BUN=Blood urea nitrogen, T-Cho=Total cholesterol, AST=Aspartate transaminase, GGT= γ -glutamyltransferase

Table 4. Carcass characteristics, chemical analysis of longissimus muscles and fatty acid composition of intermuscular lipid

Item	Experiment	Control
Carcass weight, kg	520.2±11.9	496.2±18.6
Beef marbling standard [a]	9.40±0.68	8.43±0.30
Dry matter, %	57.16±1.76	57.73±0.62
Crude fat, %	44.47±2.62	44.31±0.67
Fatty acids, % of total		
C14:0	2.26±0.24	2.46±0.11
C16:0	20.88±1.20	23.51±0.38
C16:1	5.91±0.66*	3.68±0.37
C18:0	7.18±0.74*	12.56±1.21
C18:1	59.79±1.77*	53.83±1.10
C18:2	2.75±0.12	3.27±0.29
C18:3	0.27±0.03	0.47±0.18
SFA [b]	30.61±1.96*	38.60±1.07
USFA [b]	69.39±1.96*	61.40±1.07
MUFA [b]	66.34±2.08*	57.65±1.50
PUFA [b]	3.04±0.14	3.75±0.46
USFA/SFA [b]	2.32±0.21*	1.60±0.07

Means±SE

*indicates a significant difference compared with the control group (P<0.05)

[a] Range: 1–12, 1 =None to minimum marbling, 12 = Highest marbling

[b] SFA=Saturated fatty acids, USFA =Unsaturated fatty acids, MUFA=Monounsaturated fatty acids, PUFA=Polyunsaturated fatty acids

REFERENCES

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. http://doi.org/10.1139/o59-099

Christopherson, S. W., & Glass, R. (1969). Preparation of milk fat Methyl esters by alcoholysis in an essentially nonalcoholic solution. *Journal of Dairy Science*, *52*(8), 1289–1290.

Dryden, F. D., & Marchello, J. A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. *Journal of Animal Science*, *31*(1), 36–41.

Kobayashi, M., & Ishizaki, S. (2011). Effects of raw rice bran or calcium soap of fatty acids supplementation at finishing stage of fattening on meat quality of Japanese Black steers. *Bulletin of the Chiba Prefectural Agriculture Research Center*, *11*, 77–80.

Melton, S. L., Amiri, M., Davis, G. W., & Backus, W. R. (1982). Flavor and chemical characteristics of ground beef from grass-, forage-grain- and grain-ffnished steers. *Journal of Animal Science*, *55*(1), 77–87.

Miura, Y., Yagi, I., Yamada, T., & Kondho, I. (1993). Effect of bypass fatty acid calcium on fattening cattle. *Bulletin of Beef Cattle Science*, *56*, 13–14.

Naruse, M., Morita, H., & Hashiba, K. (1996). The effects of supplementation of calcium soap of fatty acids on meat quality in dairy steers. *Research Bulletin of the Aichi Agricultural Research Center*, *28*, 335–339.

Otawara, K., Nishida, K., Fujiwara, T., & Onodera, T. (2000). Effects of bypass fatty-acid calcium on the meat production of Japanese Black steers. *Bulletin of the Iwate Agricultural Research Center*, *1*, 53–58.

Takahashi, M., Kawate, N., Sawada, K., Inaba, T., & Tamada, H. (2010). The effect of the bypass unsaturated fatty acids on the superovulation and conception rate of collected embryos of Japanese Black breeding cattle. *Japanese Journal of Large Animal Clinics*, *1*, 76–77.

Tamaki, M., & Ishigaki, I. (1992). Preference of fatty acid calcium in cattles. *Bulletin of the Okinawa Agricultural Experiment Station*, *30*, 41–45.

Tanabe, S., Amari, M., Masaki, S., Oshio, S., Kobayashi, R., Ishida, M., … Yoshida, N. (2000). *Techinical Manual for Feed Analysis*. Japan Livestock Technology Association.

PO-04-41

Carcass Commercial Cuts Percentage of Ram Raised Under Different Energy-Protein Ratio Feeding and Different Slaughtered Weight

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ABSTRACT

A study was carried out to determine the effect of dietary energy-protein ratio on the carcass commercial cuts percentage (flank, leg, loin, rib, breast, shoulder-neck and shank) of rams at different slaughter weight. The study used 24 thin tail rams aged 3-5 months and weighed 8.7 to 15.5 kg (CV = 15.01%). A Generalized Randomized (Complete) Block Design was used in this study with 4 different feeding treatments, i.e. R1 = 14.48% crude protein (CP) and 50.46% *total digestible nutrients* (TDN), R2 = 17.35% CP and 52.61% TDN, R3 = 15.09% CP and 58.60% TDN, and R4 = 17.42% CP and 57.46% TDN. Rams were grouped based on the initial body weight, i.e. B1 = 10,73 + 1,37 kg (slaughtered at 15 kg), B2 = 12,76 + 0,54 kg (slaughtered at 20 kg) and B3 = 14,91 + 0,36 kg (slaughtered at 25 kg). The results showed that slaughter weight, cold carcass weight, cold carcass percentage, and carcass commercial cuts percentage (except flank) were not significantly different (P>0.05) among feeding treatments. In average, the animals had 20 kg slaughter weight, 8909.58 g cold carcass weight, 44.18% cold carcass percentage. Whilst, the average of percentage of shoulder-neck, leg, loin, rack, breast and shank were 32.05; 34.23; 9.32; 8.70; 9.14; and 4.05%, respectively. On the other hand, the highest percentage flank was found inR1 (2.70%), followed by R3 (2.56%), R2 (2.40%) and R4 (2.26%). Cold carcass percentage increased (P<0.05) with increasing slaughter weight. The percentage of leg and shank decreased (P<0.05) with increasing slaughter weight, while the percentage of other commercial cuts are not significantly different (P>0.05) among slaughter weights. The conclusion of this study is that energy-protein ratio of the feed does not affect the percentage of commercial cuts (except flank), while the slaughtered weight affects the percentage of carcass, leg, and shank.

INTRODUCTION

The carcass is the main yield expected from the sheep. The carcass yield measurements, both relative and actual weights are important as these are the criteria used to evaluate animal productivity. The carcass is the result of a biological process affected by genetic, environmental and management factors (Cardoso et al., 2013).

Carcass weight of sheep is affected by slaughter weight, which in turn is affected by feed intake. Protein and energy are the main nutrients required by the animal. Protein is found in all living cells, where they are intimately with all phases of activity that constitute the life of cell. Dietary energy is used for production after satisfying the requirement of maintenance. A young growing animal stores protein in new tissues, while an adult stores relatively more energy in fat (McDonald et al., 1991). Energy and protein interact because dietary protein is a source of dietary energy, because dietary energy is needed for protein turnover and deposition and because deposited protein represents part of the body's energy store (Boorman, 1980).

Carcass traits are greatly modified by slaughter weight (Galvani et al., 2008). Hot carcass weight of Barki lambs increased significantly (P<0.01) with increasing slaughter weight from 30 to 60 kg (Shehata, 2013).Similiar results were reported by Galvani et al. (2008), that dressing percentage of Texel x Ile de France crossbred feedlot lambs increased linearly with increased slaughter weight (P<0.01).

The proportions of the carcass cuts are an important index for the commercial evaluation of the carcass and have different economic value. Factors such as genetics, diet, slaughter weight, sex among others, are responsible for differences in cuts between carcasses (Cardoso et al., 2013). This study was carried out to determine the effect of feed energy-protein ratio of complete feed on the carcass commercial cuts percentage (flank, leg, loin, rib, breast, shoulder-neck and shank) of ram at different slaughtered weight.

MATERIALS AND METHODS

This study used 24 thin-tailed rams, aged 3-5 months and weighed 8.7 to 15.5 kg (CV = 15.01%). The rams were kept in individual pens and fed a diet composed of rice straw (25%), and a concentrate mix 75% (fish meal, soybean meal, *Leucaena leucocepala* leaf meal, rice bran, cassava meal, mollasess, and mineral), and formulated according to treatments.

A Generalized Randomized (Complete) Block Design was used in this experiment with 4 different feeding treatments, i.e. R1 = 90.73% dry matter (DM), 14.48% crude protein (CP) and 50.46% *total digestible nutrients* (TDN), R2 = 90.82% DM, 17.35% CP and 52.61% TDN, R3 = 89.01% DM, 15.09% CP and 58.60% TDN, and R4 = 90.11% DM, 17.42% CP and 57.46% TDN. Rams were grouped based on the initial body weight, i.e. B1 = 10,73 + 1,37 kg (slaughtered at 15 kg), B2 = 12,76 + 0,54 kg (slaughtered at 20 kg) and B3 = 14,91 + 0,36 kg (slaughtered at 25 kg). Dry matter intake (DMI), CP intake, and TDN intake were recorded.

The rams were slaughtered after a 24 hour fasting period. Before being slaughtered, the animals was weighed individually. The animals were killed by cutting their jugular vena, throat and esophagus removing . The carcass was obtained after removal of the head, feet, skin, digestive tract and internal organs, except kidneys and kidney fat. The carcass was weighed (hot weight), then two hours later the carcass was reweighed (cold weight). The carcass was then halved longitudinally by a band saw, after the removal of tail, kidneys and kidney fat. Right carcass half was then cut into seven joints: flank, leg, loin, rack, breast, shoulder with neck, and shank (Figure 1). The percentage of each cut was calculate. Analysis of variance and Duncan's Multiple Range Test were used to analyze the data (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The main characteristics of sheep carcasses from different energy-protein ratio feeding are presented in Table 1. The results showed that slaughter weight, cold carcass weight, cold carcass percentage, and carcass commercial cuts percentage (except flank) were not significantly different (P>0.05) among treatments.

The non-significant difference in carcass percentage and carcass commercial cuts percentage in this study occurred because of the fact that the slaughter weight and cold carcass weight no significant differences (P>0.05). This was in accordance with the opinion of Soeparno (2005), that the weight of carcass weight affects carcass commercial cuts. Slaughter weights, carcass weights and dressing percentages in this study were not significantly different (P>0.05) among the treatments, because the energy intake was not significantly different (P>0.05) either. According to Blakely and Bade (1985), the main nutrients needed for fattening animals is energy. Rianto et al. (2006) stated that an increase in dietary energy intake will be followed by an increase in energy deposition in the body, increasing energy deposition will be used to accelerate the rate of metabolism and establish fat deposition. The dietary energy intake of sheep in this study were similar, so that the energy deposited was also relatively the same.

The percentage of flank of R4 was the lowest (P <0.05), followed by R2, R3 and R1. This was so because the weights of flank in R4was the lowest, but the carcass weight was the highest. Hasnudi (2004) reported that while the empty body weight increased, the flank weight was relatively stable, so the flank percentage was getting lower as the body weight increased.

Data in Table 2 show that carcass weight and percentage increased with slaughter weight. These findings were in agreement with the statement of Cardoso et al. (2013), that animal carcass production is influenced by slaughter weight, which in turn is affected by the feed intake. An increase in feed intake will result in higher slaughter weight.

The percentage of leg and shank were significantly different (P <0.05) among slaughter weights. The percentage of leg and shank of B3 were the lowest, followed by B2 and B1. These findings indicated that leg and shank were early mature compared with other parts of the body. This was in agreement with the statement of Tillman et al. (1991), that head and leg bones reach maturity faster than bones of shoulder, pin bones and muscles. Thus while the other parts of the grow, leg and shank stop growing at certain stage of growth, so that the percentage of leg and shank are lower than the other parts of carcass. This results is also confirmed by the results obtained by Tobing et al. (2004), that the weight of head, feet and viscera decline their growth rate at the beginning of life, while the other parts still continue to grow. Consequently, the weight of leg and shank did not increase with the increasing slaughter weight, resulting in low percentage of leg and shank in higher slaughter weight as occurred in animals of B3.

CONCLUSIONS

The conclusion of this study is that dietary energy-protein ratio does not affect the percentage of commercial cuts (except flank) of rams, while rams slaughter at higher body weight had higher carcass percentage, but lower leg and shank percentage.

Keywords: Sheep, dietary energy-protein ratio, slaughter weight, carcass commercial cuts

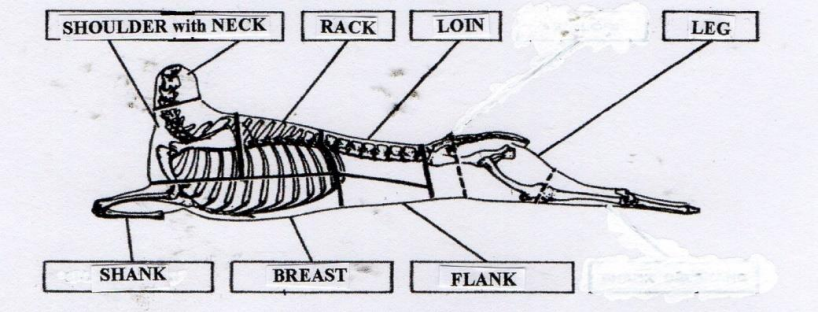

Figure 1. Carcass commercial cuts (Soeparno, 2005)

Table1. Slaughter weight, cold carcass weight, dressing percentage, carcass commercial cuts percentage, and dry matter intake, crude protein intake, and TDN intake of ram raised under different dietary energy-protein ratio

Variables	R1	R2	R3	R4
Slaughter weight (kg)	20.42[a]	19.58[a]	20.05[a]	19.97[a]
Cold carcass weight (g)	8,966[a]	8,631[a]	8,898[a]	9,142[a]
Dressing percentage (%)	43.91[a]	44.08[a]	44.38[a]	45.78[a]
Percentage of carcass commercial cuts (%)				
- Shoulder with neck	33.09[a]	31.90[a]	31.24[a]	32.46[a]
- Leg	33.83[a]	34.12[a]	34.72[a]	34.03[a]
- Loin	9.30[a]	9.22[a]	9.08[a]	9.80[a]
- Rack	8.31[a]	8.71[a]	8.73[a]	9.22[a]
- Breast	8.80[a]	9.62[a]	9.63[a]	8.37[a]
- Flank	2.70[c]	2.40[ab]	2.56[bc]	2.26[a]
- Shank	4.00[a]	4.05[a]	4.05[a]	3.87[a]
DMI (g/day)	956.35[b]	966.94[b]	827.94[a]	850.55[a]
CP intake (g/day)	138.51[b]	140.04[b]	119.91[a]	123.18[a]
TDN intake (g/day)	480.24[a]	535.09[a]	499.99[a]	345.48[a]

[a, b, c] Different letters in the same raw are significantly different (P<0,05), using Duncan test

Table2. Slaughter weight, cold carcass weight, dressing percentage, carcass commercial cuts percentage, and dry matter intake, crude protein intake, and TDN intake of ram at different slaughter weight

Variables	B1	B2	B3
Slaughter weight (kg)	15.09[a]	19.86[b]	25.06[c]
Cold carcass weight (g)	6,266.75[a]	8,918.75[b]	11,543.25[c]
Dressing percentage (%)	41.52[a]	44.93[b]	46.07[b]
Percentage of carcass commercial cuts(%)			
- Shoulder with neck	31.92[a]	31.98[a]	32.62[a]
- Leg	34.94[b]	34.25[ab]	33.33[a]
- Loin	9.11[a]	9.52[a]	9.42[a]
- Rack	8.76[a]	8.63[a]	8.84[a]
- Breast	8.34[a]	9.37[a]	9.60[a]
- Flank	2.45[a]	2.45[a]	2.54[a]
- Shank	4.49[b]	3.83[ab]	3.65[a]
DMI (g/day)	711.91[a]	913.87[b]	1,075.56[c]
CP intake (g/day)	103.10[a]	132.36[b]	155.77[c]
TDNintake (g/day)	381.95[a]	396.94[a]	616.71[a]

[a, b, c] Different letters in the same raw are significantly different (P<0,05), using Duncan test

REFERENCES

Blakely, J. and D.H. Bade, 1985. The Science of Animal Husbandry.4th Edition. Englewood Cliffts, New Jersey

Boorman, K.N. 1980. Dietary constraints on nitrogen retention.In: P.J. Buttery and D.B. Lindsay. Protein Deposition in Animals. Butterworths, London. Page: 147-166

Cardoso, M.T.M., A.V. Landim, H. Louvandini, and C. McManus. 2013. Performance and carcass quality in three genetic groups of sheep in Brazil. R. Bras. Zootec. 42 (10) : 734 – 742.

Galvani, D.B., C.C. Pires, T.P. Wommer, F. deOliveira, A.M.S. Bolzan, and P. Francios. 2008. Carcass traits of feedlot crossbreed lamb slaughtered at different live weight. Ciencia Rural, Santa Maria. 38 (6) : 1711 – 1717.

Hasnudi, 2004.The relative growth of the commercial carcass cutting of SungeiPutih and Local Sumatera sheep feed on palm oil by-product. Journal of the Indonesian Tropical Animal Agricultural. 29 (3) : 115-120.

Mc.Donald, R.A. Edward, and J.F.D. Greenhalgh. 1991. Animal Nutrition. 4th Ed. John Wiley and Sons, Inc., New York.

Rianto E., E. Lindasari and E. Purbowati. 2006. Growth and physical components of thin tailed ram carcass raised under different rice bran feeding.Animal Production 56 (8) : 28-33

Shehata, M.F. 2013. Carcass Characteristics of Barki Lambs Slaughtered at Different Live Weights. Journal of American Sci. 9 (6) : 656 – 664

Soeparno. 2005. Meat Science and Technology. GadjahMada University Press, Yogyakarta.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics..McGraw-Hill, Inc., New York

Tillman, A. D., H. Hartadi, S. Reksohadiprojo, S. Prawirakusuma and S.Ledbosukotjo. 1991. Livestock Food Science Basis. GadjahMada University Press, Yogyakarta.

Tobing, M. M., C. M. S. Lestari and S. Dartosukarno. 2004. The proportion of carcass and non carcass local male sheep using elephant grass feed with varying levels of tofu by-product. Journal of the Indonesian Tropical Animal. Special Ed. Oct 2004 : 90-97.

PO-04-45

Breeding of Napiergrass（Pennisetum purpureum）Taisu no.6

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ABSTRACT

Napiergrass（*Pennisetum purpureum*）line 7768 is one of the progeny of Tift85 DB（pearl millet）crossed with the line Mott of napiergrass. Which were selected and tested after serval different comparative experiments, i.e., regional trial, managemental trials and animal feeding trial, respectively. The results showed those of the line 7768 had significantly higher crude protein (CP) as compared to Napiergrass Taisu no.3 (NPcv.TS3) (12.0 % vs 10.8 %). There are 31.8 % and 60.7 % of acid detergent fiber (ADF) and neutral detergent fiber (NDF) of line 7768, significantly lowerer 33.2 % and 60.9 % of NPcv.TS3. The fresh yield 130.2 mt $ha^{-1}year^{-1}$ of line 7768 is significantly lower than that of NPcv.TS3. No significantly different between line 7768 and NPcv.TS3 on *in vitro* dry matter digestibility (IVDMD) value. There are not significantly different of hay feeding trial and parameters of blood analysis between feed with Timothygrass (*Phleum pratense*) and line 7768. According to the results above, line 7768 was named Napiergrass Taisu no.6 (NPcv.TS6) on May 2015.

INTRODUCTION

Napoergrass（*Pennisetum purpureum*）is one of the importmrnt forage crop in Taiwan. It has herbage yield, strong competition and persistence, and also has some defect in quality, hairy leaves and sheaths, small seeds and shedding readily. The breeding program have been conduced since 1978 and introduced germplasms from Philippines to Taiwan in 1961. Accronding to different animal inneed or market target there are selected and named Napiergrass Taisu no.1 to no.5（NPcv.TS1 - 5）since 1996 to 2011. Pearl millet（*P. americanum*）line Tift#1S-1 and Napiergrass line A146 used for parents, pass through different selected experiments and was named NPcv.TS1 in 1991 (Cheng, 1991). NPcv.TS2 produced fresh yield 270 mt/ha/yerarly, which parent were used from lines A146 and A149 of napiergrass and be name in 1999. NPcv.TS2 also was adapted machine to harvest in Taiwan. NPcv.TS3 used dwarf napiergrass 'Mott' for patent, which is both yield, quality and heigh leaf/stem ratio, and named in 2009 (Cuomo *et al*., 1996). NPcv.TS4 used NPcv.TS2 and local purpureum napiergrass for parents, which has 300 mt/ha/yerarly heighest fresh weighth among all napiergrass, also was named in 2010. NPcv.TS5 was collected purpureum napiergrass germolasms from Taiwan and named in 2011. It had higher fresh yield for ruminantss and higher anthocyanin content for ingredient. In this program objective was to breed one species has higher quality for feeding herbivores of pet.

MATERIALS AND METHODS

Line 7768 parents was line'Mott' of napiergrass, which was introduced from USA in 1986. 7768 was the progent and selection from the Mott self-pollinant and pass through the serval different comparative experiments, i.e., regional trial, managemental trials and animal feeding trial, respectively (Table 1). The methods of plant chemical content analysi of entries were by Olsen and Dean (1965) and Thomas (1985).

RESULTS AND DISCUSSION

Above the results of different regional trial, the results were showed those of (Table 2) the toppest height of last leaf collar (THC), plant height of leaf tip (PHL), fresh weight (FW) and dry weight (DW) of NPcv.TS2 was highest and shortest was line 7768 among all entries, respectively. Also, stem diameter (SD) strongest of NPcv.TS3 and thinnest was line 7768. But, tiller number (TN) 153.7 and leaf stem ratio (LS) 1.81 of line 7768 was heightest among entries, respectively.

The chemical contents analysis and *in vitro* dry matter digestibility (IVDMD) values of entries were showed on Table 3. The highest 12 % of crude protein (CP) was line 7768 and lowest 7.6 % was NPcv.TS2. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were highest 39.4 % and 69.4 % of NPcv.TS2 among all entreis, respectively. The lowest contest values 31.8 and 60.6 % from line 7768, respectively. On IVDMD value, the highest values were both NPcv.TS3 68.3 % and line 7768 68.2 % among all entries.

On the Table 4 is showed the effect of parameters of blood analysis on different forage hay by rabbit. Above the result was showed the significantly different Calcium (Ca) of blood between feeding with Timothygrass and Bermudagrass. The result showed that of the Ca of blood conteration value was higher than feeding Bermudagrass had. ca of blood conteration value of feeding with line 7768 was not significantly different with Timothygrass of 9 weeks age old.

CONCLUSION

According to the results above, line 7768 was the first napiergrass breeding method and objective for feeding herbivores of pet in Taiwan. It also was named Napiergrass Taisu no.6 (NPcv.TS6) on May 2015.

Keywords：*Pennisetum purpureum*, NPcv.TS6, Breeding, Herbivore

Table 1. Time, region and content of all experiments of line 7768

Treatment	date	Lacation	Material and meathod
Pollination and selection	Nov.,1986 - Jul.,1988	Tainan	1. Pearl millet "Tift85 DB"×Napiergrass"Mott" 2. Agronomic traits evaluation
Line comparative experiment	Aug.,1988 - Jul.,1989	Tainan	1. Mott and NPcv.TS1 with control 2. Agronomic and yield evaluation
Regional experiment	Aug.,1989 - Jul.,1993	Pintung, Kaohsiung, Shinchu, Changhua, Taitun, Hualien	1.Eight lines within trial with "Mott" and "NPcv.TS1" as control 2.Agronomic trailts, yield and chemical contents evaluation
Animal feeding trial for chicken	Jan.,2008-Dec.,2008	Hualien	Eight lines within trial to feed Hualien local chicken
	Jan.,2009-Dec.,2009	Tainan	Four lines within trial to feed TLRI chicken no.13
Animal feeding trial for rabbit	Jan.,2000-Dec.,2002	Tainan	1.Feeding trial of Line 7768 with timothygrass and bemudagrass as control 2.Blood parameters analysis
Yield trial	Jan.,2013-Dec.,2014	Tainan	Three lines within trial
DNA analysis	Jan.,2015-Apr.,2015	Tainan	Line 7768 and other 5 napiergrass cultivars within trial

Table 2. Average data of agronomic characters and yield of entries from different areas

Line	THC[#]	PHL	SD	TN	LN	LS	FW	DW
	----- cm ----		mm	no./clone	no./tiller		-- mt/ha/y --	
7718	50.1	115.4	9.3	27.9	7.3	1.20	155.0	25.8
7728	54.3	119.7	9.0	39.5	8.5	1.28	240.8	41.6
7754	38.3	79.2	6.9	60.8	8.2	1.47	173.1	31.0
7768	14.6	42.6	4.7	153.7	7.3	1.81	130.2	22.9
Mott	61.4	118.9	7.7	41.3	8.6	1.20	226.5	39.5
NPcv.TS1	44.5	83.5	8.3	45.8	9.0	1.19	163.1	29.6
NPcv.TS2	86.6	174.8	10.5	22.8	9.1	0.84	315.3	53.0
NPcv.TS3	33.5	81.5	10.5	37.8	9.3	1.61	228.5	38.0

[#] THC: toppest height of last leaf collar, PHL: plant height of leaf tip, SD: stem diameter, TN: tiller number, LN: leaf number, LS: leaf stem ratio, FW: fresh weight, DW: dry weight.

Table 3. Average data of chemical content of entries from different areas

Line	CP[#]	ADF	NDF	IVDMD
	----------		%	----------
7718	10.4	34.7	64.0	64.2
7728	9.2	37.1	67.0	63.4
7754	10.7	35.2	65.1	65.5
7768	12.0	31.8	60.7	68.2
Mott	9.2	37.3	67.2	63.4
NPcv.TS1	10.1	36.0	66.4	65.1
NPcv.TS2	7.6	39.0	69.4	58.6
NPcv.TS3	10.8	33.2	60.9	68.3

[#] CP:crude protein, ADF:acid detergent fiber, NDF:neutral detergent fiber, IVDMD:*in vitro* dry matter digestibility

Table 4. Effect of parameters of blood analysis on different forage hay by rabbit

9 Weeks age	Bermudagrass	Timothygrass	7768
Asparate aminothransferase (AST)	17.8 ± 3.4	17.6 ± 3.1	16.3 ± 2.7
Alanine aminothranferase (ALT)	31.9 ± 10.7	35.4 ± 11.3	35.4 ± 9.3
Total serum protein (TP)	5.32 ± 0.54	5.72 ± 0.26	5.59 ± 0.60
Albamin (ALB)	4.29 ± 0.63	4.56 ± 0.38	4.51 ± 0.70
Globulin	1.03 ± 0.40	1.16 ± 0.31	1.08 ± 0.19
Albumin/ Globumin (A/G)	4.64 ± 1.51	4.25 ± 1.35	4.36 ± 1.28
Blood urea niotrgen (BUN)	14.7 ± 3.8	14.8 ± 3.0	15.5 ± 2.4
Cholesterol (CHOL)	54.2 ± 13.6	58.8 ± 17.2	61.3 ± 18.0
Tryglyceride (TG)	81.3 ± 35.0	100.6 ± 31.6	102.8 ± 44.5
Calcium of blood (Ca)	14.0 ± 0.5 [b]	14.6 ± 0.4 [a]	14.4 ± 0.7 [ab]
pH value of blood	7.88 ± 0.85	7.93 ± 0.74	7.88 ± 1.03
11 Weeks age	Bermudagrass	Timothygrass	7768
Asparate aminothransferase (AST)	17.9 ± 2.7	20.7 ± 5.9	17.3 ± 2.9
Alanine aminothranferase (ALT)	28.0 ± 8.0	30.0 ± 12.8	37.9 ± 15.3
Total serum protein (TP)	5.95 ± 0.40	5.83 ± 0.58	5.83 ± 0.33
Albamin (ALB)	4.77 ± 0.47	4.69 ± 0.63	4.82 ± 0.36
Globulin	1.17 ± 0.32	1.13 ± 0.25	1.02 ± 0.27
Albumin/ Globumin (A/G)	4.36 ± 1.22	4.34 ± 1.04	5.08 ± 1.63
Blood urea niotrgen (BUN)	18.1 ± 3.3	19.4 ± 4.4	18.0 ± 2.1
Cholesterol (CHOL)	46.1 ± 6.9	54.7 ± 13.2	50.3 ± 13.6
Tryglyceride (TG)	70.6 ± 38.1	85.8 ± 30.9	80.7 ± 19.7
Calcium of blood (Ca)	14.4 ± 0.7	14.4 ± 0.8	14.5 ± 0.6
pH value of blood	7.49 ± 0.47	7.22 ± 0.79	7.28 ± 0.52

[a,b] Means is the same row with different superscript differed significantly ($P < 0.05$).

REFERENCE

Cheng. Y. K. 1991. Forage breeding in Taiwan. AJAS 4: 203-209.

Cuomo, G. J., D. C. Blouin and J. F. Beatty. 1996. Forage potential of dwarf napiergrass and a pearl millet x napiergrass hybrid. Agron. J. 88: 434-438.

Olsen, S. R. and L. A. Dean. 1965. Phosphorus, pp. 1035-1048. *In* Black, C. A. (ed) Method of Soil Analysis. part 2. Am. Soc. Agrono. Inc., Madison, Wisconsin.

Thomas, G. W. 1985. Exchangeable cation. pp. 159-165. *In* Page, A. L. (ed) "Method of Soil Analysis" part 2. Am. Soc. Agron. Inc., Madison, Wisconsin.

PO-04-46

Physical and Growth Characteristics of Japanese 'Kuchinoshima Feral Cattle', a Native Cattle Breed

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Introduction

Four types of Wagyu beef cattle (Japanese Short horn, Japanese Polled, Japanese Brown, and Japanese Black) were established by breeding foreign to Japanese native breeds. These breeds have modified growth rate and the carcass traits, which resulted in the achievement of modern Japanese Wagyu beef production. Among these breeds, Japanese Black (JB) cattle contributes the most to Wagyu beef production in Japan, and is well known to produce high-quality meat that contains a large amount of fat within the muscles (Gotoh, 2003). To make shown this fat accumulation trait, Japanese black are generally needed to fatten by the specific long-term feeding system with high concentration of concentrate feed. This intensive fattening system may be adapted only to Japanese black, since the foreign type cattle fattened by the equal feeding condition shows less fat contain and significant smaller marbling flecks in longissimus muscle than Japanese black (Albrecht et al., 2011). These results suggest that Japanese black has higher genetic potential to produce the marbling beef than foreign type cattle and some specific genes, probably derived from Japanese natives, may play an important role on it.

There are two breeds of Japanese native cattle: Mishima cattle (MC) and Kuchinoshima feral cattle (KFC). The MC and KFC are thought to have originated from a common ancestor, which had migrated from the Asian continent to Japan approximately 1500-2000 years ago, and both breeds completely diverged from the European Hereford and Holstein strains (Mannen et al., 2004; Tsuda et al., 2013). MC has been protected as a national natural monument on Mishima Island in Yamaguchi Prefecture, and their physical and genetic characteristics have been investigated. It was revealed that MC have been maintained as an isolated population on Mishima Island for about 200 years (Nagamine et al., 2008). The MC body weight ranges from 250 kg for mature females (Nagamine et al., 2008) to 517 kg for fattened steers (Morita et al., 2000) therefore, steers are extremely small compared with fattened JB steer, which can reach about 800 kg at 30 months of age. However, the features of KFC, which inhabit Kuchinoshima Island in Kagoshima Prefecture, have not been studied in detail. The KFC coat colors include black, brown and white spotted, which are characteristic of ancestral cattle that were drawn in an ancient document from the 13th century (Figs. 1 and 2) (Kawahara-Miki et al., 2011). The KFC also have smaller body sizes compared with JB, but its weight is similar to that of MC, which have 300 kg females to 500 kg males. Some genetic investigations related to KFC origin, divergence, and polymorphisms have been conducted (Shimogiri et al., 2006; Kawahara-Miki et al., 2011; Kanemaki and Ando, 2013), and Kawahara-Miki et al. (2011) suggested that KFC would be ideal for investigating adaptations associated with the domestication of wild cattle, because specific genes may exist in the KFC population that are associated with molecular function.

Like MC, KFC is one of the rarest and most endangered animal genetic resources in the world, so it is important to adequately preserve this breed. However, there is little information available on KFC characteristics, especially regarding their physical and growth characteristics. Therefore, we tracked age-related changes in KFC body measurements and analyzed allometric growth during their first 40 months of life in this study.

Materials and Methods

A total of eight KFC (four intact bulls and four steers) reared on the Iriki Experimental Farm at the Faculty of Agriculture, Kagoshima University were used in this study. All calves were separated from their dams and fed artificial colostrum immediately after birth. The calves were bottle-fed until 77 days of age and simultaneously provided with starter rations. After weaning, the calves were fed concentrates (CP: 15-16%, TDN: 70%) and roughage (timothy hay, oats hay, Italian ryegrass silage and Japanese millet silage) to meet the nutritional requirements for maintenance and growth according to the Japanese Feeding Standard for Beef Cattle (NARO, 2009). The cattle were reared with ad libitum access to water and minerals. Body measurements of all cattle (body weight, withers height, hip height, body length, shoulder length, heart girth, chest depth, chest width, abdominal girth, rump length, hip width, thurl width, pin bone width, and shank circumference) were recorded monthly from

birth until 40 months of age. These data were used to calculate the relative growth coefficients and constants in allometric equations between body weight and other body scale measurements ($\ln Y = a + b \ln X$, Y: body scale, X: body weight). Student t test were used to compare the relative growth coefficient (the value of b) to 0.333. Using these equations, Y corresponds to $\sqrt[3]{X}$ (i.e., lnY corresponds to 0.333lnX) when both elongation and hypertrophy of body parts equally contribute to body weight gain. A relative growth coefficient, that significantly greater than 0.333 indicates that elongation contributes more to body weight gain.

Result and Discussion

Changes in JB and KFC body weight with increasing age are shown in Fig. 3. The average birth weights of the KFC bulls and steers were 19.5 kg and 18.4 kg, respectively, which was more than 10 kg smaller than the standard birth weights of the JB bulls (39.0 kg) and steers (29.9 kg) (Wagyu Registry Association, 2004). The JB growth rate, which was increased for meat production, showed fast growth during the first 20 months of life followed by retarded growth in both sexes (Fig. 3). Growth rate of JB decreased every 10 months in both sexes, and their average daily gain (DG) through the first 40 months of life were 0.56 kg/d for bulls and 0.35 kg/d for steers, respectively (data not shown). In contrast, the growth rate of KFC was slower than that of JB. KFC steers showed the more gradual growth but their growth curve was similar to that of JB (Fig. 3); that is, growth rate of KFC steers decreased after 20 months of age, which resulted in a lower DG (0.22 kg/d) (data not shown). The body weight of the KFC bulls increased gradually during the experimental period, and their DG was similar to that of the JB steers (0.37 kg/d, data not shown). The body weights of the KFC bulls and steers were approximately 450 kg and 300 kg, respectively, at 40 months of age, although the body weights of the JB reached 709 kg in bulls and 452 kg in steers at the same age (Fig. 3). Therefore, growth of KFC bulls and steers was very gradual, which led to a lower adult body weight compared with JB.

Domestication and artificial selection were associated with drastic environmental changes for the wild ancestor animals and there were also thought to be 'lifestyle' changes. Those environmental changes brought about various morphological changes, such as in color, body size, fat location, head or brain size, and jaws on wild animals (Lohman, 1971; Mignon-Grasteau et al., 2005). In the present study, marked differences in growth and body weight were observed between JB and KFC. In general, JB is fattened and slaughtered at 28--30 months of age to produce marbled beef. In this fattening regime, JB need to manifest their growth potential in early stages of life and demonstrate fat uptake into skeletal muscle in later stages. Therefore, the results of the growth rate and body weight differences may reflect long-term breeding of unimproved Japanese native cattle (KFC) into improved Japanese native cattle (Wagyu) for the purpose of meat quantity and quality.

The relative growth coefficients for heart girth, chest depth, chest width, hip width, and pin bone width were significantly larger than 0.333 for both KFC bulls and steers, which indicates that trunk widening and deepening contributed to body weight gain (Table 1). The wild ancestor of four-footed livestock, such as cattle, sheep, pigs and goats, had naturally developed forequarters for exerting power when fighting with the others or enemies (Hall, 2004). In this study, the width, depth, and girth of the KFC pectoral region were main contributors to body weight gain, and well-developed pectoral regions were observed in both sexes (Fig. 4). In the hindquarters, the relative growth coefficients, which were significantly larger than 0.333, were observed in only hip width and pin bone width, but not in rump length or thurl width; these characteristics may be the leading cause of the relatively poor appearance of hip and hind limb in KFC (Fig. 4). The artificial selection of the wild ancestors of four-footed livestock for meat production has placed more emphasis on the hind quarters where the more palatable meat is located, which resulted in the difference in conformation between wild and modern animals (Hall, 2004). These developed forequarters in the KFC indicate that the typical characteristics of wild ancestor (unimproved) cattle may remain in KFC. Long-term artificial selection and breeding may also have produced elongated height and length of meat animals to increase the dorsal, ventral, and femoral meat yield. In contrast to trunk width development, there were no significant differences in the relative growth coefficients for withers height, hip height, body length, or rump length, which indicated that the contributions of height and length to increased body weight gain might be small.

In conclusion, the growth of the KFC bulls and steers was very gradual, and resulted in a lower adult body weight and smaller adult body size than JB. Our results also indicate that KFC body weight may reflect the width and depth of the trunk rather than the height and length of the body. These characteristics of body measurements observed in KFC were speculated that the characteristics were the typical of unimproved cattle. From there results, it considered that KFC should be preserved as an animal genetic resources.

Fig.1 The ox-drawn carriage in the document of Japan drawn in 13th century.

Source: Tale of Heiji picture scroll in the Japan NDL

Fig.2 The coat colors of Kuchinoshima feral cattle reared in Kagsohima University
a: brown with white spot, b: brown, c: black with white spot, d: black

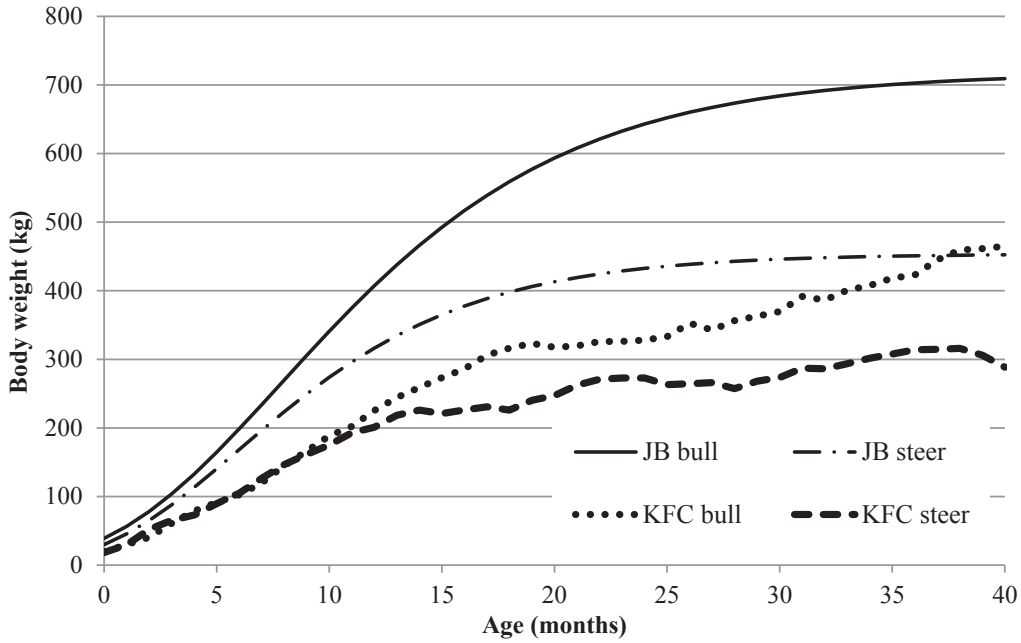

Fig.3 The age-related changes of body weight in Japanese black and Kuchinoshima feral cattle

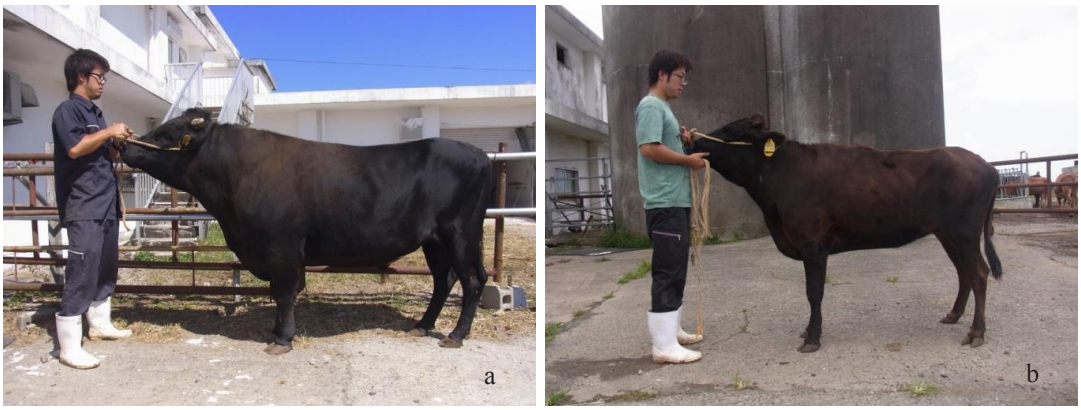

Fig.4 The appearance of Kuchinoshima feral adult bull and steer

a: Kuchinoshima feral bull (44 months of age)

b: Kuchinoshima feral steer (41 months of age)

Table 1. Relative growth coefficient and constant in allometric equation between body weights and body scale in Kuchinoshima feral cattle.

	Body weights (In *X*), Body scale (In *Y*)			
	bull		steer	
	b	a	b	a
Withers height	0.25	3.31	0.26	3.30
Hip height	0.23	3.46	0.23	3.43
Body length	0.34	2.95	0.33	2.99
Shoulder length	0.38	1.44	0.38 ‡	1.41
Heart girth	0.37 †	2.97	0.38 †	2.94
Chest depth	0.37 ‡	2.01	0.38 †	1.93
Chest width	0.44 †	1.06	0.49 †	0.84
Abdominal girth	0.36	3.14	0.37	3.09
Rump length	0.36	1.75	0.35	1.81
Hip width	0.45 †	1.09	0.49 †	0.99
Thurl width	0.36	1.61	0.36	1.62
Pin bone width	0.44 †	0.62	0.46 †	0.55
shank circumference	0.31	1.05	0.26	1.26

b: relative growth coefficient, a: constant

† shows the significant difference from 0.333 ($P<0.01$)

‡ shows the significant difference from 0.333 ($P<0.05$)

Reference

Albrecht, E., Gotoh, T., Ebara F., Xu, J.X., Viergutz, T., Nurnberg. G., Maak. S., Wegner. J. Cellular condition for intramuscular fat deposition in Japanese Black and Holstein steers. Meat Science, 89: 13-20.

Gotoh, T. 2003. Histochemical properties of skeletal muscles in Japanese cattle and their meat production ability. Animal Science Journal, 74: 339-354.

Hall, S.J.G. 2004. Changes in animals following domestication. Livestock Biodiversity Genetic resources for the farming of the future. 9-15. Blackwell Science Ltd.

Kanemaki, M., Ando, H., 2013. Characteristics of Kuchinoshima feral cattle herd bred and maintained in Nagoya University. Report of the Society for Researches on Native Livestock, 26: 79-97 (in Japanese with English summary).

Kawahara-Miki, R., Tsuda, K., Shiwa, Y., Arai-Kichise, Y., Matsumoto, T., Kanesaki, Y., Oda, S., Ebihara, S., Yajima, S., Yoshikawa, H., Kono T. 2011. Whole-genome resequencing shows numerous genes with nonsynonymous SNPs in the Japanese native cattle Kuchinoshima-Ushi. BMC Genomics, 12: 103

Lohman, T.G. 1971. Biological variation in body composition. Journal of Animal Science, 32: 647-653.

Mannen, H., Kohno, M., Nagata, Y., Tsuji, S., Bradley, D.G., Yeo, J.S., Nyamsamba, D., Zagdsuren, Y., Yokohama, M., Nomura, K., Amano, T. 2004. Independent mitochondrial origin and historical genetic differentiation in North Eastern Asian cattle. Molecular Phylogenetics and Evolution, 32: 539-544.

Mignon-Grasteau, S., Boissy, A., Bouix, J., Faure, J-M., Fisher, A. D., Hinch, G. N., Jensen, P., Neindre, P. L., Mormède, P., Prunet, P., Vandeputte, M., Beaumont, C. 2005. Genetics of adaptation and domestication in livestock. Livestock Production Science, 93: 3-14.

Morita, S., Iwamoto H, Fukumitsu Y, Gotoh T, Nishimura S, Ono Y. 2000. Histogeneous composition of histochemical fibre types in the different parts of M. longissimus thoracis from Mishima (Japanese native) steers. Meat Science, 54: 59-63.

Nagamine, Y., Nirasawa, K., Takahashi, H., Sasaki, O., Ishii, K., Minezawa, M., Oda, S., Visscher, P.M., Furukawa, T. 2008. Estimation of the time of divergence between Japanese Mishima island cattle and other cattle populations using microsatellite DNA markers. Journal of Heredity, 99: 202-207.

National Agriculture and Food Research Organization. 2009. Japanese Feeding Standard for Beef Cattle. 234pp. Japan Livestock Industry Association, Tokyo (in Japanese).

Shimogiri, T., Okumura, F., Ryuno, M., Hanada, H., Imura, Y., Kawabe, K., Okamoto, S., Maeda, Y. 2006. Genetic variability of Kuchinoshima feral cattle as indicated by amplified fragment length polymorphism (AFLP). Bulletin of the Experimental Farm, Faculty of Agriculture, Kagoshima University, 29: 13-15 (in Japanese with English summary).

Tsuda, K., Kawahara-Miki, R., Sano, S., Imai, M., Noguchi, T., Inayoshi, Y., Kono, T. 2013. Abundant sequence divergence in the native Japanese cattle Mishima-Ushi (Bos taurus) detected using whole-genome sequencing. Genomics, 102: 372-378.

Wagyu Registry Association. 2004. The standard growth curve of Japanese Black. 30-35pp. Kyoto (in Japanese).

PO-04-48

Fermentation quality of Tropical Grasses Silage treated with Lactic acid bacteria and Cellulase

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INTRODUCTION

The major constraint for dairying in the tropics is shortage of feed in terms of quality and quantity, especially in the dry season. Purple guinea grass (*Panicum maximum* cv. TD 58) and Napier grass (*Pennisetum purpureum* cv. Pak Chong 1) are now popular and widely used for ruminant feed in the tropics, including Thailand. LAB and cellulase have become significant factors in predicting the adequacy of silage fermentation and determining whether to apply additives to silage. Usually, tropical grass has a high moisture content (>80%) which causes butyric acid fermentation leading to unsuccessful ensiling (Pholsen et al., 2016). Grass wilting could inhibit undesirable microorganisms and reduce nutrient loss. However, the characteristics of LAB and cellulase, and their true function in silage making under different moisture conditions are unclear. In the present study, Fermentation quality of Tropical Grasses Silage treated with Lactic acid bacteria and Cellulase.

MATERIALS AND METHODS

Purple guinea (*Panicum maximum* cv. TD 58) and Napier (*Pennisetum purpureum* x *Pennisetum americanum* cv. Pak Chong 1) grasses were grown at the experimental farm, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Both grasses were harvested at 60 days of regrowth, 50% of each chopped grass was wilted for 6 h in the shade, and 50% for fresh silage preparation. A local selected lactic acid bacteria (LAB) *Lactobacillus casei* strain TH14, a commercial inoculant strain Chikuso 1 (CH, *L. plantarum*, Snow Brand Seed Co., Ltd, Sapporo, Japan) and two commercial cellulase enzymes (AC, *Acremonium* cellulase; MC, Maicelase, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were used as silage additives. The experimental design was a 4×15 factorial arrangement in a completely randomized design (grasses×additives) with three replications. 100-g of chopped grass were mixed well with additives, and packed into a bag silo. All silos were stored at room temperature (25 to 37 °C). At day 30 after ensiling, three bags per treatment were opened for evaluation of fermentation end-products, chemical and microorganism compositions. The microorganism counts were done using the plate count method (Kozaki et al., 1992). The 16S rRNA gene sequence analysis was determined as described by Cai et al. (1999b). Silage fermentation end-products were analyzed from cold water extracts as described by Cai (2004). The chemical composition of pre-ensiled grass and silage samples were analyzed according to AOAC (1990) for DM, OM, CP and EE, Van Soest et al. (1991) for NDF and ADF, and Faichney and White (1983) for ADL. GE was determined using an automatic adiabatic bomb calorimeter (AC 500, LECO, Michigan, USA). All data were analyzed by ANOVA with a 4×15 factorial arrangement in CRD. The significance of differences among means was tested by Duncan's new multiple range test; with an accepted significant difference when a probability was less than 0.05 (SAS, 1998)

RESULTS AND DISCUSSION

At day 30 of fermentation, fresh Guinea grass silage treated with cellulase at AC 0.1% or MC 0.1% had a significantly lower (P<0.05) pH and ammonia nitrogen content, and significantly higher lactic acid content than that of control and LAB treatments (Table 1). In wilted Guinea grass, the AC 0.1% and MC 0.1% treatments also showed the best result for fermentation quality. In addition, the AC 0.01% and MC 0.01% treatments were well preserved with lower (P<0.05) pH and higher (P<0.05) lactic acid content than both control and LAB treatments. All silages of Napier grass were good quality with significantly lower (P<0.05) ammonia nitrogen content and significantly higher (P<0.05) lactic acid content than Guinea grass silage. AC 0.1% treatments showed the highest fermentation quality than other treatments. The forages (A), additives (B) and their interaction (A×B) significantly influenced (P<0.001) silage pH, contents of lactic acid, propionic acid, butyric acid and ammonia nitrogen. Compared to the control, the pH and ammonia nitrogen content of cellulase and cellulase + LAB treatments were lower and lactic acid contents higher (P<0.01). The cellulase 0.1% treatments improved ($P<0.05$) fermentation quality more than cellulase 0.01% in both silages.

Table 1 Dry matter, pH and fermentation products of Guinea and Napier grass silages at 30 days of ensiling

Item	DM	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ammonia-N
	%				g/kg DM		
Forage means							
Fresh Guinea	19.05^{c}	4.21^{b}	1.68^{d}	0.74^{c}	0.02^{b}	0.12^{b}	1.08^{b}
Wilted Guinea	27.56^{a}	4.41^{a}	2.86^{c}	1.40^{b}	0.06^{a}	1.11^{a}	1.30^{a}
Fresh Napier	15.11^{d}	3.69^{d}	6.89^{a}	1.69^{a}	ND^{c}	0.01^{b}	0.66^{c}
Wilted Napier	26.01^{b}	4.07^{c}	5.15^{b}	0.96^{c}	ND^{c}	0.05^{b}	0.74^{c}
SEM	0.717	0.045	1.604	0.535	0.023	0.363	0.165
Additive means							
Control	22.83^{ab}	4.49^{b}	2.19^{f}	1.26	0.05^{a}	0.57^{ab}	1.32^{ab}
CH	22.87^{a}	4.45^{b}	3.06^{ef}	1.19	0.04^{a}	0.34^{bcd}	1.27^{bc}
TH14	22.69^{abc}	4.57^{a}	2.23^{f}	1.24	0.05^{a}	0.38^{bc}	1.49^{a}
AC 0.01%	21.80^{cdef}	4.11^{e}	3.46^{cdef}	1.12	0.01^{b}	0.59^{ab}	1.07^{cd}
AC 0.1%	21.22^{ef}	3.85^{g}	5.18^{ab}	1.29	ND^{b}	0.04^{d}	0.73^{f}
MC 0.01%	21.85^{bcdef}	4.30^{c}	3.60^{cdef}	1.52	0.04^{a}	0.84^{a}	1.07^{cd}
MC 0.1%	21.82^{bcdef}	3.94^{f}	4.61^{abcd}	1.11	ND^{b}	0.06^{cd}	0.76^{f}
CH+ AC 0.01%	22.39^{abcd}	3.93^{f}	4.69^{abc}	0.89	ND^{b}	0.14^{cd}	0.74^{f}
CH+ AC 0.1%	20.85^{f}	3.74^{h}	5.87^{a}	0.99	ND^{b}	0.01^{d}	0.42^{g}
CH+ MC 0.01%	21.88^{abcde}	4.19^{d}	3.83^{bcde}	1.04	0.04^{a}	0.80^{a}	0.98^{de}
CH+ MC 0.1%	22.07^{abcde}	3.86^{g}	5.53^{a}	0.95	ND^{b}	0.02^{d}	0.63^{f}
TH14+ AC 0.01%	21.69^{cdef}	3.97^{f}	4.43^{abcde}	1.3	ND^{b}	0.19^{cd}	1.01^{de}
TH14+ AC 0.1%	21.52^{def}	3.83^{g}	5.54^{a}	1.46	ND^{b}	0.03^{d}	0.71^{f}
TH14+ MC 0.01%	21.20^{ef}	4.29^{c}	3.19^{def}	1.46	0.04^{a}	0.73^{a}	1.19^{bcd}
TH14+ MC 0.1%	22.31^{abcd}	3.95^{f}	4.76^{abc}	1.15	ND^{b}	0.09^{cd}	0.84^{ef}
Significance of main effect and interaction							
Forages (A)	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Additives (B)	<.001	<.001	<.001	0.116	<.001	<.001	<.001
A x B	0.001	<.001	0.007	0.009	<.001	<.001	<.001

$^{a\ to\ g}$ Means within columns with difference superscript letters differ at $P < 0.05$.; Values are means of three silage samples; DM, dry matter; ND, not detected.

In the present study, lower LAB counts and WSC content were presented in Guinea grass compared to Napier grass. Both fresh and wilted Guinea grass silages were poor quality in control and LAB-inoculated treatments because they had low lactic acid contents, high pH and ammonia nitrogen. All Napier grass silages had significantly (P<0.05) lower pH, ammonia nitrogen and significantly (P<0.05) higher lactic acid content compared with Guinea grass silages. The most plausible explanation lies in the physiological properties of natural LAB strains and the chemical composition of Napier grass that contained a relatively high level of WSC (>2.31% of DM). The natural LAB population showed positive relationships with propionic acid, acetic acid, butyric acid and NH_3-N content, but a negative relationship with lactic acid content. The LAB inoculation had no further beneficial effect on promoting lactic acid fermentation. This could be attributed to the natural strains *L. casei* and *L. plantarum* (which were most frequently isolated from both grasses) having the ability to produce more lactic acid and more WSC than other strains (Pholsen et al., 2016). Therefore, when sufficient LAB is present on grass, there is no need to use LAB as inoculant for silage making. This indicates that in any future experiments it may be necessary to study the relationships between grass condition and fermentation quality. The results confirmed that cellulase could improve tropical silage quality; inhibiting protein degradation and promoting fiber degradation, especially in tropical grasses containing low WSC.

REFERENCES

AOAC, 1990. Of?cial Methods of Analysis, 15th edn. ed. Association of Of?cial Analytical Chemists, Washington, DC.

Cai, Y., 2004. Analysis method for silage, in: Japanese Society of Grassland Science (ed.), Field and Laboratory Methods for Grassland Science. Tosho Printing Co. Ltd., Tokyo, Japan., pp. 279–282.

Cai, Y., Benno, Y., Ogawa, M., Ohmomo, S., Kumai, S., Nakase, T., 1998. Influence of lactobacillus spp. from An inoculant and of weissella and leuconostoc spp. from forage crops on silage fermentation. Appl. Environ. Microbiol. 64, 2982–2987.

Cai, Y., Kumai, S., Ogawa, M., Benno, Y., Nakase, T., 1999. Characterization and identification of Pediococcus species isolated from forage crops and their application for silage preparation. Appl. Environ. Microbiol. 65, 2901–6.

Faichney, G., White, G., 1983. Methods for the Analysis of Feeds Eaten by Ruminants. Division of Animal Production, Ian Clunies Ross Animal Research Laboratory, Commonwealth Scienti?c and Industrial Research Organization. Melbourne, Australia.

Kozaki, M., Uchimura, T., Okada, S., 1992. Experimental Manual for Lactic Acid Bacteria. Asakurasyoten, Tokyo, Japan.

Lin, C., Bolsen, K.K., Brent, B.E., Fung, D.Y.C., 1992. Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. J. Appl. Bacteriol. 73, 375–387.

Nadeau, E.M.G., Russellt, J.R., Buxton, D.R., 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J. Anim. Sci. 78, 2980–2989.

Pholsen, S., Khota, W., Pang, H., Higgs, D., Cai, Y., 2016. Characterization and application of lactic acid bacteria for tropical silage preparation. Anim. Sci. J. n/a–n/a.

SAS, 1998. User's Guide: Statistic, Version 6, 12th ed. SAS Institute Inc., North Carolina.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–97.

PO-04-49

Silage fermentation of natural grasses in meadow steppe and typical steppe

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INTRODUCTION

Meadow steppe (MS) and typical steppe (TS) are important natural grassland in the world, they are widely distributed in temperate semi-arid continental climate region and the northern hemisphere boreal and temperate. Silage can be prepared ahead of time which preserves nutritional value, and also extend the retention time, facilitating fodder provision throughout the year, regardless of the weather (Kaiser and Curll, 1987). However, it is usually difficult to prepare a good quality of silage from natural grasses, because of their lower water-soluble carbohydrate (WSC) content and lactic acid bacteria (LAB) countsbut higher lactate buffering capacity (King et al., 2012).The present study examined the grassland population, dry matter yield, fermentation quality, and chemical composition of natural grasses in MS and TS environments. To improve fermentation quality, round bale silages of mixed natural grasses in both steppes were prepared using LAB inoculant and cellulase enzyme.

MATERIAS AND METHODS

Natural grasses were harvestedat full-bloom stage from Hulunbuir MS (48.27°N, 119.44°E), and Xilingol TS (43.46°N, 115.13°E), Inner Mongolia, China on 24 July 2014. The grasses in both steppes were harvested at full-bloom stage and the silages were prepared using round bale system (Rollant round baler, 375 RC, Harsewinkel, Germany). A commercial LAB inoculant Chikuso-1 (CH, *Lactobacillus plantarum*, Snow Brand Seed Co., Ltd, Sapporo, Japan) and a commercial cellulase enzyme (AC, acremonium cellulase produced from *Acremonium*, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were used as silage additives. The LAB were inoculated at 20 mg/kg as 1.00×10^5 colony forming unit (cfu)/g on a fresh matter (FM) basis. Cellulase was added at 10 mg/kg of FM. Silage treatments were designed as control, CH, AC and CH + AC. These bales were stored at temperature 20 to 26°C. Three replicates per treatments were opened at 60 days of ensiling, fermentation quality and chemical composition were analyzed. Statistical analyses of chemical composition and silage fermentation were performed by one-way ANOVA using the general linear model (GLM) procedure of SAS version 9.0 (SAS Institute Inc., Cary, NC, USA). The differences between means were assessed by Tukey's multiple comparison tests at a significant level of *P.*

RESULTS AND DUSICUSSION

MS and TS contained thirty-three and nine species of natural grasses, respectively. *Stipa Baicalensis* in MS and *Stipa grandi* in TS were the dominant grasses with the highest dry matter (DM) yield. The crude protein (CP), neutral detergent fiber (NDF) and water-soluble carbohydrate (WSC) of the mixed natural grasses in both steppes were 8.0 to 9.0, 66.0 to 69.0 and 2.0 to 2.2% on a DM basis, respectively.

All silages treated with LAB and cellulase were well preserved with lower pH, butyric acid and ammonia-N content, and higher lactic acid than those of control in four kinds of silages. Compared with CH- or AC-treated silages, the CH + AC-treated silages had higher lactic acid content (Table 1). These results are likely explained by the WSC content of the materials and by the numbers and physiological properties of epiphytic LAB. WSC is also an important factor that influences the fermentation quality of silage (McDonald and Henderson, 1964), the low WSC content of the mixed grasses could hardly provide enough substrate for LAB fermentation, and it could inhibit the growth of harmful bacteria (McEniry et al., 2014). Added cellulase may degrade the cytoderm and increase the available sugars, thereby providing a substrate for lactic acid fermentation.

The results confirmed that combination with LAB and cellulas emay result in beneficial effects by improving the natural grass silage fermentation in both grasslands.

REFERENCES

Kaiser, A. G. and M. L. Curll. 1987. Improving the efficiency of forage conservation from pastures. Pages 397-411

in Wheeler JL, Pearson CJ, Robards GE (eds), Temperate Pastures: Their Production, Use and Management. CSIRO Publishing Publishing, Melbourne.
King, C., J. McEniry, and P. O'Kiely. 2012. A note on the fermentation characteristics of red clover silage in response to advancing stage of maturity in the primary growth. Irish J Agr Food Res. 51:79-84.
McDonald, P. and A. R. Henderson. 1964. Determination of water-soluble carbohydrates in grass. J. Sci. Food Agric. 15:395-398.
McEniry, J., C. King, and P. O'Kiely. 2014. Silage fermentation characteristics of three common grassland species in response to advancing stage of maturity and additive application. Grass Forage Sci. 69:393-404.
Saidin, S., M. R. A. Kadir, E. Sulaiman, and N. H. Abu Kasim. 2012. Effects of different implant-abutment connections on micromotion and stress distribution: Prediction of microgap formation. J. Dent. 40:467-474.

PO-04-50

Effect of molasses and cellulase on the silage fermentation of kudzu, sugarcane top and their mixture

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INTRODUCTION

Kudzu (*Pueraria lobata*) belongs to a native leguminous plan, which contain relatively high nutrient value as animal feed (Chen *et al*., 2015). Sugarcane top (SCT) is a major by-product of the sugar cane industry. It is the top of sugarcane 2-3 cane stalk and green leaves which be cut section and discarded. It accounts for about 20% of sugarcane plants, and has abundant in resources, concentration of origin and low cost (Chen *et al*., 2015). Therefore, kudzu and sugarcane are suitable for making premium cattle and sheep feed (Bao *et al*., 1984; Corley *et al.*, 1997; Valentim *et al*., 2005). The objective of this study was to investigate the effects of wild lactic acid bacteria (LAB), molasses and cellulase on silage fermentation of kudzu, sugarcane top and their mixture.

MATERIALS AND METHODS

Kudzu (*Pueraria lobata*) was planted at the *quantou* herbage base of Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agricultural Sciences (Fuzhou, China) in 2013. The harvesting stage of kudzu was at early-bloom stage of first cutting on October 31, 2013. Sugarcane was planted at the experimental field of the Sugarcane Research Institute, Fujian Agriculture and Forestry University (Fuzhou, China) in 2013. The test used the abandon tail of the sugarcane harvest on October 31, 2013. Wild LAB was made as Fermented method of grass juice. Commercial molasses (Guangxi Xuanwudongrun trado Co., Ltd., Naning, China) and cellulase (R-10, Shanghai Bide Biotechnology Inc., Shanghai, China) were used as additives to ensiling materials. Silages were prepared by using Kudzu 100% (100K), kudzu 90% + sugarcane top 10% (9K1S), kudzu 80% + sugarcane top 20% (8K2S), kudzu 70% + sugarcane top 30% (7K3S), kudzu 60% + sugarcane top 40% (6K4S) and sugarcane top 100% (100S). Each treatment was treated with control, 5% molasses (M) and 0.1% cellulase (C) based on a fresh matter. The materials were immediately added with additives and then packed into plastic bag. The additives were plashed homogeneously to the chopped materials of 300 g with 3 replicates of each treatment. The plastic bags were sealed by a vacuum sealer and kept in laboratory at ambient temperature for 50 d. For statistical analysis, the data of fermentation quality and chemical composition were analyzed by two- ways ANOVA analysis of variance using SPSS 17.0 software package (SPSS Statistics 17.0). The values were significant at $P<0.05$.

RESULTS AND DISCUSSION

Fresh kuzdu and sugarcane top prior ensiling showed both similar counts with 10^4 to 10^5 LAB cfu/g FM, 10^5 to 10^6 aerobic bacteria, 10^5 yeast, and 10^3 to 10^4 mold. The DM of fresh Kudzu and sugarcane top were similar, ranging from 20.92 to 21.65%. Kudzu had higher LBC (669.22 mE/kg), and CP (28.15%), while lower WSC (18.61%) than sugarcane top (325.49 mE/kg LBC, 8.86% CP, 18.61% WSC,) based on DM.

Fermentation quality of kudzu silage, sugarcane top silage and their mixed silage are shown in Table 1. The mixture ratio had significantly effects on pH value, lactic acid (LA) content, acetic acid (AA) content and propionic acid (PA) content ($P<0.01$, $P<0.05$). The additive significantly influenced pH value, LA content, AA content, PA content and ammonia-N content ($P<0.01$) of silages. Mixture ratio and additives had significant interactions on contents of LA, AA, PA, ammonia-N and pH value ($P<0.01$, $P<0.05$). Compared to 100K, pH value was significantly decreased by other five mixture ratios ($P<0.05$), LA content was significantly increased by 6K4S and 100S ($P<0.05$), PA content was significantly decreased by 6K4S ($P<0.05$), AA content and ammonia-N content were significantly decreased by 100S ($P<0.05$). All silages treated with additives had lower ($P<0.05$) pH value, higher ($P<0.05$) LA content than control, and silage treated with the molasses had the lowest pH value (4.07) and highest LA content (7.32). The results confirmed that molasses and 6K4S mixture ratio could best at reducing pH value and inhibit nutritive loss, and result in improve silage quality.

Table 1 Fermentation quality of kudzu silage, sugarcane top silage and their mixed silage

Item		pH ~~value~~	Lactic acid (% DM)	Acetic acid (% DM)	Propionic acid (% DM)	Butyric acid (% DM)	Ammonia-N (% DM)
MR	100K	4.62±0.24^{a}	5.06±1.97^{b}	2.57±1.50^{b}	2.85±2.22^{a}	ND	0.17±0.06^{a}
	9K1S	4.50±0.28^{b}	3.80±6.23^{b}	2.87±1.12^{ab}	2.55±2.20^{a}	ND	0.15±0.02^{ab}
	8K2S	4.30±0.24^{c}	5.23±3.02^{b}	3.57±1.89^{a}	2.59±1.83^{a}	ND	0.15±0.02^{b}
	7K3S	4.22±0.17^{cd}	6.37±4.53^{b}	2.91±0.98^{ab}	2.71±1.67^{a}	ND	0.15±0.02^{ab}
	6K4S	4.15±0.22^{d}	8.31±3.09^{a}	2.53±1.15^{b}	1.61±1.22^{b}	ND	0.15±0.05^{ab}
	100S	3.63±0.38^{e}	8.33±5.14^{a}	1.71±0.67^{c}	2.12±1.52^{ab}	ND	0.14±0.02^{b}
A	CK	4.46±0.45^{a}	4.33±1.99^{d}	1.99±0.99^{b}	1.37±0.88^{b}	ND	0.17±0.04^{a}
	LAB	4.28±0.32^{b}	5.11±2.58^{c}	2.12±0.68^{b}	1.14±1.05^{c}	ND	0.13±0.01^{c}
	M	4.07±0.30^{c}	7.32±4.23^{a}	2.91±2.00^{a}	2.77±1.64^{a}	ND	0.15±0.01^{b}
	C	4.31±0.43^{b}	6.53±3.48^{b}	3.00±1.46^{a}	2.32±1.84^{a}	ND	0.18±0.05^{a}
MR		**	**	**	*		
A		**	**	**	**		**
MR×A		**	**	**	**		*

1) MR, mixture ratio; A, additive; 100K, 9K1S, 8K2S, 7K3S, 6K4S and 100S mean six mixture ratios (10:0, 9:1, 8:2, 7:3, 6:4 and 0:10 respectively) of kudzu and sugarcane top silages; CK, control; M, molasses; C, cellulose; ND, not detect.

2) Values are mean ± standard deviation; $^{a-e}$Means within columns with different superscript letters differ ($P < 0.05$). * means $p<0.05$, ** means $P<0.01$.

REFERENCES

Bao GY. 1984. Comprehensive utilization of sugarcane top. *Sugarcane Industry* **12,** 38-41.

Chen XZ, Yiu Y, Gao CF, Zhang XP, Li WY, Dong XN, Weng BQ. 2015. Effect of additives on shelf-life and quality of sugarcane tips for forage. *Fujian Journal of Agricultural Science* **30,** 745-749.

Corley RN, Woldeghebriel A, Mushy MR. 1997. Evaluation of the nutritive value of kudzu (*Pueraria lobata*) as a feed for ruminants. *Animal Feed Science and Technology* **68,** 183-188.

Valentim JF, Andrade C. 2005. Fropical kudzu (*Pueraria phaseoloides*): successful adoption in sustainable cattle production systems in the western Brazilian Amazon. *Tropical Grasslands* **39,** 221-221.

PO-04-54 Evaluation of Protein Utilization from Broken Job's tears and Job's tears bran in Weanling Pigs

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ABSTRACT

The objective of this study was to evaluate protein utilization in brown broken job's tears, broken job's tears and job's tears bran in weanling pigs. First, the metabolic and endogenous nitrogen excretions were determined, using twelve weanling pigs with average body weight of 6.93 kg. They were fed cassava flour based semi-purified, non-protein diets. It was found that the tested piglets fed the protein-free diets excreted 835.97 mg of metabolic fecal nitrogen/h/d (119.86 mg MFN/kgBW$^{0.75}$per day) and 881.92 mg of endogenous urinary nitrogen/h/d (128.18 mg EUN/kgBW$^{0.75}$per day). In the experiment, twelve castrated male, crossbred (Large white x Landrace x Duroc) weanling pigs averaging 6.88 kg body weight were subjected to Completely Randomized Design. The pigs were randomly divided into 3 groups (brown broken job's tears, broken job's tears and job's tears bran). Feces and urine samples were collected quantitatively from the last 3 days (5th to 7th days of week) of each experimental period. The results showed that weanling pigs fed with brown broken job's tears and broken job's tears yielded the higher true nitrogen retentions than those fed with job's tears bran. The true nitrogen retentions were 597.69, 593.04 and 545.00 mg/kgBW$^{0.75}$per day, respectively ($P < 0.01$). Similarly, the true net protein utilizations were 86.20, 86.10 and 80.61 %, respectively ($P < 0.01$). Furthermore, the true biological values of the three groups were almost the same, with the values of 90.98, 90.76 and 91.17 %, respectively ($P > 0.05$).

INTRODUCTION

Types of plant ingredients for processed feeds can be classified by their sources. The first group is the by-product of the vegetable oil manufacturing and processing, such as soybean meal, full fat soybean, sunflower meal and peanut. The second type is raw materials and by-products of the cereal processing, such as wheat, rice barn and broken rice. The last group is made of cereal grains such as millet and corn. Different ingredients contain different types and amounts of proteins. Soybean meals, full-fat soybeans and sunflower meal contain globulin. Rice barn contains albumin and globulin. Wheat barn contains gliadin and glutenin; both can be call gluten. The broken rice contains gliadin protein called kafirin. Different types of proteins can be digested and utilized by the piglets differently, especially with different proportions in feed formulas. The protein utilization of the ingredients is an essential parameter for formulating the feeds. Feed formula with accurate and balanced ingredients can help the piglets to obtain appropriate amount of proteins and increase their growth performance.

Loei Province is a significant part of upper northeast of Thailand that grows crops, such as cassava, corns as feedstuff and sugarcane. Loei grows job's tears the most in Thailand. Besides, job's tears is also imported from Lao PDR via border crossings in Thali and Chiang Khan Districts of Loei. There are job's tears mills to purchase from the local farmers and import from Lao PDR. From November to February of 2015, 9,842 tons of job's tears were imported from Lao, totaling 179,652,549 baht (Loei Office of Commercial Affairs, 2015). In Loei, Job's tears is milled throughout the year. Job's tears is used for feeds. It belongs to a grass family called "Gramineae" in Coix genus. Its scientific name is *"Coix lacryma-jobi linn"* in Panicoideae sub-family, the same as corn (maize) and sorghum (Tatham et al., 1996). Its common names include job's tears, pigeon barley, and adley. For exporting, it is called pearl barley. Evidently, it was native Southeast Asia and spread to the west by the Arabs since 1500 BC. Today job's tears is widespread throughout the world. Thailand imported job's tears for the first time in 1960, at Phrabuddhabaht self-plantation of Buddhabaht district in Saraburi, Chaibadal self-plantation of Chaibadal district in Lobburi, and at Kornburi district in Nakhon Rachasima. Later, it was brought to Chaiyaphum and Loei. And in 1982, when the price increased, it was expanded to Phayao, Chiangrai, Chiangmai, Petchaboon, Udonthani, Nongkhai, Sakonnakorn, and Khon Kaen. When the price droped, the growing area shrinked. And in 2003, Loei grew job's tears the most, followed by Phayao and Petchaboon. About 85- 90 % of the crop is exported to Japan, Korea and Taiwan. Only 10-15 % is used domestically (Department of Academic Agriculture, 2005). After milling, the products include job's tears and job's tears bran plus broken job's tears with a ratio of 40:10-15, respectively (Somkiet, 2004). Brown job's tears and brown broken job's tears are produced and exported to China.

Rich with proteins, by-products of the milling process can be used for animal feed. The nutrients composition of brown broken job's tears, broken tears and job's tears bran are shown in **Table 1**. Even though job's tears has been used as animal feeds for a long time, the true nitrogen retention, true net protein utilization and true biological of job's tears by-products have not been examined. To improve the feed formulation that can provide more accurate protein contents and reduce protein loss, the three values needs to be found. Consequently, this study was aimed to evaluate protein utilization in brown broken job's tears, broken job's tears and job's tears bran in weanling pigs.

MATERIALS AND METHODS

Animals

The study evaluates protein utilization of brown broken job's tears, broken job's tears and job's tears bran in weanling pigs. Twenty four castrated male pigs (initially 6.90 kg BW) were used as experimental animals. The pigs were kept in metabolism cages (dimension of 45 x 60 x 45 cm) to collect feces and urine, in constant lighting and temperature-controlled room. The pigs were divided into two groups. There were 12 pigs in the first groups, castrated male pigs (initially 6.88 kg BW), for the protein utilization study, they were fed with 10 % protein diet (protein sources from brown broken job's tears, broken job's tears and job's tears bran). The second group comprised 12 castrated male pigs (initially 6.93 kg BW), for investigation of endogenous nitrogen losses, including metabolic fecal nitrogen and endogenous urinary nitrogen excreted from feces and urine. This group was fed with protein free diet.

Experimental diets

The experimental diets consisted of protein free diet and 10% protein diet. Protein free diet was formulated to meet or exceed all NRC (1998) nutrient requirements of 5 to 10 kg pigs, except proteins and amino acids. Protein free diet was used to evaluate endogenous nitrogen loss. Protein diet was used to evaluate protein utilization. There were 3 sub formulations with different protein sources: brown broken job's tears, broken job's tears and job's tears bran. They contained 10 % protein. The formulation met or exceeded metabolizable energy, vitamins and minerals requirement of 5 to 10 kg pigs according to NRC (1998) recommendation as showed in **Table 2**.

Experimental design

The experimental period of this study lasted 4 weeks. In the preliminary period, all animals were adapted to housing and experimental conditions for 7 days. The pigs were fed equally. In 1^{st} week, the pigs were given 280 g/h/d, then 310, 330 and 370 g/h/d of diet in 2^{nd}, 3^{rd} and 4^{th} week, respectively. The pigs were given the same amount of feed twice a day at 6.00 a.m. and 5.00 p.m. Water was available freely at all times.

Data collection

The feed given to the pigs was weighed every day. The feces and urine weights were collected on the last 3 days of each week (1^{st}, 2^{nd}, 3^{rd} and 4^{th} week, respectively). The feces and urine samples were weighed and collected in H_2SO_4 solution (3% H_2SO_4 for feces and 10% H_2SO_4 for urine) and stored at –10°C according to Schneider and Flatt (1975). Samples of feed, feces and urine were analyzed for nitrogen contents by Kjeldal method according to Jowaman (1980) and A.O.A.C. (1975) to find the true nitrogen retention, true net protein utilization and true biological value.

Parameters calculations

- Protein utilization

True nitrogen retention = $[NI – (FN – FNm) – (UN – UNe)] / BW^{0.75}$

True net protein utilization = $\{[NI – (FN – FNm) – (UN – UNe)] / NI\} \times 100$

True biological value = $\{[NI – (FN – FNm) – (UN – UNe)] / [NI - (FN – FNm)]\} \times 100$

- Abbreviations;

NI = Nitrogen intake, *FN* = Fecal nitrogen

UN = Urinary nitrogen, *FNm* = Metabolic fecal nitrogen

UNe = Endogenous urinary nitrogen, *BW* = Body weight of pig

Statistical analysis

Completely Randomized Design was used in this study. There were three treatments: protein source in diets, brown broken job's tears, broken job's tears and job's tears bran. There were four replications of each treatment. The data were processed by the analysis of variance (ANOVA) procedure, that is suitable for the Completely Randomized Design. The Duncan's New Multiple Range Tests were used to test the significance of treatment effects (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

For endogenous nitrogen loss of pigs fed protein free diet, on the 0 to 28 days of experimental periods, the metabolic fecal nitrogen loss of pigs fed protein free diet was 835.97 mg/h/d (119.86 mg/kg $BW^{0.75}$/d). The endogenous urinary nitrogen loss was 881.92 mg/h/d (128.18 mg/kg $BW^{0.75}$/d) as shown in **Table 3.** For protein utilization of brown broken job's tears, broken job's tears and job's tears bran, the results showed that the pigs fed brown broken job's tears and broken job's tears yielded higher true nitrogen retention than those with job's tears bran. The retentions were 597.69, 593.04 and 545.00 mg/kg $BW^{0.75}$/d, respectively. They were significantly different ($P < 0.01$). Similarly, true net protein utilizations were 86.20, 86.10 and 80.61 %, respectively ($P < 0.01$). Moreover, the results of this study showed that the values of biological value of brown broken job's tears, broken job's tears and job's tears bran were close. They were not statistically significantly different ($P > 0.05$). The values were 90.98, 90.76 and 91.17 %, respectively as showed in **Table 4**.

The results of this study found that the brown broken job's tears and broken job's tears yield the true net protein utilization and true biological value as high as the broken rice does. The values were 82.37 and 90.59 %, respectively (Amphol, 2002). Also, the job' s tears bran yields the true net protein utilization and true biological as high as the rice bran does. The values were 73.44 and 92.15 %, respectively (Amphol, 2002).

CONCLUSION

For brown broken job's tears, broken job's tears and job's tears bran, the true nitrogen retentions were 597.69, 593.04 and 545.00 mg/kg $BW^{0.75}$/d, respectively. While the true net protein utilizations were 86.20, 86.10 and 80.61 %, respectively. The true biological values were 90.98, 90.76 and 91.17 %, respectively. In conclusion, this study indicated that brown broken job's tears, broken job's tears and job's tears bran can be used as feedstuff in weanling pigs diets.

Keywords: Broken Job's tears, Job's tears bran, Protein utilization

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Table 1. Nutrient compositions of by products of job's tears milling

Nutrient composition (%)	By products of job's tears milling		
	Brown broken job's tears	broken job's tears	Job's tears bran
Dry matter	88.20	88.28	90.09
Crude protein (N x 6.25)	13.84	13.20	16.32
- Amino acids			
Histidine	0.79	0.75	1.41
Isoleucine	0.71	0.66	0.71
Leucine	2.80	2.70	2.20
Lysine	0.59	0.38	2.13
Methionine	0.26	0.21	0.17
Cystine	0.55	0.53	0.65
Methionine + Cystine	0.81	0.74	0.82
Phenylalanine	1.69	1.52	1.66
Tyrosine	1.08	1.29	1.36
Phenylalanine + Tyrosine	2.77	2.81	3.02
Threonine	0.10	0.09	0.15
Tryptophan	0.10	0.09	0.20
Valine	0.60	0.53	0.68
Crude fat	7.50	2.37	20.26
Crude fiber	1.49	0.26	3.35
Ash	2.13	0.72	5.51
Nitrogen free extract	63.24	71.73	44.65
Digestible energy [2]	4433	4478	4284
Metabolism energy [3]	4300	4351	4133

[1] Analysis by Central Lab (Thailand) Co, Ltd.; [2] Noblet and Perez (1993), DE (Kcal/kg) = 4151 – 122 (% Ash) + 23(% CP) + 38(% EE) – 64(% CF); [3] Noblet and Perez (1993), ME (Kcal/kg) = DE x (1.003 – (0.0021 x % CP))

Table 2. Ingredients and nutrient composition of experimental diets

Items	Experimental diets			
	Protein free diet	Protein diets		
		Brown broken job's tears	Broken job's tears	Job's tears bran
Ingredients, %				
Brown broken job's tears	-	72.25	-	-
Broken job's tears	-	-	75.75	-
Job's tears bran	-	-	-	61.30
Cassava flour	56.65	11.58	5.00	27.22
Rice bran hull	24.40	5.39	6.53	4.10
soybean Oil	12.58	5.00	6.92	1.50
Dicalcium phosphate[1]	3.80	2.90	2.85	3.50
Calcium carbonate[2]	1.07	1.38	1.45	0.98
Common salt	0.50	0.50	0.50	0.50
Premix[3]	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Nutrients calculated,%				
Dry matter	92.31	89.76	89.89	90.93
Calculated ME, Mcal/kg	3,500	3,500	3,500	3,500
Crude protein (Nx6.25)	0.89	10.00	10.00	10.00
Crude fat	12.58	10.42	8.72	13.92
Crude fiber	8.80	3.27	2.85	3.69
Ash	8.17	6.36	5.55	8.16
Calcium	1.25	1.20	1.20	1.20
Available phosphorous	0.80	0.80	0.80	0.80

[1]Contained 23.31 % calcium and 17.48 % phosphorous; [2]Calcium content 38.00 %; [3]Mineral and vitamins premix provided the following per kilogram of diets; manganese, 65.99 mg; iron, 182.37 mg; copper, 11.00 mg; selenium, 0.55 mg; zinc, 183.70 mg; iodine, 0.26 mg; magnesium, 238.70; vitamin A, 4,036 IU; cholecalciferol, 806 IU; vitamin E, 29.70 mg; vitamin K, 0.91 mg; vitamin B_1, 1.84 mg; vitamin B_2, 6.46 mg; nicotinic acid, 27.50 mg; vitamin B_6, 2.75 mg; vitamin B_{12}, 0.03 mg; pantothenic acid, 18.34 mg; folic acid, 0.55 mg; biotin, 0.11 mg; and chorine, 916.30 mg

Table 3. Endogenous nitrogen loss of the pigs fed protein free diet

Items	Experimental period, days				Mean
	0 to 7	7 to 14	14 to 21	21 to 28	
Number of pigs, head	3	3	3	3	3
Initial weight, g	6.93	7.60	8.20	9.03	7.94
Final weight, g	6.82	7.42	7.83	8.38	7.61
Feed intake, g/h/d	280.00	310.00	330.00	370.00	322.50
Endogenous nitrogen loss					
Metabolic fecal nitrogen[1]	580.97	724.43	916.95	1121.53	835.97
Metabolic fecal nitrogen[2]	96.88	110.02	129.20	143.34	119.86
Endogenous urinary nitrogen loss[1]	754.00	867.67	888.00	1018.00	881.92
Endogenous urinary nitrogen loss[2]	125.73	131.80	125.13	130.05	128.18

[1] mg/h/d, [2] mg/kg $BW^{0.75}$/d

Table 4. Protein utilization of pigs fed protein form by products of job's tears milling

Items	Experimental diets			SEM
	Brown broken job's tears	Broken job's tears	Job's tears bran	
Number of pigs, head	4	4	4	-
Initial weight, g	6.90	6.88	6.85	0.08
Final weight, g	10.25	10.18	10.28	0.15
Feed intake, g/h/d	322.50	322.50	322.50	0.00
Protein utilization				
True nitrogen retention[1]	597.69[a]	593.04[a]	545.00[b]	8.42
True net protein utilization, %	86.20[a]	86.10[a]	80.61[b]	0.78
True biological value, %	90.98	90.76	91.17	0.74

[1] mg/kg $BW^{0.75}$/d

[ab] Mean within the same row with no common superscript differ significantly ($P < 0.01$)

REFERENCES

Amphol Jupamatta. 2002. Utilization of plant protein sources for weaning pigs. A thesis for the degree of Master of Science in Animal Science. Graduate School, Khon Kaen University. Khon Kaen, Thailand.

A.O.A.C. (1975). Official methods of analysis (12th Ed.). Association of Official Analytical Chemists, Washington, D. C.

Department of Academic Agriculture (2005). Job's tears. DOA Plant Knowledge Bass. Retrived from http ://www.doa.go.th/pl_data/02_LOCAL/oard3/duay/body.html.

Jowaman Khajarern. 1980. Manual of feed analysis. Faculty of Agriculture, Khon Kaen University. Khon Kaen, Thailand.

Loei Office of Commercial Affairs (2015). The import volume of agricultural products from Lao PDR to Loei Province. Loei Office of Commercial Affairs. Loei, Thailand.

National Research Council. 1998. Nutrient Requirements of Swine (10th Ed.). National Academy Press, Washington, D. C.

Schneider, B. H., and W. P. Flatt. 1975. The Evaluation of Feeds through Digestibility Experiments. The University of Georgia Press, Athens.

Somkiat (2004). Retrived from http://www.trf.or.th/research/abstract.asp? PROJECTID = PDG4720005.

Steel, J. C., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). Mc Graw-Hill Book Co., New York.

Tatham, A. S., R. J. Fido, C. M. Moore, D. D. kasarda, D. D. Kuzmicky, J. N. Keen and P. R. Shewry (1996). Characterisation of major prolamins of Tef (Eragrostis tef) and Finger millet (Eleusine coracana). J. Cereal Sci. 24: 65-71.

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Super-Dosing Effect of Dietary Phytase on Laying Performance and Egg Quality in Laying Hens

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Introduction

Phosphorus (P) is the most expensive essential mineral for poultry. In cereal grain-based diets, however, 50 to 85% of P is bound to phytate and is poorly utilized by poultry (Selle and Ravindran, 2007). This poor utilization of P has been concerns on environmental and economic aspects. Phytase is a digestive enzyme catalyzing the release of P from the phytate complex, which increases P utilization in diets (Ravindran et al., 1998). In addition, phytase also increases utilization of other nutrients including amino acids and various cations such as Ca, Fe, Mg, K, and Mg (Ravindran et al., 1998). Therefore, phytase is used extensively in poultry diets.

Currently, the recommended inclusion level of phytase in poultry diets is 500 FTU/kg (Selle and Ravindran, 2007; Cowieson et al., 2009). However, recently higher levels of phytase inclusion in diets have been gaining great attention because it may release more P from phytate, and concurrently it may also increase utilization of other nutrients at a greater extent than when it is included at the recommended level. It is reported that more than 1,000 FTU/kg phytase improved nutrient utilization in diets fed to broiler chickens as compared to recommended levels (Cowieson et al., 2006). However, limited information for laying hens fed high levels of phytase in diets has been available. The objective of this experiment, therefore, was to determine super-dosing effect of dietary phytase on laying performance and egg quality in laying hens.

Materials and methods

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Birds, diets and experimental design

A total of 200 42-wk-old Hy-Line Brown laying hens were allotted into 1 of 5 dietary treatments with 5 replicates in a completely randomized design. Each replicate consisted of 4 consecutive cages with 2 hens per cage. Dietary treatments included the negative control diet [NC; 3.91% Ca and 0.26% available P (AP)], NC + 10,000 FTU/kg phytase (SD10), NC + 20,000 FTU/kg phytase (SD20), NC + 30,000 FTU/kg phytase (SD30). The supplemental phytase was a Phyzyme XP TPT (Danisco Animal Nutrition, Wiltshire, UK). The positive control diet (PC) was also prepared to contain 3.91% Ca and 0.38% AP. All other nutrients and energy were included to meet or exceed NRC (1994) requirement estimates for laying hens. The experimental diets were given to the hens on an ad libitum basis for 6 weeks. A 16-h lighting schedule was used during the entire experiment.

Sample collection and chemical analyses

Laying performance including hen-day egg production, egg weight, egg mass, and broken and shell-less egg production rate was recorded daily. However, feed intake (FI) and feed conversion ratio (FCR) were recorded weekly. The data for laying performance were summarized for 6 weeks of feeding trial. Egg quality was assessed with all eggs that were collected at the conclusion of the experiment. Eggshell strength was determined using a Texture analyzer (model TAHDi 500, Stable Micro System, Godalming, UK) and was displayed as unit of compression force per unit eggshell surface area. Eggshell thickness was measured at three different regions (top, middle and bottom) using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan). Eggshell color was determined using the eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea). The Hunter values for lightness (L*), redness (a*), and yellowness (b*) were determined using the Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). Egg yolk color was estimated by using the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland). Haugh units (HU) were measured using a micrometer (model S-8400, B.C. Ames Co., Waltham, MA), and the HU values were calculated from the egg weight (W) and albumen height (H) based on the following equation: $HU = 100 \log (H - 1.7W^{0.37} + 7.6)$ as described by Eisen et al. (1962).

Statistical analysis

All data were analysed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Each replicate was considered an experimental unit in the analysis of laying performance and egg quality. Outlier data were examined according to the method of Steel et al. (1997), using the UNIVARIATE procedure of SAS; however, no outliers were identified. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $P < 0.05$.

Results

Laying performance

Results indicated during overall experimental period, laying performance including egg weight, egg mass, broken and shell-less egg production rate, FI, and FCR was not affected by dietary treatments. However, the SD20 treatment had a greater ($P < 0.05$) hen-day egg production than NC, PC, and SD10 treatment groups. The SD30 treatment did not have different egg production rate compared with NC, SD10, and SD20 treatment groups.

Egg quality

Egg quality including eggshell strength, eggshell thickness, eggshell color, egg yolk color, and HU was not affected by dietary treatments.

Conclusion

In conclusion, 20,000 FTU/kg phytase in diets fed to laying hens has a positive effect on egg production rate, but more or less than 20,000 FTU/kg phytase in diets has no beneficial effects on laying performance and egg quality in laying hens.

Table 1. Super-dosing effect of dietary phytase in diets on laying performance in laying hens[1]

Items	Dietary treatments[2]					SEM	P-value
	NC	PC	SD10	SD20	SD30		
Hen-day egg production (%)	94.4[bc]	93.6[c]	94.9[bc]	97.3[a]	96.6[ab]	0.85	0.02
Egg weight (g)	68	68	67	68	66	0.7	0.22
Egg mass (g/d)	65	64	63	67	65	1.1	0.27
Broken and shell-less egg production (%)	0.03	0.02	0.02	0.03	0.06	0.013	0.33
Feed intake (g/d)	132	128	132	132	132	2.7	0.78
Feed conversion ratio (g of feed/g of egg)	2.03	2.02	2.09	1.99	2.05	0.039	0.44

[a-c]Means with different superscripts within a row in dietary treatments are different ($P < 0.05$).

[1]Data are least squares means of 5 observations per treatment.

[2]Dietary treatments = NC, negative control [3.91% Ca and 0.26% available P (AP)]; PC, Positive control (3.91% Ca and 0.38% AP); SD10, NC + 10,000 FTU/kg phytase; SD20, NC + 20,000 FTU/kg phytase; SD30, NC + 30,000 FTU/kg phytase.

Table 2. Super-dosing effect of dietary phytase in diets on egg quality in laying hens[1]

Items		Dietary treatments[2]					SEM	P-value
		NC	PC	SD10	SD20	SD30		
Eggshell strength (kg/cm^2)		3.4	3.2	3.2	3.1	3.2	0.16	0.65
Eggshell thickness (μm)		436	428	445	433	433	5.40	0.15
Eggshell color (Color fan)		11.8	10.7	11.2	11.4	11.0	0.39	0.33
Eggshell color (Hunter color)	L*	53.0	53.0	53.7	53.1	54.2	0.59	0.50
	a*	19.7	20.0	19.7	19.3	19.0	0.40	0.24
	b*	25.4	25.3	25.6	25.0	24.9	0.30	0.40
Egg yolk color (Roche color fan)		7.8	7.4	7.6	8.0	7.2	0.30	0.26
Haugh unit		96.7	97.0	98.1	97.5	98.7	1.06	0.45

[1]Data are least squares means of 5 observations per treatment.

[2]Dietary treatments = NC, negative control [3.91% Ca and 0.26% available P (AP)]; PC, Positive control (3.91% Ca and 0.38% AP); SD10, NC + 10,000 FTU/kg phytase; SD20, NC + 20,000 FTU/kg phytase; SD30, NC + 30,000 FTU/kg phytase.

REFERENCES

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Supplementation of corn-soy-based diets with an Eschericia coli-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Cowieson, A. J., M. R. Bedford, P. H. Selle, V. Ravindran. 2009. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401-418.

Eisen, E. J., B. B. Bohren, and H. E. Mckean. 1962. The haugh units as a measure of egg albumen quality. Pout. Sci. 41:1461–1468.

NRC, 1994. Nutrient Requirements of Poultry. 9th Rev. ed. Natl. Acad. Press, Washington, DC.

Ravindran V., W. K. Bryden, S. Cabahug, and P. H. Selle, 1998. Impact of microbial phytase on the digestibility of protein, amino acids and energy in broilers. Pages 156-165 in: Proceedings of the Maryland Nutrition Conference for Feed Manufacturing, Baltimore, MD.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw Hill BookCo., NewYork.

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Dietary Supplementation of Activated IGF-I during Gestation and Lactation Affects Sow and Piglet Performance

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INTRODUCTION

The involvement of IGF-I on cell growth and development, skeletal muscle deposition and immunity has been well studied (Velloso, 2008; Ellis et al., 2011). Pigs naturally produce IGF-I, but stress brought by metabolic changes (e.g. gestation, parturition progress, lactation and weaning) and health challenges can impair IGF-I production (Mejia-Guadarrama et al., 2002). Increasing IGF-I levels through dietary supplementation may have the potential in improving the performance of the sow and its litter.

Activated IGF-I (aIGF-I) is a proprietary product mainly composed of "free" IGF-I. Activated IGF-I has undergone a patented double pass electron beam irradiation (29 kGy) process to ensure product safety. Dietary supplementation of aIGF in sows claimed to increase IGF-I level in blood and milk which improves sow and piglet performance (Casebolt, 2014). Preliminary studies have shown that dietary supplementation of aIGF-I in sows reduced backfat loss during lactation and weaning to estrus interval (WEI); while increased weaning weight and survival of piglets (Song et al., 2014a and 2015b; Reyes et al., 2015). Currently, data available is still limited on the application of aIGF-I in sows. Therefore, the objective of the study is to determine the effect of supplementing aIGF-I during gestation and lactation on sow and piglet performance.

MATERIALS AND METHODS

The experiment was conducted from April 28 to October 26, 2015 in a commercial swine farm in Batangas, Philippines. A total of 39 sows (Yorkshire x Landrace, 3.21 ± 0.28 of average parity) were fed basal gestation and lactation diets (Table 1) added with 0 (n=19) or 1 kg/ton (n=20) aIGF-I (Gbh Laboratories, MN, USA). Gestating diet was provided after insemination until d 100 of gestation (2.00 kg ADFI in both treatments). At d 101, sows were moved to individual farrowing stalls (2.90 m x 0.85 m) and were fed lactating diets until d 30 of lactation. Sow backfat depth was measured at the P_2 position (mean value from both sides of the last rib, 6.0-6.5 cm away from the dorsal midline) using an ultrasound scanner (Renco lean meter®, MN, USA). Measurement was done at d 100 of gestation and d 30 of lactation. Water was freely accessible all throughout the experiment. The farrowing stalls had a piglet creep area provided with a heat lamp. Commercial creep diets were offered to piglets at d 7 postpartum. The piglets were not cross-fostered.

Gathered data were checked for outliers, normality (Wilk-Shapiro test) and equality of variance (F-test). Count data expressed in percentage namely stillbirth, mummified and preweaning mortality were transformed using arcsin function. Individual sow and its litter served as an experimental unit. Data that did not satisfy the assumptions of ANOVA were subjected to T-test; while data that passed were subjected to ANOVA following a randomized complete block design, with parity as blocking factor. All the data gathered were subjected to general linear model (GLM) analysis using SAS 9.1.3 (SAS Inst. Inc., Cary, NC, USA) and MS Excel. Results are given as mean ± standard error of the mean. The α -level used to determine significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Dietary supplementation of aIGF-I reduced ($p<0.01$) backfat loss and WEI of sows (Table 2). Reese et al. (1984) indicated a direct relationship between backfat loss of sows during lactation and delayed estrus expression. In addition, uterine development, follicular maturation, farrowing rate and embryo survival can deteriorate at next cycle of sows that lost huge quantities of backfat (Roongsitthichai and Tummaruk, 2014). Earlier studies have shown negative correlation between circulating IGF-I level and lactation weight loss in sows (Quesnel et al., 1998). Underlying mechanisms however, were not yet clear.

On the other hand, ADFI of sows during lactation did not differ ($p>0.05$) between treatments. Addition of 1.0 kg/ton aIGF-I have negligible effect on energy level of basal diets, which could be the reason why ADFI was not affected.

The pregnancy parameters (number of piglets born alive and dead and piglet birth weight) of sows were not influenced ($p>0.05$) by aIGF-I supplementation. Findings were consistent to previous feeding trials in sows for 1 production cycle (Song et al., 2014a and 2015b). On the contrary, increased levels of circulating IGF-I in sows were found to increase uterine capacity by increasing the size and vascular strength of uterus and enhance fetal-maternal diffusion of nutrients (Ford et al., 2002). These may increase embryo survival and viability. In the present study, one cycle may not be enough to influence these parameters.

Feeding diets with aIGF-I to sows reduced mortality at 24 h after birth ($p=0.06$) and at weaning ($p<0.05$). In addition, BW was higher at 24 h post-farrowing ($p=0.06$) and at adjusted 30-day weaning ($p<0.01$) in piglets from sows fed diets with aIGF-I. The results were in agreement to previous experiments (Song et al., 2014a and 2015b; Reyes et al.,2015). The increase in body weight at 24 h post-farrowing may have contributed to the performance at weaning. Heavier piglets often have stronger suckling reflex that stimulates the sow to produce more milk (King' ori, 2005), which directly influences litter performance (Kim et al., 2015). The improvement in piglet viability can also be attributed to the claim of Casebolt (2014), where aIGF-I supplementation can increase IGF-I level in sow's milk. Furthermore, the study of Monaco et al. (2005) on transgenic sows that over express IGF-I in milk, increased IGF-I level in colostrum by 26 folds while 50-90 folds in mature milk. This resulted to improved intestinal maturation and growth performance of piglets.

Collectively, results indicated that addition of 1 kg/ton aIGF-I in gestating and lactating diets reduced WEI and backfat loss of sows at lactation, but did not affect pregnancy parameters. In addition, dietary supplementation of aIGF-I in sows increased growth and survivability of piglets until weaning. Improvement in performance can possibly be due to the increase of IGF-I level in blood and milk of sows. To verify these changes, measurement of IGF-I in blood and milk of sows can be done in different time intervals. More research is also needed to determine if feeding diets with aIGF-I for more than 1 production cycle can affect pregnancy parameters of sows.

Table 1. Composition of experimental diets (as-fed basis)

Item	Gestating	Lactating
Ingredients (%)		
Yellow corn	26.40	44.30
Wheat	20.00	-
Soybean meal (US, 46 %)	11.30	21.40
Wheat pollard (Hard)	12.00	15.00
Rice bran D1	10.00	5.00
Copra cake	10.00	5.00
Coconut oil	1.00	2.35
Molasses	2.50	2.00
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.03	0.03
Other micro-ingredients	6.80	4.95
Total	100.00	100.00
Calculated nutrient contents		
ME (Mcal/kg)	3.14	3.44
CP (%)	14.68	16.10
EE (%)	5.20	6.00
CF (%)	6.09	2.65
Ca (%)	1.05	1.20
Total P (%)	0.75	0.73
Avail. P (%)	0.50	0.50
Total Lys (%)	0.84	0.97
Total Met + Cys (%)	0.55	0.58
Total Thr (%)	0.60	0.63
Total Trp (%)	0.18	0.21

[1]Supplied per kg of diet: Vitamin A (12, 500 IU), Vitamin D_3 (2,250 IU), Vitamin E (50 mg), Vitamin K_3 (2.25 mg), Vitamin B_1 (50 mg), Vitamin B_2 (2.25 mg), Vitamin B_6 (3.5 mg) Vitamin B_{12} (0.025 mg), Niacin (37.50 mg), Pantothenic Acid (17.50 mg), Folic Acid (2.5 mg), Biotin (0.25 mg), Antioxidant (25 mg)

[2]Supplied per kg of diet: Iron (125 mg), Manganese (25 mg), Iodine (0.175 mg), Selenium (0.30 mg), Zinc (125 mg), Copper (7.50 mg).

Table 2. Performance of sows fed diets with and without Activated IGF-I[1]

Item	Activated IGF-I (kg/ton)		SEM	p-value
	0	1.0		
Parity	3.21	3.20	0.28	0.985
Weaning days (d)	34.00	33.60	0.39	0.615
ADFI at lactating (kg)	5.34	5.32	0.06	0.691
Initial backfat depth (mm)	14.06	13.94	0.50	0.691
Backfat loss (mm)	3.43	1.57	0.30	0.001
WEI (days)	6.00	4.53	0.24	0.002
Total piglets born (n)	10.16	11.00	0.42	0.328
Piglets born alive (%)	98.10	99.31	0.65	0.363
Mummified (%)	0.73	0.35	0.53	0.538
Stillborn (%)	1.12	1.23	0.31	0.919
Preweaning mortality (%)				
24 h post-farrowing	2.80	0.42	0.60	0.058
Weaning	19.75	8.14	2.36	0.016
BW of piglets (kg)				
At birth	1.51	1.54	0.04	0.718
24 h post-farrowing	1.58	1.71	0.04	0.063
Adjusted 30-day weaning	6.49	7.31	0.18	0.006

[1]SEM (standard error of mean); ADFI (average daily feed intake), WEI (weaning to estrus interval), BW (body weight)

REFERENCES

Alexander A.N. and Carey H.V. 1999. Oral IGF-I enhances nutrient and electrolyte absorption in neonatal piglet intestine. *Am J Physiol* 277:G619-G625.

Brocardo M.G., Chillaci R., Galeano A., Radrizzani M., White V., Guerrico A.G., Santa-Coloma T.A. and Roldan A. 2001. Early effects of insulin-like growth factor-1 in activated human T lymphocytes. *J Leukoc Biol* 70:297-305.

Casebolt, B.D. 2014. immuTEIN®: Concentrated Source of Activated IGF-I. [PowerPoint Slides]. Gbh Laboratories, Llc. 9122 Cottonwood Ln N, Maple Grove MN 55369 , USA.

Dunshea F.R., Chung C.S., Owens P.C., Ballard J.F., Walton P.E.. 2002. Insulin-like growth factor-I analogues increase growth in artificially-reared neonatal pigs. *Br J Nutr* 87:587-593.

Ellis S., Y. Wu, H. Courtland, D. Cannata, H. Sun, M. Beth-On, C. Liu, H. Jasper, H. Domene, L. Karabatas, C. Guida, J. Pljakic, L. Cardoso, C. Rosen, J. Frystyk and S. Yakar. Unbound (bioavailable) IGF1 enhances somatic growth. *Disease Models and Mechanisms* 4:649-658.

Ford S.P., K.A. Vonnahme and M.E. Wilson. 2002. Uterine capacity in the pig reflects a combination of uterine environment and conceptus genotype effects. *J Anim Sci* 80:E66-E73.

Hartke J.L., Monaco M.H., Wheeler M.B. and Donovan S.M. 2005. Effect of a short-term fast on intestinal disaccharidase activity and villus morphology of piglets suckling insulin-like growth factor-I transgenic sows. *J Anim Sci* 83:2404-2413.

Kim J., J. Seo, W. Kim, H.M. Yun, S.C. Kim, Y. Jang, K. Kim, B. Kim, S. Park, I. Park, M.K. Kim, K.S. Seo, H.B. Kim, I.H. Kim, S. Seo and M. Song. 2015. Effects of palm kernel expellers on productive performance, nutrient digestibility, and white blood cells of lactating sows. *Asian Australas J Anim Sci* 28(8):1150-1154.

King' ori A.M. 2012. Sow lactation: colostrum and milk yield: a review. *J Anim Sci Adv* 2(6):525-533.

Mejia-Guadarrama C.A., A. Pasquier, J.Y. Dourmad, A. Prunier and H. Quesnel. 2002. Protein (lysine) restriction in primiparous lactating sows: Effects on metabolic state, somatotropic axis, and reproductive performance after weaning. *J Anim Sci* 80:3286-3300.

Monaco M.H., Gronlund D.E., Bleck G.T., Hurley W.L., Wheeler M.B. and Donovan S.M. 2005. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increases pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. *Trans Res* 14:761-773.

Reese D.E., E.R. Peo Jr. and A.J. Lewis. 1984. Relationship of lactation energy intake and occurrence of postweaning estrus to body and back fat composition in sows. *J Anim Sci* 58:1236-1244.

Reyes F.C.C., Cardona J.K.M., Angeles E.P., Regaspi A.F.S., Magpantay V.A., Cabigting S.A., Bagui N.J.G., Briones B.H.I., Plata L.M. 2015. Effect of Activated Insulin-like Growth Factor I (IGF-I) on reproductive performance of lactating sows. Proceedings of the 52nd Philippine Society of Animal Science Scientific Seminar and Annual Convention, Palawan, Philippines. p10.

Roongsitthichai A. and Tummaruk P. 2014. Importance of backfat thickness to reproductive performance in female pigs. *Thai J Vet Med* 44(2): 171-178.

Shen W.H. and Xu R.J. 2000a. Stability of insulin-like growth factor-I in the gastrointestinal lumen in neonatal pigs. *J Pediatric Gastroenterology Nutr* 30:299-304.

Song R., K. Friesen, R. Fongemie and A. Morris. 2014a. Effect of feeding betaGRO to sows, nursery and grow-finish pigs (breed to slaughter). Retrieved March 21, 2015 from *http://www.nutriquest.com.*

Song R., K. Friesen, R. Fongemie and A. Morris. 2014b. Effect of feeding betaGRO to sows and nursery pigs (mating through nursery). Retrieved March 21, 2015 from *http://www.nutriquest.com.*

Quesnel H., A. Pasquier, A. Mounier, I. Louveau and A. Prunier. 1998. Influence of feed restriction in primiparous lactating sows on body condition and metabolic parameters. *Reprod Nutr Dev* 38:261-274.

Velloso C.P. 2008. Review: Regulation of muscle mass by growth hormone and IGF-I. *Br J Pharmacol*. 154:557-568.

Xu R.J., D. J. Mellor, M. J. Birtles, B.H. Brier and P. D. Gluckman. 1994. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. *Biol Neonate* 66:280-287.

PO-04-58 Effects of Energy Values Evaluated by Supplementation of Lysophospholipids on Growth Performance and Nutrient Digestibility in Growing Pigs

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Objective

Energy as an important part of the diets comes from carbohydrates, lipids, and protein in the feed. The cost of energy-supplying ingredients in pig's diet contributes the largest portion of total feed cost. Therefore, accurate estimation of both energy requirements for pigs and feed energy values may reduce the cost of pig production (Kill et al., 2013).

Lipid has the highest energy content among the nutrients, which is emulsified by lecithin in bile and hydrolyzed by lipase. The absorption of fat in the gastrointestinal tract depends on the emulsification for micellar formation, since they are insoluble in water (Church, 1988).

Digestion of lipids in gastrointestinal tract (GIT) is composed of physical processes and enzymatic hydrolysis sequentially. The physical processes are solubility by emulsification that is lead to the aliphatic molecule to the form of droplets in the aqueous environment of the digestive tract (Church, 1988).

Lysophospholipids are a sort of emulsifiers that can be produced by removing one of the fatty acids groups of phospholipids in an enzymatic reaction. They are major component of all cell membranes.

This research was conducted to estimate the energy values of some feed ingredients as affected by lysophospholipids supplementation in growing pigs and to determine the effect of energy increment of ingredients supplemented with lysophospholipids on growth performance of growing pigs.

Methodology

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea.

A total of 200 growing pigs (Yorkshire × Landrace × Duroc) with an initial body weight (BW) of 32.16 ± 1.20 kg were randomly allotted to 4 treatments on the basis of BW. There were 5 replicates in each treatment with 10 pigs per replicate. The experimental diets were fed for 42 d in two phases: phase Ⅰ (d 0 to 21) and phase Ⅱ (d 22 to 42). For a feeding trial, pigs were housed in partially slatted and concrete floor pens. All pens were equipped with a self-feeder and a nipple drinker to allow ad libitum access to feed and water.

As shown in Table 13, 2 × 2 factorial design was used (ME, LipidolTM/3,350kcal, 0%, 0.1% vs. ME, LipidolTM/3,298kcal, 0%, 0.1%). ME values of ingredients (corn, SBM, DDGS and animal fat) in this feeding trial was calculated from NRC (2012). For the groups of ME 3,298 kcal/kg, ME of ingredients was those measured in metabolism experiment (Expt 1), which is equal to 3,350 kcal/kg.

Diets were formulated to meet or exceed the requirement of NRC (2012) and experimental diets formula and chemical compositions were presented in tables 14 and 15, respectively.

For the analysis of nutrient digestibility, Chromium oxide (Cr2O3, 0.25%) was used as an indigestible marker in the each phase diets to calculate digestibility coefficients. All pigs in all pens were fed diets mixed with chromic oxide from d 14 to 21 and d 35 to 42 of experiment. Fecal grab samples were collected from the floor of each pen during the last 4 d of each phase. The feces collected were pooled to represent one pen and dried in an air forced drying oven 60℃ for 72 h, and ground in a 1 mm screen wiley mill for chemical analysis.

At the d 21 and d 42 of experiment, a 10-mL blood sample was collected by jugular vein puncture from 2 randomly selected pigs in each pen using a disposable vacutainer tube containing sodium heparin as an anticoagulant (Becton Dickinson, Franklin, NJ). Serum automatic biochemical analyzer (Fuji Dri-chem 3500i, Japan) applied to measure concentrations of total protein (TP), blood urea nitrogen (BUN), total cholesterol (TCHO), glucose (GLU), triglyceride (TG), albumin and globulin. After centrifugation (3,000 ? g for 20 min), plasma samples were separated and stored at –20℃ and later analyzed for concentrations blood parameters.

Also, proximate analysis in experimental diets and fecal samples (5 samples/treatment) was done by the method of (AOAC, 2007). The gross energy (GE) of ingredients and diets were measured using a bomb calorimeter (Model

1241, Parr Instrument Co., Molin, IL). Experimental diets and fecal samples were analyzed for chromium (Cr) concentration by using spectrophotometer (Model V -550, Jasco Co., Japan).

Data were analyzed as a 2 × 2 factorial arrangement of treatments. The main effects of energy level of diet, LipidolTM and their interaction were determined by GLM (general linear model) procedure of SAS. The pen was used as the experimental unit for the analysis of growth performance, nutrient digestibility and economic efficiency data. For the analysis of blood metabolites, the mean of 2 pigs from each pen was used as the experimental unit, and 1 randomly selected pig for each pen was used as the experimental unit. Statistical significance and tendency were considered at p

Result

Growth performance of pigs fed LipidolTM was presented in Table 2. Pigs fed high-energy diets had higher ADG ($p=0.014$) than pigs fed low-energy diets during phase I. There was no effect of LipidolTM supplementation on ADG ($p>0.05$), however the ADG of pigs fed LipidolTM tended to be greater ($p=0.096$). No interactions were observed between energy levels treatments and LipidolTM for ADFI and F/G.

During phase II, ADG of pigs fed high-energy diets was higher than pigs fed low-energy diets ($p=0.039$). Also, dietary addition of LipidolTM improved ADG of pigs ($p=0.047$). During the overall period, there were energy and LipidolTM effects o0n ADG and feed conversion ratio.

Pigs fed high-energy diets had higher GE and fat digestibility significantly compared with other treatments. Pigs fed low-energy diets supplementated LipidolTM had lowest GE and fat digestibility between treatments significantly in phase I and II.

There was no significant difference in the phase I, but pigs fed LipidolTM supplementation diets tended to improve digestibilities of DM, GE, CP and fat ($0.05<p\leq0.10$). Digestibilities of GE and fat were improved in pigs fed high-energy diets ($p<0.05$). Pigs fed LipidolTM were significantly improved digestibility of DM in phase II ($p<0.05$), however, there was no effect of high-energy diets on digestibility of DM ($p>0.05$). Digestibilities of GE and fat were significantly improved by high-energy diets or LipidolTM supplementation diets ($p<0.05$). LipidolTM supplemented diets significantly improved digestibility of CP ($p<0.05$).

The overall concentration of blood TCHO, TG, TP, BUN, GLU, albumin and globulin were not affected by dietary treatments ($p>0.05$).

Conclusion

In conclusion, no differences were observed between energy levels and LipidolTM for ADFI and F/G. HoweverF/G tended to be greater in pigs fed LipidolTM . The overall result showed that there was no interaction among the treatments.

Table 1. Formula and chemical composition of experimental diets.

	Phase 1				Phase 2			
Energy	3,350	3,350	3,298	3,298	3,350	3,350	3,350	3,350
Lipidol	-	+	-	+	-	+	-	+
Ingredients (%)								
Corn	56.26	56.16	57.87	57.77	61.22	61.12	59.53	59.43
SBM	22.82	22.82	22.81	22.81	21.39	21.39	21.43	21.43
DDGS	11.44	11.44	11.02	11.02	8.71	8.71	9.05	9.05
Animal fat	2.23	2.23	1.03	1.03	1.04	1.04	2.26	2.26
Molasses	3.97	3.97	3.99	3.99	4.00	4.00	4.00	4.00
Thr-98.5%	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.03
L-Lysine (78%)	0.37	0.37	0.37	0.37	0.34	0.34	0.34	0.34
Met 50%	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13
Tryptophan 10%	-	-	-	-	0.38	0.38	0.47	0.47
Choline chloride (50%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Limestone	1.22	1.22	1.21	1.21	0.78	0.78	0.77	0.77
MDCP	0.75	0.75	0.76	0.76	1.08	1.08	1.09	1.09
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix[1]	0.15	0.15	0.15	0.15	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lipidol	-	0.10	-	0.10	-	0.10	-	0.10
Total	100	100	100	100	100	100	100	100
Chemical composition (%)								
ME (kcal/kg)	3,350	3,350	3,298	3,298	3,350	3,350	3,350	3,350
CP	18.00	18.00	18.00	18.00	17.00	17.00	17.00	17.00
Ca	0.70	0.70	0.70	0.70	0.65	0.65	0.65	0.65
Av. P	0.31	0.31	0.31	0.31	0.30	0.30	0.30	0.30
SID Lys	0.98	0.98	0.98	0.98	0.92	0.92	0.92	0.92
SID Met+Cys	0.55	0.55	0.55	0.55	0.52	0.52	0.52	0.52
SID Thr	0.59	0.59	0.59	0.59	0.54	0.54	0.54	0.54
SID Trp	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16

[1]Supplied per kg diet: 150 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.336 mg Se.

[2] Supplied per kg diet: 10,000 IU Vit A, 2,500 IU Vit D3, 50 IU Vit E, 1.5 mg Vit K3, 1.5 mg Vit B1, 5 mg Vit B2, 3 mg Vit B6, 0.025 mg Vit B12, 15 mg pantothenic acid, 35 mg niacin, 0.15 mg biotin, 1 mg folic acid.

Table 2. Effect of dietary energy level and Lipidol supplementation on growing performance of growing pigs

ME, Kcal/kg	3,350		3,298		SEM[1]	*p*-value[2]		
Lipidol	-	+	-	+		ME	E	ME×E
Phase Ⅰ (d 0~21)								
ADG, g	741	750	702	730	10.37	0.014	0.096	0.390
ADFI, g	1,563	1,558	1,552	1,566	18.17	0.916	0.818	0.621
F/G	2.11	2.08	2.21	2.15	0.05	0.101	0.297	0.714
Phase Ⅱ (d 22~42)								
ADG, g	830	874	798	828	16.22	0.039	0.047	0.706
ADFI, g	1,953	1,949	1,941	1,957	13.78	0.894	0.691	0.468
F/G	2.36	2.24	2.43	2.36	0.05	0.065	0.070	0.603
Overall (d 0~42)								
ADG, g	786	812	750	779	7.23	0.001	0.003	0.809
ADFI, g	1,758	1,753	1,746	1,761	14.36	0.894	0.731	0.500
F/G	2.24	2.16	2.33	2.26	0.03	0.006	0.030	0.869

[1]Standard error of means.
[2]ME: energy level, E: emulsifier (Lipidol), ME×E: energy level×emulsifier.

Table 3. Effect of dietary energy level and Lipidol supplementation on apparent total tract digestibility of nutrients of growing pigs

ME, kcal/kg	3,350		3,298		SEM[1]	*p*-value[2]		
Lipidol	-	+	-	+		ME	E	ME×E
Phase I (d 21)								
DM	80.80	81.96	79.21	81.05	0.77	0.130	0.073	0.670
GE	81.40	82.32	78.98	80.90	0.76	0.023	0.081	0.526
CP	76.99	77.34	75.42	76.65	0.78	0.224	0.080	0.709
Fat	65.89	67.07	64.65	65.63	0.54	0.026	0.066	0.859
Phase II (d 42)								
DM	80.02	81.29	78.59	80.50	0.72	0.143	0.042	0.665
GE	80.02	81.94	77.43	79.66	0.84	0.012	0.028	0.859
CP	75.17	76.42	74.65	76.02	0.65	0.414	0.046	0.414
Fat	65.19	66.84	63.95	65.21	0.66	0.045	0.043	0.766

[1]Standard error of means.

[2]ME: energy level, E: emulsifier (Lipidol), ME×E: energy level×emulsifier.

Table 4. Effect of dietary energy level and Lipidol supplementation on blood metabolites of growing pigs

ME, kcal/kg	3,350		3,298		SEM[1]	*p*-value[2]		
Lipidol	-	+	-	+		ME	E	ME×E
Phase I (d 21)								
TCHO, mg/dL	99.70	97.00	100.20	97.80	5.83	0.912	0.665	0.980
TG, mg/dL	52.70	56.60	55.40	54.10	5.05	0.984	0.799	0.611
GLU, mg/dL	88.50	91.80	93.10	93.60	5.20	0.544	0.718	0.790
BUN, mg/dL	11.06	12.11	10.60	11.55	0.81	0.548	0.243	0.953
TP, mg/dL	6.42	6.15	6.35	6.40	0.53	0.865	0.836	0.763
Albumin, g/dL	3.46	3.37	3.45	3.39	0.08	0.949	0.344	0.849
Globumin, g/dL	2.96	2.78	2.90	3.01	0.08	0.300	0.667	0.081
Phase II (d 42)								
TCHO, mg/dL	98.70	93.80	95.30	94.20	6.51	0.819	0.648	0.772
TG, mg/dL	60.40	65.50	59.70	61.00	4.77	0.590	0.508	0.693
GLU, mg/dL	91.70	92.30	91.50	94.20	4.94	0.865	0.741	0.833
BUN, mg/dL	10.98	11.26	9.97	11.07	0.44	0.185	0.129	0.362
TP, mg/dL	6.37	6.36	6.47	6.44	0.50	0.858	0.968	0.984
Albumin, g/dL	3.75	3.70	3.89	3.79	0.10	0.282	0.480	0.813
Globumin, g/dL	2.62	2.66	2.58	2.65	0.11	0.835	0.647	0.901

[1]Standard error of means.

[2]ME: energy level, E: emulsifier (Lipidol), ME×E: energy level×emulsifier.

References

AOAC. 2007. Official methods of analysis (18th ed.). Association of Official Agricultural Chemists. Washington, DC.

Church, D. C. 1988. The ruminant animal digestive physiology and nutrition. Reston. New Jersey.

Kil, D. Y., B. G. Kim, and H. H. Stein. 2013. Feed Energy Evaluation for Growing Pigs. Asian Australas. J. Anim. Sci. 26: 1205-1217.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

PO-04-59

Effects of nutrient levels and feed Composition in Weaner Diets on Growth Performance of Growing-Finishing Pigs

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Objective

Feeding is the most important aspect of swine production that contributes for their performance and health status. The results of previous studies have indicated that pigs subjected to early dietary AA restrictions or low quality protein sources can exhibit compensatory growth in growing phase(Chiba et al., 2002; Fabian et al., 2004), utilize nutrients more efficiently (Chiba et al., 2002; Fabian et al., 2004), have better carcass traits and reduce N excretion (Fabian et al., 2004). We hypothesized that the early fast growth performance cannot guarantee the final body weight at the end of fattening period. The present experiment was designed to study the effect of nutrient levels and ingredient compositions on the performance, digestibility of nutrients and intestine healthy in pigs.

Methodology

A total of 150 weaned piglets (Landrace × Yorkshire × Duroc; initial body weight (BW): 7.40 ± 0.78 kg; 25 ± 2 days of age) of mixed sex were randomly allotted to 3 treatments on the basis of BW and sex. There were five replicates pens in each treatment with 10 pigs per pen. Dietary treatments included 3 levels of feed composition in a completely randomize design. Treatment diets were fed in a meal form in 3 starter (d 0 to 7, phase I; d 8 to 21, phase II, d 22 to 35, phase III), 2 growing (d 36 to 63, phase I; d 64 to 99, phase II) and 2 finisher phases (d 100 to 132, phase I; d 133 to 151, phase II). All diets met or exceeded the nutrient requirements as suggested by NRC (1998).

The project underwent proper ethical standards and the experiments were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. These experiments were conducted at the facility of Kangwon National University farm and the piglets were housed in partially slotted and concrete floor pens with a pen size of 1.90 m × 3.0 m. All pens were equipped with a self-feeder and nipple drinker to allow ad libitum access to feed and water.

Individual weanling piglets weight and feed disappearance from each pen were recorded at the beginning of the experiment and at the end of every phase to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F). To evaluate the effects of dietary treatments on the apparent total tract digestibility (ATTD) of nutrients, 0.25% chromic oxide (an inert indigestible indicator) was added to all four phases (Phase I, 0 to 7 days; phase II, 8 to 21 days, phase III, 22 to 35 days and grower phase) diets of each experiments. Pigs were fed diets mixed with chromic oxide from d 0 to 7, 14 to 21 and 28 to 35 days and fecal grab samples were collected from each pen on the last 3 d of each experiment to determine the ATTD of dry matter (DM), gross energy (GE) and crude protein (CP). The fecal samples were pooled within pen and dried in a forced air oven at 60 °C for 72 h, and ground in a Wiley mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) using a 1-mm screen and used for chemical analysis.

To study the effects of dietary treatments on small intestinal morphology and microbiota of ileal and cecal digesta, representative piglets from each group (2 per pen) reflecting the average BW of the pen were selected and sacrificed by electrocution at d 35 of each experiment. The digesta from the ileum and caecum were collected in sterile plastic bottles for microbial analysis. The samples collected for microbial analysis were immediately placed on ice until analyses were conducted. The samples of the intestinal segment from the region of duodenum, jejunum and ileum were collected after removing the content and flushing with physiological saline. The samples were then submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2% paraformaldehyde and 1.5% acrolein and then brought to the laboratory to study the morphological changes.

Experimental diets and excreta samples were analyzed in triplicate for DM (Method 930.15) and CP (Method 990.03) using AOAC (2007) methods. Gross energy of diets and feces were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL), and chromium concentration was determined with an automated

spectrophotometer (Jasco V-650; Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979).

The microbiological assay of ileum and cecum digesta was carried out by culturing in different media as suggested by Choi et al. (2011).

One gram of the composite cecum or ileum sample was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in the samples were then conducted by plating serial 10-fold dilutions. For the determination of *Lactobacillus* spp. (using MRS agar + 0.200 g/l NaN3 + 0.500 g/l l-cystine hydrochloride monohydrate), *Clostridium* spp. (TSC agar) and coliforms (violet red bile agar) were used.

Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Yoon et al., 2012). A total of ten intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip of the villi to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi. All morphological measurements (villus height and crypt depth) were made in 10-μm increments by using an image processing and analysis system (Optimus version 6.5 software, Media Cybergenetics, North Reading, MA).

Data generated in the present study were subjected to statistical analysis using the GLM- one-way analysis of variance test using the SAS statistical software package version 8.2 (SAS Inst. Inc., Cary, NC, USA). Pens were considered the experimental unit for growth performance, and piglets were experimental units for measuring the digestibility of nutrients and all intestinal sampling. P-values ≤0.05 were considered statistically significant.

Results

Effects of nutrient levels and ingredient composition in diets on growth performances in weanling pigs are presented in table 1. Higher average daily gain and greater ($p<0.05$) average daily feed intake were observed in T1 in phase I. No significant differences ($p>0.05$) in feed conversion ratio were observed among treatments. The greater ADG and ADFI ($p<0.05$) were observed for T1 in phase II. There were no differences ($p>0.05$) in ADFI and FCR but ADG was higher ($p<0.05$) in T1 treatment in phase III. The overall performance showed that nutrient levels and ingredient composition in diets had significant effects ($p<0.05$) on ADG, ADFI and FCR of weanling pigs. Greater ADG, ADFI and lower FCR were observed in T1 among the treatments.

Growth performances in growing pigs are presented in table 2. The greater ADG ($p<0.05$) was observed in T1 but no differences ($p>0.05$) in ADFI and FCR in growing I phase. The higher ADG and lower FCR ($p<0.05$) were showed in T3 during growing II. In the phase I of finishing period, greater ADG and lower FCR ($p<0.05$) were showed in T3 treatment. There were no differences ($p>0.05$) in ADG, ADFI and FCR among the treatments in finishing II. The overall result showed the greater ADG and lower FCR ($p<0.05$) in T3 treatment but no effects on ADFI was observed.

Apparent nutrient digestibility in weanling pigs presented in table 3. Greater gross energy (GE) and crude protein (CP) digestibility ($p<0.05$) were observed in T1 during phase I but no effect ($p>0.05$) on dry matter (DM) digestibility was observed. Digestibility of CP was higher ($p<0.05$) in T1 during phase II. There were no effects ($p>0.05$) of nutrient levels and ingredient composition in diets on DM, GE and CP digestibility during phase III.

Effects of nutrient levels and ingredient composition in diets on fecal microbial populations in weanling pigs are presented in table 4. There was significant effect ($p<0.05$) on *Clostridium* spp. and it was lower in T1 treatment. There had no effects ($p>0.05$) on total anaerobic bacteria, *Lactobacillus* spp. and *E. coli* in all phases.

Effects of nutrient levels and ingredient composition in diets on small intestinal morphology in weanling pigs are showed in table 5. Greater duodenal villus height ($p<0.05$) was observed in T1 treatment but no effects ($p>0.05$) on jejunal and ileal villus height and crypt depth. Villus height and crypt depth ratio was affected ($p<0.05$) by nutrient levels and ingredient composition in diets. Greater duodenal ratio and lower jejunal and ileal ratio between villus height and crypt depth were observed in T1 among the treatments.

Conclusion

In conclusion, the high quality weaner diet improved the performance of weanling piglets; however, weaned piglets fed low quality diet after weaning, showed greater growth performance in growing and finishing period.

Table 1. Effects of nutrient levels and ingredient composition in diets on growth performance in wealing pigs

Treatments	T1	T2	T3	SEM[4]
Phase Ⅰ (d 0~7)[1]				
ADG, g	312[a]	297[ab]	279[b]	4.79
ADFI, g	403[a]	391[ab]	379[b]	3.43
FCR	1.29	1.31	1.37	0.02
Phase Ⅱ (d 8~21)[2]				
ADG, g	428[a]	401[b]	357[c]	8.13
ADFI, g	704[a]	660[b]	603[c]	11.11
FCR	1.65	1.65	1.69	0.01
Phase Ⅲ (d 22~35)[3]				
ADG, g	623[a]	589[b]	548[c]	8.67
ADFI, g	1,009	1,013	983	6.48
FCR	1.62[c]	1.72[b]	1.79[a]	0.02
Overall (d 0~35)				
ADG, g	473[a]	447[b]	410[c]	7.11
ADFI, g	752[a]	731[b]	694[c]	6.98
FCR	1.59[c]	1.64[b]	1.69[a]	0.01

[1]Whey:DSP (%); T1=40:0, T2=26:5, T3=14:10.

[2]Whey:DSP (%); T1=20:0, T2=10:5, T3=0:10.

[3]Fish meal:DSP (%); T1=3:5, T2=0:0, T3=0:0.

[4]Standard error of means.

[abc]Values with different superscripts in the same row differ significantly ($p<0.05$).

Table 2. Effects of nutrient levels and ingredient composition in diets on growth performance in weanling pigs

Treatments	T1	T2	T3	SEM[4]
Growing Ⅰ (d 36~63)				
ADG, g	745[a]	716[b]	702[c]	5.10
ADFI, g	1,507	1,463	1,457	9.50
FCR	2.02	2.04	2.07	0.01
Final wt, kg	44.84	43.08	41.40	
Growing Ⅱ (d 64~99)				
ADG, g	814[b]	841[a]	855[a]	5.58
ADFI, g	2,208	2,178	2,183	8.08
FCR	2.71[a]	2.59[b]	2.55[c]	0.01
Final wt, kg	74.13	73.36	72.20	
Finishing Ⅰ (d 100~132)				
ADG, g	800[b]	814[ab]	834[a]	5.62
ADFI, g	2,639	2,617	2,581	20.16
FCR	3.30[a]	3.21[ab]	3.09[b]	0.03
Final wt, kg	100.52	100.24	99.72	
Finishing Ⅱ (d 133~151)				
ADG, g	842	850	848	7.49
ADFI, g	2,838	2,762	2,716	29.84
FCR	3.37	3.25	3.21	0.04
Final wt, kg	116.52	116.39	115.83	
Overall (d 36~151)				
ADG, g	798[b]	805[ab]	811[a]	2.16
ADFI, g	2,264	2,226	2,208	11.67
FCR	2.84[a]	2.77[ab]	2.72[b]	0.02
Initial wt, kg	23.98	23.04	21.74	
Final wt, kg	116.52	116.39	115.3	

[1]Standard error of means.

[abc]Values with different superscripts in the same row differ significantly ($p<0.05$).

Table 3. Effect of nutrient levels and ingredient composition in diets on apparent nutrient digestibility in weanling pigs

Treatments	T1	T2	T3	SEM[4]
Phase Ⅰ (d 7)[1]				
DM	85.69	84.95	84.56	0.23
GE	85.33[a]	85.02[ab]	83.64[b]	0.29
CP	79.11[a]	78.87[ab]	77.67[b]	0.25
Phase Ⅱ (d 21)[2]				
DM	86.47	85.80	85.43	0.22
GE	86.04	85.72	84.49	0.29
CP	80.12[a]	79.94[ab]	78.76[b]	0.24
Phase Ⅲ (d 35)[3]				
DM	86.45	85.77	85.46	0.23
GE	86.13	85.60	84.07	0.29
CP	80.22	79.83	79.48	0.24

[1]Whey:DSP (%); T1=40:0, T2=26:5, T3=14:10.

[2]Whey:DSP (%); T1=20:0, T2=10:5, T3=0:10.

[3]Fish meal:DSP (%); T1=3:5, T2=0:0, T3=0:0.

[4]Standard error of means.

[ab]Values with different superscripts in the same row differ significantly ($p<0.05$)

Table 4. Effects of nutrient levels and ingredient composition in diets on fecal microbial populations in weanling pigs

Treatments	T1	T2	T3	SEM[4]
Phase Ⅰ (d 7)[1]				
Total anaerobic bacteria	8.30	8.20	8.12	0.10
Lactobacillus spp.	7.66	7.52	7.45	0.09
Clostridium spp.	6.18[a]	6.33[ab]	6.49[b]	0.10
E. coli	5.89	5.91	5.97	0.07
Phase Ⅱ (d 21)[2]				
Total anaerobic bacteria	8.34	8.22	8.19	0.11
Lactobacillus spp.	7.60	7.49	7.39	0.14
Clostridium spp.	5.98[a]	6.18[ab]	6.31[b]	0.12
E. coli	5.79	5.87	5.90	0.11
Phase Ⅲ (d 35)[3]				
Total anaerobic bacteria	8.37	8.26	8.21	0.09
Lactobacillus spp.	7.53	7.42	7.34	0.09
Clostridium spp.	5.81[a]	6.08[ab]	6.23[b]	0.08
E. coli	5.63	5.73	5.81	0.13

[1]Whey:DSP (%); T1=40:0, T2=26:5, T3=14:10.

[2]Whey:DSP (%); T1=20:0, T2=10:5, T3=0:10.

[3]Fish meal:DSP (%); T1=3:5, T2=0:0, T3=0:0.

[4]Standard error of means.

[ab]Values with different superscripts in the same row differ significantly ($p<0.05$)

Table 5. Effects of nutrient levels and ingredient composition in diets on small intestinal morphology in weanling pigs

Treatments	T1	T2	T3	SEM[4]
Villus height (A), μm				
Duodenum	602[a]	584[ab]	571[b]	5.42
Jejunum	402	425	439	5.27
Ileum	308	321	336	4.76
Crypt depth (B), μm				
Duodenum	314	320	324	2.97
Jejunum	243	247	253	2.62
Ileum	164	167	162	2.38
A/B[1]				
Duodenum	1.92[a]	1.83[b]	1.76[b]	0.02
Jejunum	1.65[b]	1.72[a]	1.73[a]	0.01
Ileum	1.88[b]	1.93[ab]	2.07[a]	0.03

[1]Villus height and crypt depth ratio.

[2]Standard error of means.

[ab]Values with different superscripts in the same row differ significantly ($p<0.05$).

References

AOAC. 2007. Official Methods of Analysis. 18th edn Association of Official Analytical Chemists. Gaithersburg, MD, USA.

Chiba, L. I., A. J. Lewis, and E. R. Peo. 1991. Amino acid and energy interrelationships in pigs weighing 20 to 50 kilograms: I. Rate and efficiency of weight gain. J. Anim. Sci. 69:694–707.

Choi, J. Y., Kim, J. S., Ingale, S. L., Kim, K. H., Shinde, P. L., Kwon, I. K., Chae, B. J. 2011. Effect of potential multimicrobe probiotic product processed by high drying temperature and antibiotic on performance of weanling pigs. J. Anim. Sci. 89:1795–1804.

Fabian, J., L. I. Chiba, L. T. Frobish, W. H. McElhenny, D. L. Kuhlers, and K. Nadarajah. 2004. Compensatory growth and nitrogen balance in grower-finisher pigs. J. Anim. Sci. 82:2579–2587.

Fenton T. W and M. Fenton. 1979. An improved method for chromic oxide determination in feed and feces. Can. J. Anim. Sci. 59:63–634.

National Research Council. 1998. Nutrient Requirements of Swine. 10th Edn. National Academy Press, Washington, DC.

Yoon J. H, S. L Ingale, J. S Kim, K. H Kim, S. H Lee, Y. K Park, I. K Kwon, and B. J Chae. 2012. Effects of dietary supplementation of antimicrobial peptide-A3 on growth performance, nutrient digestibility, intestinal and fecal microflora and intestinal morphology in weanling pigs. Anim. Feed. Sci. Tech. 177:98–107.

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Effect of dietary melamine concentrations on performance and tissue melamine residue in broiler chickens

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Introduction

Melamine (1, 3, 5-triazine-2, 4, 6-triamine) is a 6-nitrogen-containing organic compound and contains 67% nitrogen on a molecular weight basis. Melamine is widely used for various industries such as varnishes, paints, glues, flame retardants, plastic packages, and food containers. Melamine is rarely metabolized by animals and is rapidly excreted within 24 h. However, long-term exposure to high levels of dietary melamine led to increased water intake, increased urine output, diarrhea, proteinuria, kidney stone, and bladder cancer (Hau et al., 2009). Melamine can be hydrolyzed to ammeline, ammelide, and cyanuric acid. Melamine can interact with the isomeric form of cyanuric acids to create crystals in kidney, resulting in renal failure in animals and humans (Brown et al., 2007; Ingelfinge, 2008).

Recently, it was confirmed that melamine was illegally adulterated pet food (United States, 2007) and milk power (China, 2008) in order to increase the protein content (Ingelfinger, 2008; Wu et al., 2009). Consequently, there has been a worldwide increase in the concern of melamine in human food and animal feed.

There is a lack of experiments studying the effects of various melamine levels of diets on physiological responses in broiler chickens. The objective of the current experiment, therefore, was to investigate the effect of dietary melamine concentrations on performance and tissue melamine residue in broiler chickens.

Materials and methods

Birds, diets, and experimental design

A total of 1,008 1-d-old Ross 308 broiler chickens [initial body weight (BW) = 46.0 ± 1.52] were obtained from a local hatchery (Yangji hatchery, Pyeongtaek, Republic of Korea) and were housed in 84 battery cages (76 cm × 78 cm × 45 cm = width × length × height for each cage) in an environmentally controlled room. Birds were randomly allotted to 1 of 7 dietary treatments with 12 replicates, each replicate consisting of 12 birds, in a completely randomized design. Dietary melamine concentrations were set to 0, 250, 500, 750, 1,000, 5,000, or 10,000 mg/kg by adding purified form of melamine (≥ 99.0%) at the expense of the sand. All diets were formulated to meet or exceed the NRC (1994) requirements for broiler chickens during 35-d. The experimental diets were given to the birds on an ad libitum basis for 35 d. The diets were fed in mash form. The room temperature was maintained at 30°C during the first wk and then gradually decreased to 24°C at the end of the experiment. A 24-h lighting schedule was used throughout the experiment. The BW gain (BWG) and feed intake (FI) were recorded at the end of the experiment. Feed efficiency (G:F, g/kg) was calculated by dividing BWG with FI.

Sample collection and chemical analyses

At the end of the experiment, 6 birds from each treatment were euthanized by CO_2 asphyxiation and immediately dissected, and then kidney and breast samples were collected for melamine residue analysis. The kidney and breast samples was then frozen at -20℃ before the analysis. The melamine concentrations were determined using high-performance liquid chromatography (HPLC) in an accredited laboratory.

Statistical Analysis

All data were analysed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Each replicate was considered an experimental unit in the analysis of growth performance, whereas 6 birds from each treatment served as the experimental unit for tissue melamine residue. Outlier data were checked using the UNIVARIATE procedure of SAS (Steel et al., 1997), but no outlier was identified. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing concentrations of melamine in diets. Significance for statistical tests was set at $P < 0.05$.

Results

Growth performance

Results indicated that the BW, BWG, and FI for birds fed diets containing 10,000 mg/kg melamine were less ($P < 0.01$) than those fed diets 0, 250, 500, 750, 1,000, and 5,000 mg/kg melamine. However, the G:F and mortality were not influenced by dietary treatments. Orthogonal polynomial contrast test revealed that increasing melamine concentrations of diets decreased BW, BWG (linear and quadratic, $P < 0.01$), FI (linear, $P < 0.01$), and G:F (quadratic, $P < 0.05$).

Tissue melamine residue

Kidney and breast melamine residue for 10,000 mg/kg treatment were greater ($P < 0.01$) than other treatment groups. Increasing melamine concentrations of diets increased (linear, $P < 0.01$) melamine residues in the kidney and increased (linear and quadratic, $P < 0.05$) melamine residues in the breast.

Linear regression analysis of melamine concentrations in breast at each melamine concentrations in diets

According to food safety issue (WHO), melamine concentrations of human food should be limited less than 2.5 mg/kg. Thus, based on linear regression analysis between dietary melamine concentrations and breast melamine residue ($y = 0.0063x - 1.5623$, $R^2 = 0.89$), the upper limit of melamine concentrations of diets for broiler chickens was 645 mg/kg.

Conclusion

In conclusion, 10,000 mg/kg melamine is toxic to broiler performance. A linear increase in melamine residue in the kidney and breast was observed by increasing melamine concentrations in diets. Dietary melamine concentrations for broiler chickens should be limited approximately less than 640 mg/kg in terms of human food safety.

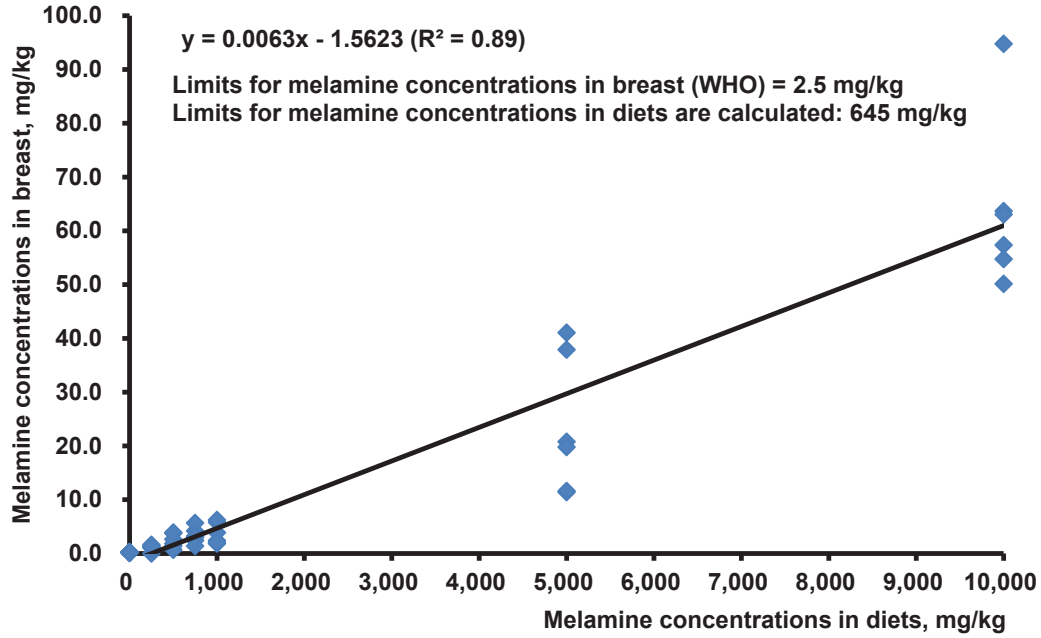

Figure 1. Linear regression analysis of melamine concentrations in breast at each melamine concentrations in diets

Table 1. Effect of dietary melamine concentrations on growth performance in broiler chickens[1]

	Dietary treatments (Melamine concentrations, mg/kg)								P-value[2]		
Items	0	250	500	750	1,000	5,000	10,000	SEM	T	L	Q
BW[3], g	1,881[a]	1,899[a]	1,865[a]	1,902[a]	1,878[a]	1,868[a]	1,666[b]	27.6	<0.01	<0.01	<0.01
BWG[3], g	1,835[a]	1,853[a]	1,819[a]	1,856[a]	1,832[a]	1,822[a]	1,620[b]	27.4	<0.01	<0.01	<0.01
FI[3], g	3,044[a]	3,078[a]	2,990[a]	3,072[a]	3,013[a]	2,987[a]	2,748[b]	36.9	<0.01	<0.01	0.06
G:F[3], g/kg	625	623	628	628	629	632	615	4.5	0.19	0.11	0.02
Mortality, %	4.5	2.3	3.0	0.8	2.3	5.3	3.0	1.81	0.65	0.60	0.43

[ab]Means with different superscripts within a row in dietary treatments are different ($P < 0.05$).

[1]Data are least squares means of 12 observations per treatment.

[2]T: overall effects of treatments; L: linear effects of increasing melamine concentrations in diets; Q: quadratic effects of increasing melamine concentrations in diets.

[3]BW, body weight; BWG, body weight gain; FI, feed intake; G:F, feed efficiency.

Table 2. Effect of dietary melamine concentrations on tissue melamine residue in broiler chickens[1]

	Dietary treatments (Melamine concentrations, mg/kg)								P-value[2]		
Items	0	250	500	750	1,000	5,000	10,000	SEM	T	L	Q
Kideney, mg/kg	0.2[b]	1.0[b]	1.7[b]	2.6[b]	2.7[b]	31.4[ab]	66.1[a]	7.87	<0.01	<0.01	0.83
Breast, mg/kg	0.2[c]	1.1[c]	2.4[c]	3.3[c]	3.7[c]	23.8[b]	64.0[a]	3.19	<0.01	<0.01	0.03

[a-c]Means with different superscripts within a row in dietary treatments are different ($P < 0.05$).

[1]Data are least squares means of 6 observations per treatment.

[2]T: overall effects of treatments; L: linear effects of increasing melamine concentrations in diets; Q: quadratic effects of increasing melamine concentrations in diets.

References

Brown, C. A., K.-S. Jeong, R. H. Poppenga, B. Puschner, D. M. Miller, A. E. Ellis, K.-I. Kang, S. Sum, A. M. Cistola, and S. A. Brown. 2007. Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J. Vet. Diagn. Invest. 19:525-531.

Hau, A. K., T. H. Kwan, and P. K. Li. 2009. Melamine toxicity and the kidney. J. Am. Soc. Nephrol. 20:245-250.

Ingelfinger, J. R. 2008. Melamine and the global implications of food contamination. N. Engl. J. Med. 359:2745-2748.

NRC, 1994. Nutrient Requirements of Poultry. 9th Rev. ed. Natl. Acad. Press, Washington, DC.

Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw Hill BookCo., NewYork.

World Health Organization (WHO). Expert meeting to review toxicological aspects of melamine and cyanuric acid. http://www.who.int/foodsafety/fs_management/Exec_Summary_melamine.pdf

Wu, Y. N., Y.-F. Zhao, and J.-G. Li. 2009. A survey on occurrence of melamine and its analogues in tainted infant formula in china. Biomed. Environ. Sci. 22:95-99.

PO-04-71

ACE inhibitory activity of original peptides derived from "milky miso" on hypertensive rats and people

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Introduction

Because of the local community demand in Japan to minimize the risk of hypertension in elderly people, we attempted to take advantage of the functional properties of skim milk and soybeans by developing a new product named milky miso (MM). This product simply achieved and consists of skim milk and miso paste, which it was found to be effective in improving rats bone conditions and decreasing the incidence of lifestyle-related diseases, especially which occurs for elderly women[1)]. In the previous results obtained from the same product demonstrated that MM had clinical benefits on young rats. In current study, we put onto consideration a special attention, which has been given to the isolation of bioactive peptides with ACE-inhibitory activities.

The present study aimed to isolate two peptides and to elucidate their functions and the bioavailability that present in MM. Particularly the purpose was to identify the crucial aspects that have to be taken into consideration for an efficient production of bioactive peptides from MM. With association to other physiological functions in this article also a superior interest has been given to the specific action against ACE mechanism in vivo.

Materials and methods

1. Materials

The used skim milk in this study was similar to the product we used in a previous research. Skim milk was obtained from Yotsuba Co. Ltd. (Sapporo, Japan) in the form of a powder that contained 3.8% H_2O, 36.3% proteins, 51.5% carbohydrates, 7.8% minerals and 0.6% fat [1)].

The milky miso (MM) was prepared according to our previous method, which consists of 80% steamed soybeans and 20% skim milk powder [1)].

2. Purification of ACE inhibitory peptide from digested milky miso and peptide synthesis

The milky miso hydrolysate was separated by gel permeation chromatography. The peptic hydrolysate of MM applied to a Superdex TM 30 prep grade (GE Healthcare Biosciences, AB, Uppsala, Sweden) column (1.6 × 90 cm) and eluted with a solution of 20 mM sodium acetate (pH 7.0), 150 mM NaCl and 0.5 mM NaN_3 at a flow rate of 0.45 ml/min. Eluted fractions were collected and desalted with 50% CH3CN using a SEP-PAK Plus C18 cartridge (Waters Co., Milford, MA, USA). Next, the active fractions (30 to 36) were subjected to reverse phase (RP) high-performance liquid chromatography (HPLC) using a Comosil 5PE-MS (Nacalai Tesque, Inc. Kyoto, Japan). Fractions were collected every minute. The active fractions were further separated by RP-HPLC using a 1–50% gradient of acetonitrile in 0.1% TFA at a flow rate of 0.5 ml/min and the same column. The amino acid sequences of the active fractions finally obtained were analyzed using a protein sequencer, Procise 492 (Applied Biosystems, Foster City, CA, USA).

3. ACE inhibitory activity assay

The ACE inhibitory activity was measured according to the method of Cushman and Cheung[2)], with slight modifications as described in a previous publication[3,1)]. This assay is based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (Hip–His–Leu) catalyzed by ACE.

4. Antihypertensive activity after oral administration in SHR

Animals used in this study were maintained according to the guidelines of the Institutional Animal Care and

Utilization Committee, in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. A group of sixteen spontaneously hypertensive rats (SHR) were used for the current work. SHRs (aged 8 weeks) were purchased from Charles River Japan, Inc. (Yokohama, Japan). They were kept in a room with a 12 h light–dark cycle (lights on between 7 and 19h). Temperature and humidity were controlled at 23 ± 1 °C and 50 ± 10%, respectively. Diet (CRF-1, Charles River Japan) and tap water (0.2 μm filtered) were available[1)]. Four rats were used for each treatment including a control group (water 3ml/rat), milky miso group (1.5g/3ml/rat), ordinary miso group (1.5g./3ml/rat) and salt fed group (5% salt solution 3ml/rat). The milky miso and ordinary miso and salt were dissolved in water and orally administered to SHRs at a dose with a metal oral syringe. The systolic blood pressure (SBP) of the rats was measured at 0, 3, 6, 9 and 24 h after administration of the samples using the tail-cuff method with a programmable electro-sphygmomanometer (BP-98A; Softron Co., Ltd., Tokyo, Japan) after warming the rats in a chamber maintained at 38.7 °C for 15-20 min.

5. Human and angiotensin II content

In a local clinical facility, we fed milky miso to a number of hypertensive people who engaged in this research as experiment panellists. There were 33 people, mixed gender (7 men and 26 women), age average was 42 years-old. The people were fed 15g milky miso/person/day.

Results and discussions

1. Purification of ACE inhibitory peptides from milky miso.

In the first experiment by gel filtration chromatography we used the following purified fraction for fractions with high ACE inhibitory activities that were obtained in elution time 30-36 min (Fig. 1). We detected tremendous number of low molecular weight fractions ranged from 367 to 6500 Da. In first elution, fractions having strong activity at 20 to 30 percent acetonitrile concentration was obtained was purified by a linear gradient elution of acetonitrile concentration of 0-50% using reverse-phase column fractions (Fig. 2-A). In the second HPLC reverse phase pattern, the first purified samples were collected and the fraction was more purified by elution a linear gradient of 10-30% acetonitrile concentration (Fig. 2-B). As a result, high activity was obtained at about 12-15% acetonitrile concentration. Also, we were quite successful to fractionate the peak fraction of the target, which was purified by elution a linear gradient of 10-20% acetonitrile concentration (Fig. 2-C).

We have further purified the same fractions by isocratic elution of 10% acetonitrile concentration in order to obtain a complete single peak obtained from the other peaks. As a results in the isocratic elution, fraction A and B were obtained, at elution time of about 45 minutes out of 55 min (Fig. 3). Fractions A and B were collected and subjected to amino acid sequencing process to identify the amino acids that build the frame of those active peptides. Fraction A is a dipeptidyl consist of Tyr-Pro and the location of this peptide in the residue 146-147 in α s1 -Casein, with a molecular weight 278 Da. The Tyr-Pro peptide had showed a great ACE inhibition activity that exemplifies 679.052 mg/ml (2.4mM). This peptide was also discovered in the residue 45-46 and 58-59 in κ -casein. More interestingly, the Tyr-Pro peptides detected in the primary structure of β -casein and the residue located in the 60-61 and 180-181.

Fraction B was determined and found as Val and Try, and this peptide exists along the primary structure of α S2-casein, at the residue 183-184, with a molecular weight 280.3 Da. As estimated that this peptide is hydroponically stronger than A peptide, the IC_{50} was valued as excellent as 6.167 mg/ml (22μM). Furthermore, this crucial peptide was detected in primary structure of β -casein at 59-60 residues, again along the β -lactoglobulin at 41-42 residues, and also in the lactoferrin at 81-82 and 399-400 residues. We found that these peptides positioned in other skim milk proteins. In the current research to this point we have just found 2 peptides and we specified their locations, and these peptides proofed their physiological ability to increase the inhibition of ACE activity. However, we expect that milky miso has and rich in many undiscovered peptides that can increase the ACE inhibition activity.

2. Hypotensive effect to SHR

When milky miso was orally administered to SHR, it reduced SBP compared with control (Fig. 4). We did in vivo study on milky miso, we used water solution, salty solution and ordinary miso as standard solution to differentiate the effect of milky miso on the blood pressure of RATS. Generally ordinary miso contains 5% salt the samples of milky miso had a salt concentration of 5% as well. The measurements took at zero time, 3 hours, 6, 9 hours and 24 hours. These data suggest that milky miso could significantly reduce blood pressure of rats.

A 27.0 mmHg decrease was observed at maximum after 6 h of administration. This hypotensive effect was still observed at 9 h and even 15 h after administration, but SBP at 24 h returned to the value before administration. This showed that milky miso was a temporally effective hypo tensor, and that very effective if hypertensive people consume such product once every 24 h. That is to say, the blood pressure regulatory system concerned with ACE was restored to almost the pre administration state of the peptide[4]. In the current research, in the test rats, the values of SBP showed clearly that milky miso samples were the lowest values within the measurements, and specially at 6 hours even though the salt concentration was as the same as in control samples. Many short peptides (di- or tripeptides) from different food sources have been reported to have ACE inhibitory activity [5-10]. From the nutritional point of view, small molecular weight peptides can be more easily absorbed into the body than long chain ones [11-12]. Peptides A and B are residues of the digest milk protein with koji and that exist in different locations on the primary structure of casein, albumin and lactoferrin, might be absorbed easily and retained in the blood stream. This had helped restoring SBP readings in a very short time as ACE flows in the blood stream most likely these peptides inhibited ACE activity. Data of this work, suggests that the in vitro and in vivo results were considered to be linked to each other.

3. Hypertensive effects to human

We suggest that it would be meaningful to consider digested skim milk includes a bioactive fragment in addition to some soy proteins even though plant proteins are not considered as complete proteins. So no matter if the proteins are complete or not, it is important that after digestion we can obtain biologically active peptides that enhance and maintain a healthy society. By this, we were optimistic for considering this novel product and apply this research on the human especially hypertensive people. Since Japanese people consume miso on a daily babes, and as no chemical, no risk, no artificial additives were used in this novel product, we did not hesitate to check its effect on human. In this study, we investigated how milky miso contributes to the hypotensive effect in vivo.

Angiotensin II level was determined in human and the hypertensive relative activity of milky miso was estimated. This part of this study is very important, because it is done on a human that reflects a real physiological effect. The length of this experiment was 5 months. The people ate 15g milky miso/person/day. Till September 17th people subjected to angiotensin II analysis, and till this date the panellists did not have milky miso. However, the angiotensin II level was reduced after we started giving the milky miso and incorporate it in the diets that interdicted to those panellists. As a result milky miso was very good element in reducing the angiotensin II level. Since we start introducing milky miso the angiotensin II was reduced from 6.6 to 4.5 pg/ml after 30 days of administration (Fig. 5). The level was restored for other three months and the changes are very slight and non-significant. Hypertension (high blood pressure) affects one in five adults in Japan and one in 4 adults in the world. More indexes pointed out that approximately extra 25 % of adults have hypertension values considered to be on the high end of normal level [1].

This silent killer is a dangerous epidemic that destroys society especially the elderly people because it has no symptoms. Clinically, hypertension cannot be cured, but it can be controlled through lifestyle changes and prescriptive medication. While medications to treat hypertension are available, research has shown that modest lifestyle and dietary changes can help treat and often reduce or prevent high blood pressure. Recently the American Heart Association and the National Cancer Institute recommended hypertensive people to take an eaten plan called dietary approaches to stop hypertension (DASH) into consideration. DASH to lower blood pressure is rather unique plan for all hypertensive people[13, 14]. As DASH plan has been introduced to people, we considered of bringing our opinion to reality as we produced a novel product made from milky miso.

This product showed to have an ACE inhibitory activity due to some bioactive peptides. We delighted to introduce the results as showed great inhibition of ACE. We do not really consider milky miso as DASH diet but we do think it would be quite similar as being suggested by the results applied on rats and hypertensive people.

Conclusions

This document mainly proposed to contribution of providing new understanding about milk- and soybean-derived products with bioactivities. In conclusion, apparently, we detected tremendous number of low molecular weight fractions ranged from 367 to 6500 Da. Results suggest that unique micro flora have been contributed to produce biological effector components in fermented milk and soy product rich in ACE-inhibitory activities. In particular, original contributions are in relation to the mechanisms by which milk- and soy derived bioactive peptides are generated during the fermentation course, by expressing their bioactivities, and their fate in the biological action

in vivo. As we isolated some dipeptides, we suggest that milky miso is a valuable product that rich in many biologically active peptides. The results of this study showed that the SBP values were decreased in hypertensive rats after 6 hours of administration. Also milky miso product showed a great reduction in angiotensin II after 30 days of administration to hypertensive people. Research on the mechanisms of functional constituents of endogenous food is an ideal objective and the continuation to discover more valuable components is a must. Such research may contribute to promote a healthy society in South-East Asian countries and the whole world in general.

Acknowledgements

This research was funded in part by Grants from Japan Live stock Technology Association and Japan Dairy Association.

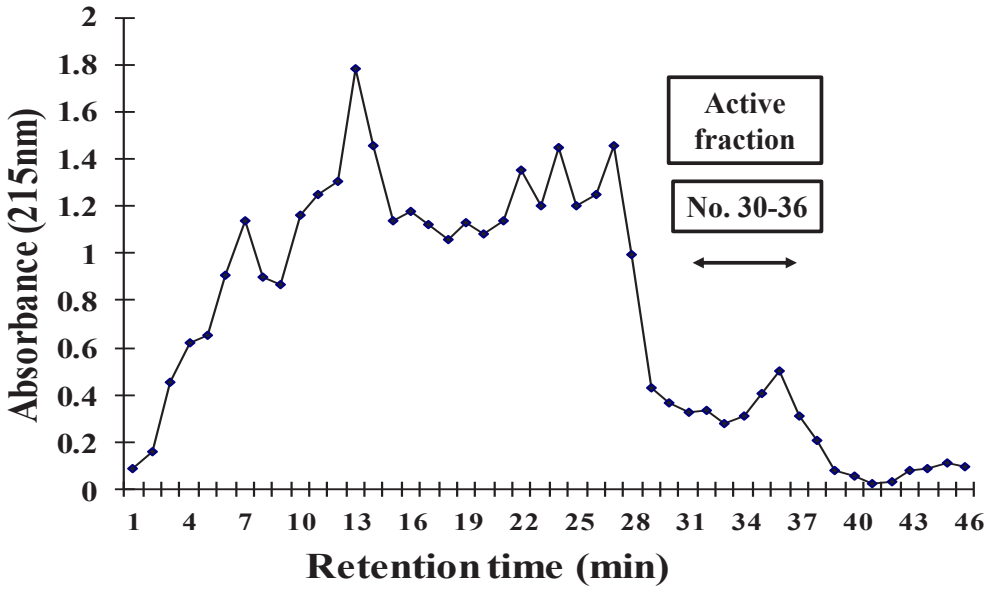

Fig. 1. Purification of ACE inhibitory peptide from milky miso. The hydrolysate was separated by gel permeation chromatography. It was applied to a Surpedex TM 30 prep grade (1.6cm I.D×95 cm) and eluted with 20mM acetate buffer (pH7.0),150mM NaCl,0.5mM NaN_3 at a flow rate of 0.2ml/min. High active fractions was indicated by a horizontal arrow.

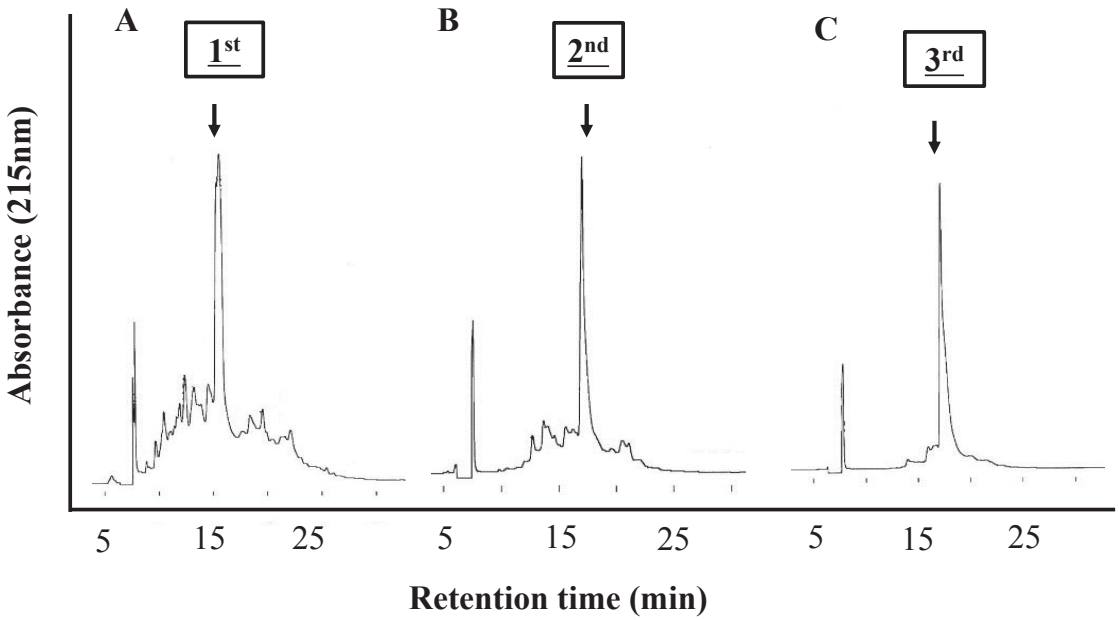

Fig. 2. Chromatograms of RP-HPLC using PE-MS. The active fraction from gel filtration chromatography at 30-36 min of retention time was separated with 3 iterations of RP-HPLC using Cosmosil 5PE-MS (4.6 × 250 mm, Nacalai Tesque). It was applied to the column and eluted with 0-50% (for 1st) or 10-30% (for 2nd) and 10-15 % (for 3rd) CH_3CN in 0.1% TFA at a flow rate of 0.5 ml/min. Active peaks are indicated with arrows.

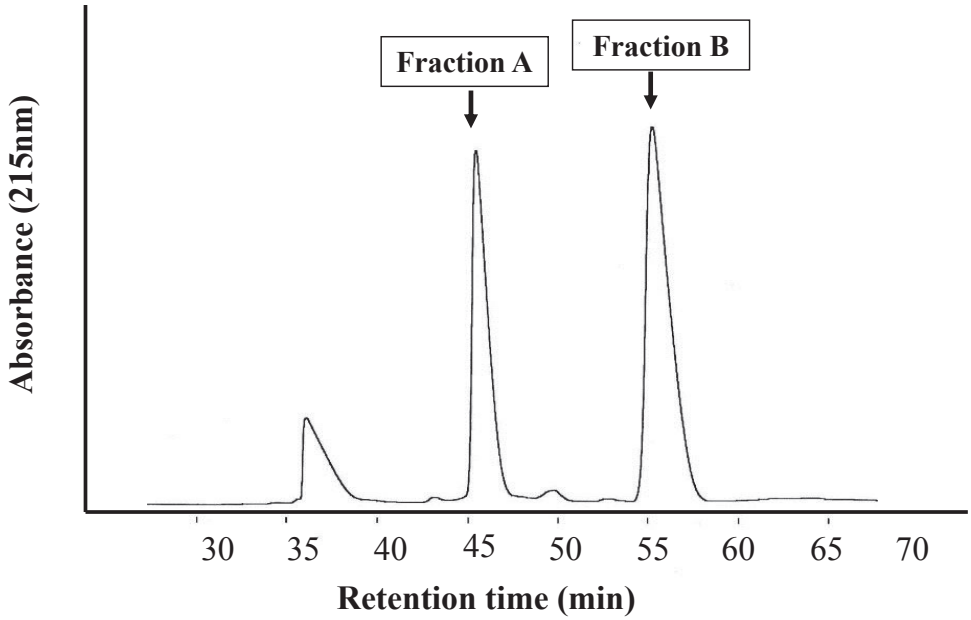

Fig. 3. Final chromatogram of the active fractions of 3rd iterations for purifying peptide A and B. The active fraction from 3rd iterations was further purified by isocratic elution of 10% acetonitrile concentration, 0.1% TFA at a flow rate of 0.5 mL/min. The column used for RP-HPLC was Comosil 5PE-MS (4.6 × 250 mm, Nacalai Tesque, Inc. Kyoto, Japan).

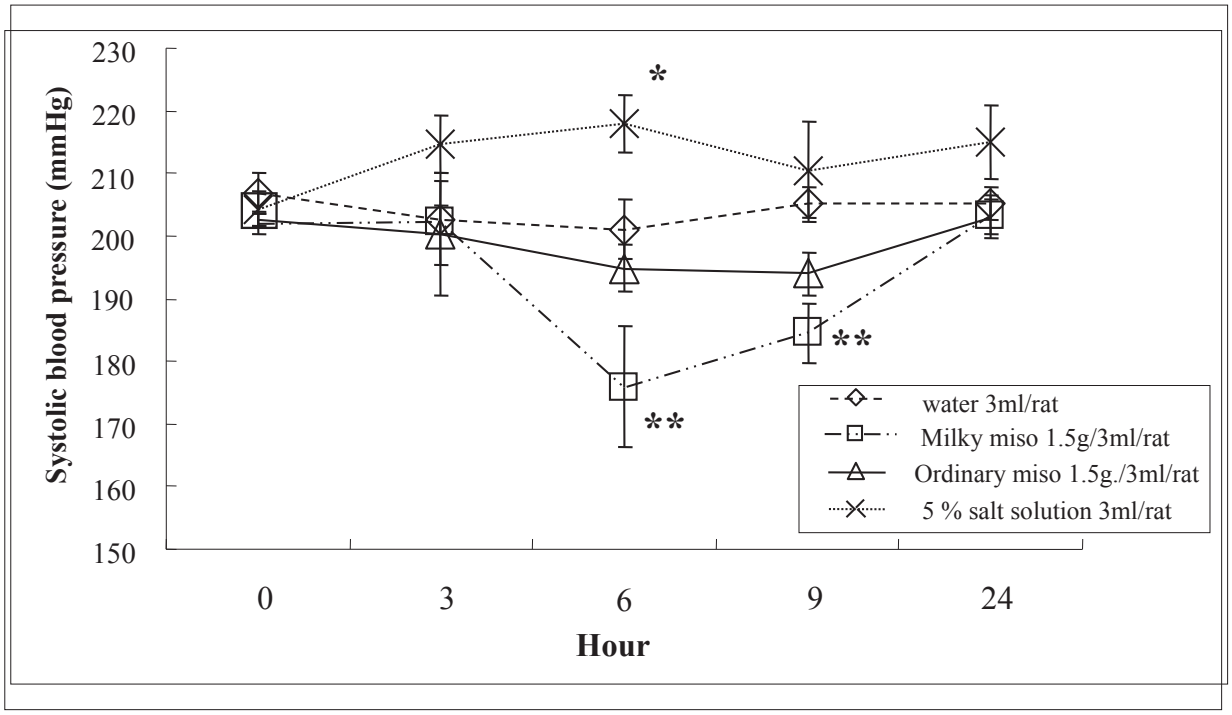

Fig. 4. Effect of single oral administration of milky miso and other meatrials on SHR. Changes of systolic blood pressure (SBP) from zero time were expressed with means, and the vertical bars represent the standard deviations (*: $p<0.05$, **: $p<0.01$).

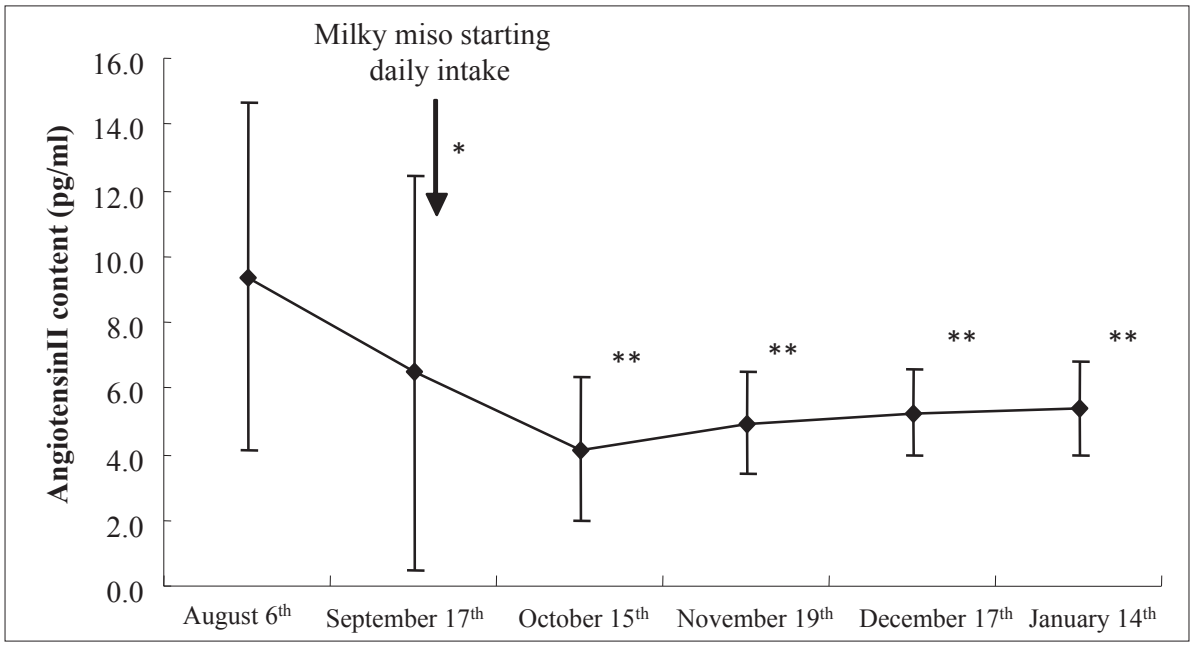

Fig. 5. Determination of angiotensin II level in human and the hypertensive relative activity of milky miso were estimated. Experiment length was 5 months, and milky miso was introduced at the second month of the experiment. Changes of angiotensin II level from august 6th were expressed with means, and the vertical bars represent the standard deviations (*: $p<0.05$, **: $p<0.01$).

References

1) Muguruma, M., Ahhmed M. A., Kawahara, S., Kusumegi, K., Hishinuma, T., Ohya, K. and Nakamura. T. (2012). A combination of soybean and skimmed milk reduces osteoporosis in rats. *Journal of Functional Foods*, 4, 810-818.
2) Cushman, D. W. and H. S. Cheung. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacol*ogy, 20, 1637-1648.
3) Katayama, K., Tomatsu, M., Kawahara, S., Yamauchi, K., Fuchu, H., Sugiyama, M., Kawamura, Y. and Muguruma, M. (2004). Inhibitory profile of nonapeptide derived from porcine troponin C against angiotensin I-converting enzyme. *Journal of Agriculture and Food Chemistry,* 52, 771-775.
4) Katayama, K., Jamhari, Mori, S., Kawahara, S., Mikae, K., Kodama, Y., Sugiyama, M., Kawamura, Y., Nakayama, T., Maruyama, M. and Muguruma, M. (2007). Angiotensin-I Converting Enzyme Inhibitory Peptide Derived from Porcine Skeletal Muscle Myosin and Its Antihypertensive Activity in Spontaneously Hypertensive Rats. *Journal of Food Science*, 72, S702-S706.
5) Yokoyama, K., Chiba, H. and Yoshikawa, M. (1992). Peptide inhibitors for angiotensin I converting enzyme from thermolysin digest of dried bonito. *Bioscience Biotechnology and Biochemistry*, 56, 1541-1545.
6) Fujita, H., Yokoyama, K. and Yoshikawa, M. (2000). Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *Journal of Food Science*, 65, 564-569.
7) Yamamoto, N., Ejiri, M. and Mizuno, S. (2003). Biogenic peptides and their potential use. *Current Pharmaceutical Design, 9*, 1345-1355.
8) Muguruma M., Ahhmed M.A., Katayama K., Kawahara S., Maruyama M. and Nakamura T. (2009). Identification of pro-drug type ACE inhibitory peptide sourced from porcine myosin B: Evaluation of its antihypertensive effects *in vivo*. *Food Chemistry*, 114, 516-522.
9) Yamada A., Sakurai T., Ochi D., Mitsuyama E., Yamauchi K. and Abe F. (2015) Antihypertensive effect of the bovine casein-derived peptide Met-Lys-Pro. *Food Chemistry*, 172, 441-446.
10) Gobba C.D., Tompa G. and Otte J. (2014) Bioactive peptides from caseins released by cold active proteolytic enzymes from *Arsukibacterium ikkense. Food Chemistry*, 165, 205-215.
11) Craft I. L., Geddes D, Hyde C. W., Wise I. J. and Matthews D. M. (1968) Absorption and malabsorption of glycine and glycine peptides in man. *Gut,* 9, 425-437.
12) Adibi S. and Phillips E. (1968) Evidence for greater absorption of amino acids from peptide than from free form in human intestine. *Clinical Research,* 16, 446-448.
13) American Heart Association: http://newsroom.heart.org/news/1103 (2013)
14) American National Cancer Institute: http://www.hypertensioninstitute.com/dash-1-2.php (2013)

PO-04-73

Molecular basis of maternal blood IgY transfer into egg yolks of birds

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Nagoya University Graduate School of Bioagricultural Sciences

Objective

In avian species, maternal IgY is selectively incorporated into the egg yolks of maturing oocytes. The incorporated IgYs are transferred to the embryonic circulation through yolk sac membrane to give passive immunity to newly-hatched chicks. Of the three Ig classes (IgA, IgM and IgY) in birds, only IgY is actively transferred into the egg yolks (Kitaguchi et al., 2008b). The concentration factor of egg yolk IgY against blood IgY was approx. 2-fold in chickens (Kitaguchi et al., 2008a) and quail (Murai et al., 2016). These evidences suggest existence of a selective IgY transport system in maternal ovary. Thus, IgY is believed to be incorporated into the oocytes by receptor-mediated endocytosis at the oocyte plasma membrane, but the relevant receptor in the maternal IgY transfer is still unidentified.

An understanding of the structural requirements of IgY and other Igs for selective transport into egg yolks is of importance for gaining insight into the molecular basis of maternal IgY transfer. In our previous study, the intact chicken IgY or its Fc fragment was incorporated into yolks of Japanese quail (*Coturnix japonica*) more effectively than Fab and $F(ab')_2$ fragments (Kitaguchi et al., 2008b). The IgY heavy chain consists of four constant domains, and the Fc fragment mainly contains two constant domains on the C-terminal, the Cu3 (C upsilon 3) domain and the Cu4 domain (Suzuki and Lee, 2004). Thus, our results suggest that the Cu3 and Cu4 domain are required for selective IgY transport into egg yolks.

To elucidate mechanism of maternal IgY transfer in birds, we investigated critical amino acid residues required for efficient IgY transport into the egg yolks by mutational analyses of selected residues located along the Fc domains of IgY. Recombinant wild-type IgY-Fc and its mutants were synthesized, and their uptakes into the egg yolks of quail were determined. In addition, we tried to detect IgY receptor involved in maternal IgY transfer from ovarian tissues by utilizing cross-linker reagent.

Methodology

Experiment 1

The cDNA encoding the IgY u-heavy chain was isolated from a quail splenocyte cDNA library. The gene encoding the Fc regions (heavy chain residues at 333–566) was isolated by PCR from the template cDNA, and the PCR product was ligated into the KpnI-XhoI cloning site of pSecTag2 A mammalian expression vector (Invitrogen), with a C-terminal 6×His tag. The constructed expression vector was used to synthesize recombinant wild-type quail IgY-Fc (designated WT). The amino acid residues located on the Cu3 domain of IgY-Fc were individually substituted for alanine or other amino acid residues by site-directed mutagenesis (Fig. 1). The generated WT and mutant constructs were then transiently transfected into CHO-S cells by FreeStyle™ MAX reagent (Invitrogen) with standard protocols. The expressed WT and mutants were purified by His Spin Trap affinity columns (GE Healthcare). The purified proteins were labeled with digoxigenin (DIG). The regularly laying quail were each injected intravenously with 20 μg of WT or mutants in a volume of 200 μL of PBS. The injected IgY-Fc in uptakes into egg yolks were measured by ELISA.

Experiment 2

Ovary was removed from the euthanized chickens and placed into ice-cold PBS. The yellow follicles (F2-F6) were isolated, and the theca layer containing interna and externa was striped, and a sheet of granulosa cell layer sandwiched between the basal lamina and oocyte plasma membrane was separated from yolk as described previously (Gilbert et al., 1977). The separated sheets were incubated with or without 1 mM disuccinimidyl suberate (DSS; crosslinker) in Hank's balanced salt solution on ice for 2 hr. The separated sheets were washed several times in ice-cold PBS, and the samples were preserved at -80°C until use. Plasma membrane fraction samples were isolated from the frozen sheet samples by commercially available kit (Minute Plasma Membrane Protein Isolation Kit; Intervet Biotechnologies), and they were solubilized in 1% SDS buffered at pH 6.8 with 70 mM Tris-HCl. Solubilized endogenous IgY and its complex were detected by a Western blotting analysis utilizing goat anti-chicken IgY conjugated with HRP (Bethyl).

Results

Experiment 1

The uptakes of the four mutants, D361A, L362A, Y363A and I364A, were markedly lower than that of the WT; in particular, the uptakes of the L362A, Y363A and I364A mutants were reduced to undetectable levels (Fig. 2A), suggesting importance of LYI motif for efficient IgY uptake. In contrast, the uptakes of the Y363F and Y363W mutants were slightly higher than that of the WT, suggesting the necessity of an aromatic side-chains at the Y^{363} residue. The level of incorporation of the G365A mutant into egg yolks was more than twice that for the WT.

The reproducibility of the elevated IgY-Fc uptake and the contribution of the amino acid side chain at the G365 residue was examined. The replacement of the G365 residue with polar or nonpolar amino acid residues (G365S, G365F, G365M, G365N, G365H and G365V) increased IgY-Fc uptake into egg yolks compared to the WT; in particular, the uptake of the G365S mutant was increased by 2.6-fold compared to that of the WT (Fig. 2B). The replacement of the G365 residue with a charged amino acid residue (G365K and G365D) or proline residue (G365P) lowered the IgY-Fc uptake compared to the WT. These results emphasize the existence of a novel IgY transport system in ovarian follicles of birds. We speculate that this novel IgY transport system is controlled by a membrane receptor that recognizes the Cu3 domain of IgY.

Experiment 2

Under non-reducing condition, IgY signals were detected at 180 kDa in both DSS-treated (DSS (+)) and DSS-non-treated (DSS (-)) samples (Fig. 3). The IgY band signal was spread across 180 kDa toward to higher molecular range in the DSS (+) sample, whereas such signal was not detected in the DSS (-) sample. Under reducing conditions, clear bands for IgY heavy chain were detected around 70 kDa, but no differences were observed in higher molecular range between DSS (+) and DSS (-) samples. We cannot be certain whether the IgY signal >180 kDa in the DSS (+) sample is IgY and its receptor complex or not. Roth et al. (1976) reported that IgY binds specifically to chicken plasma membrane prepared from ovarian follicles dissected free from the overlying connective tissue and follicular epithelium. Kitaguchi et al. (2010) also reported IgY binding signal to the inner layers of ovarian follicular tissues, when whole-mount sections of quail ovarian follicle were incubated with the IgY-Fc. To elucidate the precise molecular mechanism of ovarian IgY transport, it will be necessary to identify the protein interacting with IgY when the ovarian inner layers were incubated with cross-linker.

Conclusion

Important amino acid residues (Y^{363} and G^{365}) of IgY required for maternal IgY transfer into egg yolks were identified. The detection of IgY signal >180 kDa in the DSS-treated ovarian follicular tissue enhanced existence of selective maternal IgY transport through a specific IgY receptor.

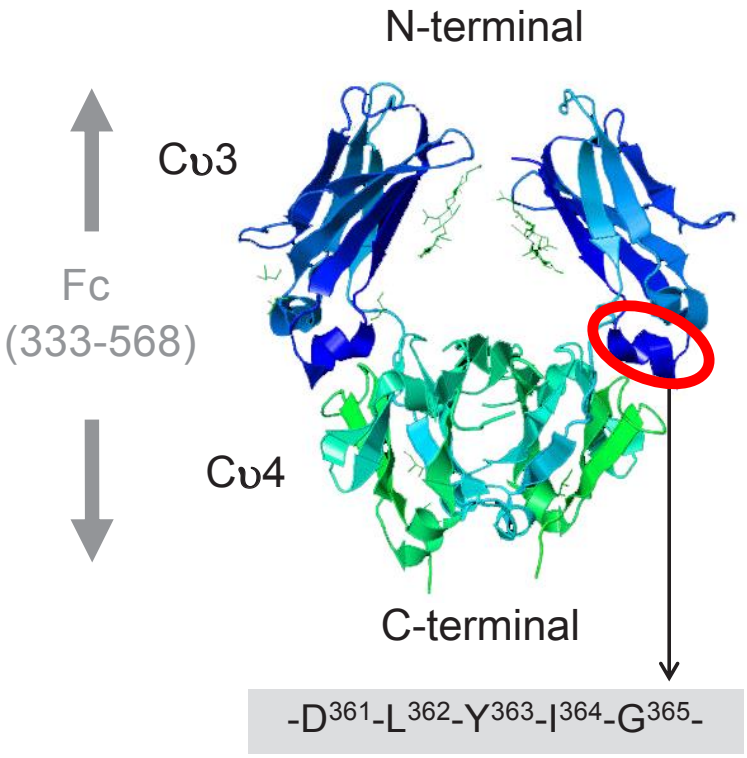

Figure 1. Structure of IgY-Fc and alignment of amino acid sequences of quail IgY. Residues D^{361}-L^{362}-Y^{363}-I^{364}-G^{365} of IgY-Fcυ3 were targeted for alanine-scanning mutagenesis. The crystal structure of chicken IgY-Fc (PDB entry 2W59) was drawn using Cn3D 4.3.

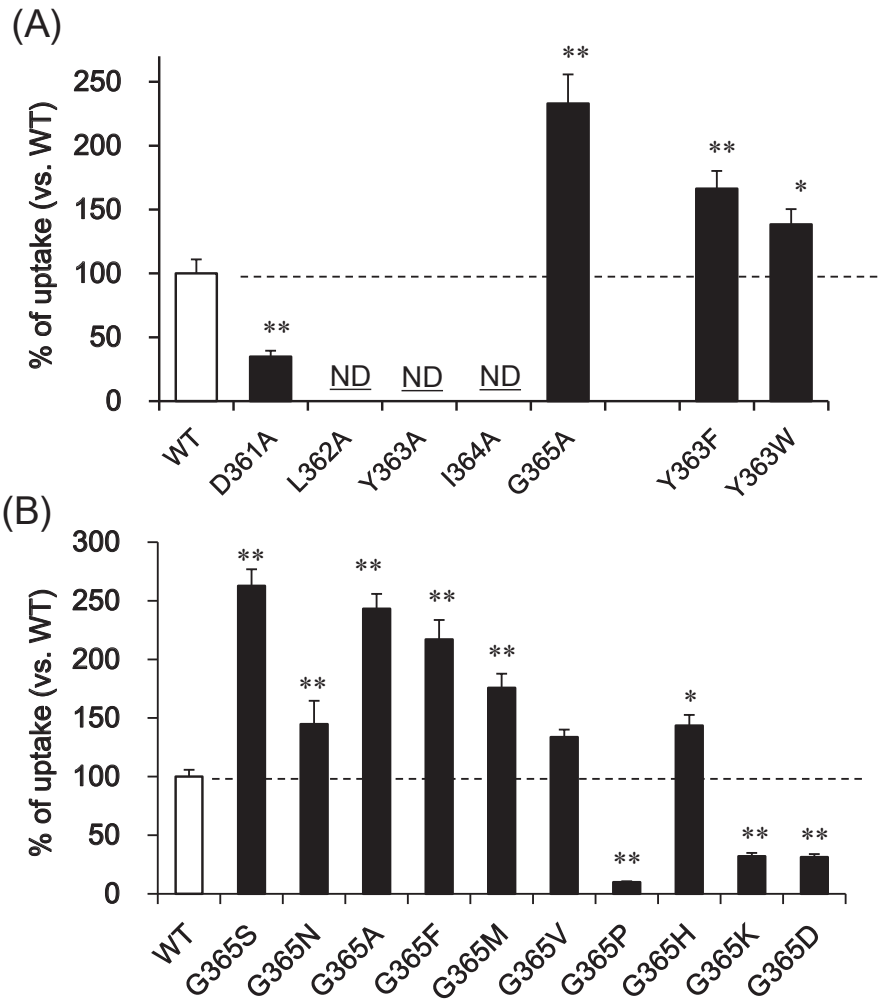

Figure 2. **Uptakes of recombinant wild-type IgY-Fc (WT) and its mutants with single amino acid substitution into egg yolks of quail.** Forty μg of seven IgY-Fc mutants was injected intravenously into laying quail, and their uptakes into egg yolks were measured by ELISA. The averaged uptakes into eggs laid on 2 and 3 days after the injection were compared between WT and mutants. (A) Uptake of mutants with substitution at 361-365 residues. (B) Uptake of mutants with substitution at 365 residue. ND, not detected. The vertical bar indicates the mean ± SEM of six quails. Means having asterisks are significantly different from the WT (*, $P< 0.05$, **, $P< 0.01$).

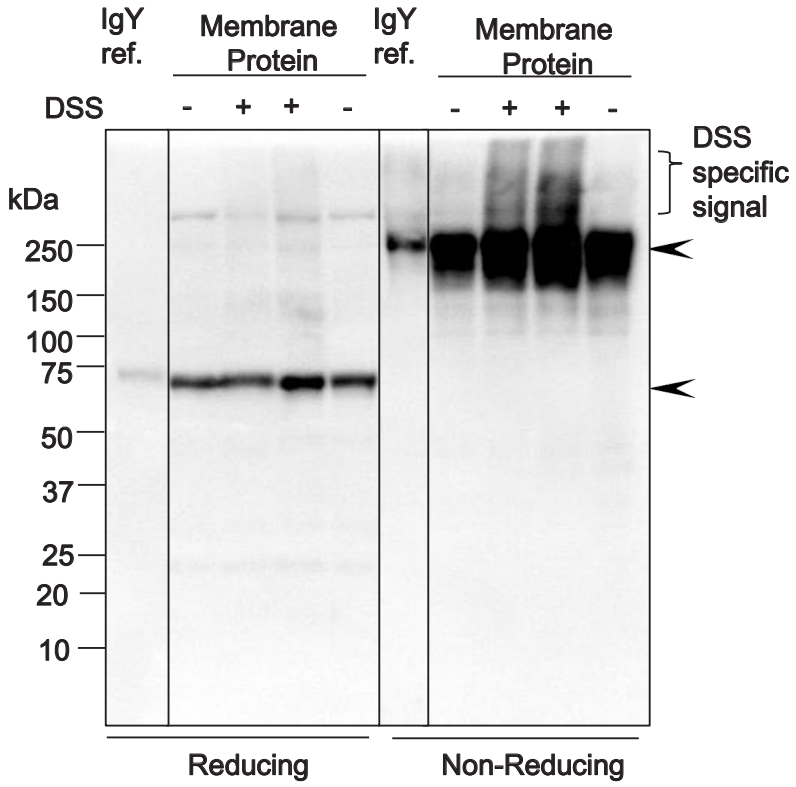

Figure 3. Detection of IgY complex from fractionated plasma membrane of chicken ovarian follicles treated with cross-linker (DSS). Isolated inner layer tissue of chicken ovarian follicles were incubated with or without cross-linker (DSS) in Hank's balanced salt solution. Fractionated plasma membranes were lysed, and they were separated by SDS-polyacrylamide gradient gels under reducing or non-reducing conditions. The blotted membrane was incubated with rabbit anti-chicken IgY antibody. The IgY signals were detected by an enhanced chemiluminescence system. The molecular maker size is indicated on the left. IgY ref: reference IgY sample. Arrows indicate IgY.

References

Gilbert, A. B., Evans, A. J., Perry, M. M. and Davidson, M. H. (1977) A method for separating the granulosa cells, the basal lamina and the theca of the preovulatory ovarian follicle of the domestic fowl (*Gallus domesticus*). J. Reprod. Fertil., 50: 179-181.

Kitaguchi, K., Bae, H.-D., Sasanami, H., Kobayashi, M., Horio, F. and Murai, A. (2010) Microscopic detection of IgY-Fc binding signal in the inner layers of ovarian follicular tissues in quail. Anim. Sci. J., 81: 580-585.

Kitaguchi, K., Minoura, M, Noritake, M., Mizutani, M., Kinoshita, K., Horio, F. and Murai, A. (2008a) Determination of immunoglobulin Y concentration in yolk extract prepared by water dilution method: comparisons among three strains of chickens. J. Poult. Sci., 45: 82-87.

Kitaguchi, K., Osada, K., Horio, F. and Murai, A. (2008b) Exclusion of polymeric immunoglobulins and selective immunoglobulin Y transport that recognizes its Fc region in avian ovarian follicles. Vet. Immunol. Immunopathol., 121: 290-299.

Murai, A., Kakiuchi, M., Hamano, T., Kobayashi, M., Tsudzuki, M., Nakano, M., Matsuda, Y. and Horio, F. (2016) An ELISA for quantifying quail IgY and characterizing maternal IgY transfer to egg yolk in several quail strains. Vet. Immunol. Immunopathol., 175: 16-23.

Roth, T. F., Cutting, J. A. and Atlas, S. B. (1976) Protein transport: a selective membrane mechanism. J. Supramol. Struct., 4: 527-548.

Suzuki, N. and Lee, Y. C. (2004) Site-specific *N*-glycosylation of chicken serum IgG. Glycobiology 14: 275-292.

PO-04-79

Effects of rice mixing ratio on productive performance in broiler chickens

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We investigated the effects of rice mixing ratio on productive performance in broiler chickens. Two hundreds Chunky birds were divided into four experimental groups and a control group. Thirty, fifty, seventy-five and a hundred percent of the corn in the formula diet for the experimental groups was replaced by whole paddy rice (Table 1,2) and fed during the later term of fattening. The formula diet for the control group contained 65% of corn with no rice. The following results were obtained: 1) All rice mixing ratio suited the birds' taste and the condition of the birds' health was good. 2) The average body weight was significantly higher in the 30% group than in the control group ($p<0.05$). The average body weights of all experimental groups were higher than the control group. 3) The feed conversion rate tended to be worse with increasing the rice mixing ratio (Table 3). 4) The meat yield and the abdominal fat ratio were not different among the five groups. 5) The ratios of gizzard weight to live body weight were significantly higher in the all experimental groups than in the control group ($p<0.05$) (Table 4). 6) The fatty acid composition in the thigh meat was not different among the five groups. 7) The total amino acid yield was significantly higher in the 30% group than in the control group ($p<0.05$) (Table 5). 8) Benefit per a bird was better with increasing the rice mixing ratio. It in all the experimental groups were higher than in the control group (Fig 1). We suggest that rice mixing ratio does not significantly influence the productive performance and increase the economic benefit in broiler chickens.

Table 1. Composition (%) of diets

Ingredient	30% rice	50% rice	75% rice	100% rice	control
Whole paddy rice	19.5	32.5	48.8	65.0	0.0
Corn wih fish meal	46.4	33.2	16.6	0.0	66.3
Soybean meal	22.0	22.0	22.0	22.0	22.0
Wheat bran	2.0	2.0	2.0	2.0	2.0
Fish meal （CP60%）	3.9	4.2	4.5	4.8	3.5
Soybean oil	4.0	4.0	4.0	4.0	4.0
Calcium carbonate	0.9	0.9	0.9	0.9	0.9
Tribasic calcium phosphate hydrate	0.8	0.8	0.8	0.8	0.8
Sodium Chloride	0.3	0.3	0.3	0.3	0.3
Vitamin Mixture	0.2	0.2	0.2	0.2	0.2
total	100.0	100.0	100.0	100.0	100.0

Table 2. Nutrient composition and unit price

Ingredient	30% rice	50% rice	75% rice	100% rice	control
Crude protein (%)	18.0	17.8	17.7	17.5	18.2
Crude fat (%)	6.9	6.7	6.5	6.2	7.3
Crude fiber (%)	3.8	4.7	5.8	6.9	2.4
Crude ash (%)	4.0	4.6	5.3	5.9	3.2
Calcium (%)	0.96	0.96	0.96	0.96	0.96
Phosphorus (%)	0.61	0.61	0.60	0.60	0.62
Metabolizable energy (kcal/g)	3.08	3.00	2.90	2.80	3.20
Unit price (yen/kg)	63.2	61.2	58.6	56.0	66.3

Table 3. Growth traits of experimental chickens

Traits	30% rice	50% rice	75% rice	100% rice	control
Rate of raising (%)	97.5	100.0	100.0	100.0	97.5
Final body weight (g)	3,674^{a}	3,659^{ab}	3,666^{ab}	3,639^{ab}	3,610^{b}
Feed intake (g)	5,585	5,730	5,806	5,941	5,638
Feed conversion ratio	1.98	2.04	2.07	2.13	2.05
Production score	393.5	392	389	376.17	377

^{a,b}Means within the same column with no common superscripts differ significantly ($P < 0.05$).

Table 4. Carcass traits of experimental chickens

Traits	30% rice	50% rice	75% rice	100% rice	control
Mmeat yield (%)	44.0	43.8	44.0	43.4	43.2
Gizzard ratio (%)	1.23^{c}	1.35^{b}	1.43^{ab}	1.50^{a}	1.02^{d}
Abdominal fat ratio (%)	2.47^{ab}	2.47^{ab}	2.78^{a}	2.41^{b}	2.70^{ab}
Thigh meat color	3.15^{a}	2.56^{b}	2.70^{ab}	2.40^{b}	2.81^{ab}
Breast meat color	2.65^{a}	2.36^{ab}	2.00^{bc}	1.80^{c}	2.61^{a}

^{a,b,c}Means within the same column with no common superscripts differ significantly ($P < 0.05$).

Table 5. Meat quality of experimental chickens in thigh meat

Traits	30% rice	50% rice	75% rice	100% rice	control
Oreic acid (%)	37.3	37.7	39.0	39.0	37.6
Mono-unsaturated fatty acids (%)	41.6	43.0	44.2	44.6	42.9
Glutamic acid (μmol/g)	2.10	1.94	2.02	2.31	2.01
Total amino acid (μmol/g)	56.1^{a}	53.4^{ab}	50.9^{ab}	53.9^{ab}	50.3^{b}

^{a,b}Means within the same column with no common superscripts differ significantly ($P < 0.05$).

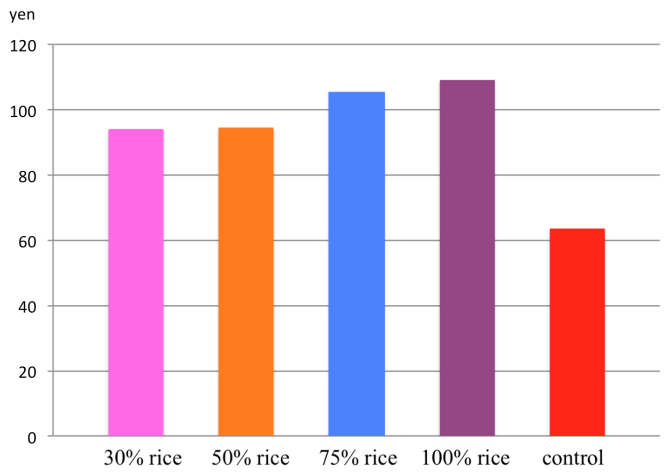

Fig.1 Benefit per a bird

PO-04-80 Efficacy of Supplementary Penergetic® T poultry on Production Performance, Oocyst Quantity and Intestinal Morphology of Broilers Infected with Eimeria tenella

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Introduction

Broiler chicken industries in Thailand have been expanded tremendously. There was approximately thousand million Baht a year of exporting values for chicken meat of Thailand. This would be partly the results of applying new technology and knowledge for improving chicken rearing (Khonyoung et al., 2010). In the current situation, infectious diseases make losses and low efficiency of broiler production. An anti-coccidial agent usually supplemented in diet for broiler chickens, especially during the beginning until a week before slaughtering age, in order to control and prevent. Consequently, it resulted in drug residues in body and products of the broiler chickens, then affecting to consumer health. This would lead to unacceptable products of consumers both national and international levels, especially European Union (EU) which is highly strict for importing chicken products by allowing only the products without drug residues. Therefore, the use of an alternative innovation without drug residues and with efficacy for control and prevent coccidiosis would be a crucial solution for broiler chicken industries.

Penergetic® T poultry is an innovation product derived from transferring of energy from monensin (anti-coccidial drug) to calcium carbonate ($CaCO_3$), which is hypothesized that dietary supplementation of this innovation product can cure, control and prevent coccidiosis in chickens as closely the use of monensin. The principle of energy transfer is adapted from "Homeopthy" principle which is body could be cure itself after stimulating by obtained energy to be back to normal situation of body. This homeopathy principle is rather new discipline and can be applied for the use of natural substances such as herbs, organic or inorganic substance (salt and minerals) and products derived from animals, consequently lower chance of allergy from these substances. Thus, the aim of the current study was to investigate efficacy ofPenergetic® T poultry supplementation in diet for broiler chickens on anti-coccidial properties.

Materials and Methods

Two-week-male and female chickens (Cob500) were divided into 3 groups. Forty-eight chickens of groups 1-3 were received 1 ml of *E. tenella* suspension (30,000 oocysts/ml). The chickens in each group will be housed in 4 cages with twelve of each. The chickens in the first group served as the control group, which were offered a basal diet containing no monensin andPenergetic® T poultry. The chickens in the second group was received the basal diet supplemented with Penergetic® T poultry 250 ppm and for the third group, the chickens were received the basal diet supplemented with Monensin 500 ppm. All studied chicken were fed *ad libtum* throughout 6 weeks of this experiment. They were individually infected with *E. tenella* at 14 day old chickens by the use of sporulated oocysts of 30,000 oocysts/ml/chicken. Production performance was measured weekly while one chicken from each cage was randomly selected for measuring cecal lesion score, oocyst quantity in cecum and intestinal morphology at week 0, 1, 2, 3 and 4 post infection. Statistical analysis was performed by ANOVA with repeated measurement.

Results

There were 28.87 ± 3.55 ºC (range: 23-37 ºC) for environmental temperature and 91.26 ± 0.44% (range: 91-92%) for relative moisture during the experiment study. From the Table 1, growth performance of the studied chicks was demonstrated. At wk 1-2 of the chicken age or pre-inoculation period, there were no differences ($P>0.05$) for ADFI among all studied groups. Howevere, at the wk 3-6 of the chicken age or post-inoculation period, the chickens offered the Penergetic® T poultry had a trend of higher feed intake ($P=0.0739$) when compared with the chickens offered the diet supplemented with the Monensin. At wk 1-2 of the chicken age or pre-inoculation period, the chickens offered the Penergetic® T poultry and the Monensin had a lower growth rate ($P<0.05$) when compared with the chickens offered the Control diet. At post inoculation (wk 3-6), there were no differences

(P>0.05) for ADG among all studied groups. However, the chickens offered the Penergetic ® T poultry diet had highest value of growth rate at post inoculation period. At wk 1-2 of the chicken age or pre-inoculation period, the chickens offered the Penergetic ® T poultry and the Monensin had the trends of higher FCR (P=0.0626 and P=0.0864, respectively) when compared with the chickens offered the Control diet. However, at post inoculation (wk 3-6), there were no differences (P>0.05) for FCR among all studied groups.

From the Table 2, lesion score and oocysts/g (log + 1) was showed. Both caecal lesion and oocysts/g (log + 1), there were no differences (P>0.05) among three experimental groups at each week after coccidia inoculation. The chickens in all groups had a similar pattern of value changes after inoculation; high lesion score (more severe) and high oocysts/g at wk 1 post coccidia inoculation and then gradually decreased at wk 2, 3 and 4 post coccidia inoculation.

From the Table 3, intestinal morphology was illustrated. The ratio of the Villous height and Crypt depth (VH:CD) at the jejunum of the chickens offered the Penergetic® T poultry had higher VH:CD (P<0.05) at the wk 0 of the study (the start of inoculation) when compared with those offered the Control and Monensin diets. However, there were no differences (P>0.05) among three studied groups for wk 1 to 4 after coccidia inoculation. The ratio of the Villous height and Crypt depth (VH:CD) at the caecum of the chickens offered the Penergetic® T poultry had higher VH:CD (P<0.05) at the wk 1 after coccidia inoculation when compared with those offered the Control and Monensin diets. However, there were no differences (P>0.05) among three studied groups for wk 0 and 2 to 4 after coccidia inoculation.

Discussion

From the results of the current study, the growth rate and feed conversion ratio of the chickens obtained the diets containing the Monensin and also the Penergetic® T poultry might get side effects of monensin, resulting anorexia and consequently lower growth rate (Bartov, 1994) at the wk 1-2 of the chicken age. However, after the coccidian inoculation for 4 weeks, there were no differences for growth performance among three studied groups. This would implied that the chickens obtained the diets containing both the Monensin and the Penergetic® T poultry would much do compensated for growth with the higher rate, especially the chickens offered the Penergetic ® T poultry diet. These would be explained by higher caecal VH:CD of the chickens obtained the Penergetic ® T poultry, indicating lower damages of caecal VH:CD at 1 week post inoculation which is the most severe of coccidia infection (highest value of caecal lesion score and quantities of caecal oocysts). These is in agreement with earlier study (Silva et al., 2009) reporting that the ratio of VH:CD is an important indicator for nutrient absorption. Under the present study, the production performance was not the main tested parameters as low number of the chickens in each group, so it is hardly to make conclusion for the effects of dietary treatment on the growth performance. In addition, the present study found no differences of caecal lesion score and quantities of caecal oocysts which might be explained by less sensitive to detect difference by the use of lesion score and might be low quantity of oocysts produced after inoculation, respectively. From the study of Mitchaothai et al. (2015), the rice hull bedding had a crucial part for transmission and re-infection of *E. tenella* in broiler chickens. In the earlier report (Grahum and Brandly 1914), infective period of coccidiosis is 5-7 days after infection. These two last reasons together would be explained for low quantities of *E. tenella* oocysts in caecum. For efficacy of the Monensin, it is seem to be no efficacy of the Monensin for control or prevent *E. tenella* infection in the current study. This is hardly to give explanation. However, there might be partly effect of monensin-resistanct *E. tenella*.

Conclusion

Supplementation of the Penergetic ® T poultry in diet for broiler chickens at 250 ppm would lower damage of caecal villi at 1 week after coccidia infection, but no effects on caecal lesion score, quantities of oocysts in caecum and Newcastle disease titer.

Acknowledgement

The current research project was funded by Behn Meyer Chemical (T) Co., Ltd. and Mahanakorn University of Technology.

Table 1 Growth performance of the experimental chickens

Parameter	Treatment		
	Control	Penergetic® T poultry	Monensin
ADFI, g/day			
Day 1-14	28.56±1.42	29.15±1.35	28.73±1.41
Day 15-42	126.27±5.68	137.61±7.91	124.53±8.01
ADG, g/day			
Day 1-14	33.14±1.30[a]	27.49±1.35[b]	26.83±1.46[b]
Day 15-42	72.80±4.42	78.73±9.52	70.49±1.38
FCR			
Day 1-14	0.95±0.08	1.07±0.06	1.08±0.06
Day 15-42	1.83±0.15	1.93±0.12	1.83±0.15

Table 2 Lesion score and oocysts/g (Log+1) of the experimental chickens

Parameter	Treatment		
	Control	Penergetic® T poultry	Monensin
Lesion caecal score*			
Week 0 after infection	0.00±0.00	0.00±0.00	0.00±0.00
Week 1 after infection	2.00±0.00	1.50±1.00	1.75±0.96
Week 2 after infection	1.00±0.00	0.75±0.50	1.00±0.00
Week 3 after infection	1.00±0.00	0.75±0.46	1.00±0.00
Week 4 after infection	0.67±0.49	0.75±0.45	1.00±0.60
Caecal oocysts/g (Log+1)			
Week 0 after infection	0.00±0.00	0.00±0.00	0.00±0.00
Week 1 after infection	5.80±0.49	6.85±0.41	5.23±1.44
Week 2 after infection	3.16±2.16	4.62±0.22	4.44±0.77
Week 3 after infection	2.38±2.60	2.66±1.69	2.26±1.95
Week 4 after infection	1.27±1.58	0.92±1.37	1.23±1.33

* 0 = normal, 4 = severe

Table 3 Intestinal morphology of the experimental chickens

Parameter	Treatment		
	Control	Penergetic® T poultry	Monensin
Jejunum: Villous height:Crypt depth			
Week 0 after infection	5.15±0.78[b]	9.61±0.85[a]	5.29±0.68[b]
Week 1 after infection	3.19±1.24	3.50±1.34	2.79±0.86
Week 2 after infection	4.34±2.94	7.14±2.26	4.56±1.13
Week 3 after infection	6.83±1.63	6.80±0.72	4.26±0.45
Week 4 after infection	5.86±1.90	6.34±0.89	5.62±2.09
Cecum: Villous height:Crypt depth			
Week 0 after infection	2.48±0.71	1.63±0.21	1.78±0.73
Week 1 after infection	1.68±0.41[b]	4.55±1.63[b]	2.05±0.35[a]
Week 2 after infection	1.90±0.34	1.98±0.33	2.08±0.19
Week 3 after infection	2.35±0.47	2.95±0.61	3.13±0.93
Week 4 after infection	2.20±0.47	2.53±0.51	2.70±0.41

References

Bartov, I. 1994. Effect of growth promoters on monensin toxicity in broiler chicks. *Br. Poult. Sci*. 35(1): 123-133.

Conway, D.P. and McKenzie, M.E. 2008. Anticoccidial drug. In: Poultry Coccidiosis. Blackwell Publishing Professional. p. 119-121.

Khonyoung, D., Chunchay, N. and Hanthongchai, V. 2010. Use of dietary Thai herbal supplementation on growth performance and coccidia control in broiler. Available from: http://librae.mju.ac.th/goverment/20111119104834_librae/23558.pdf.

Grahum, R. and Brandly, C.A. 1914. Coccidiosis of Poultry. Extension Service in Agriculture and Home Economics, University of Illinois, Illinois. p. 4-5.

Mitchaothai, J., Jaipeng, P., Paditporn, K. and Lertpatarakomol, R. 2015. Effect of rice husk bedding on *Eimeria tenella* transmission in broiler chickens. Accepted for Proceedings of The 5th International Conference on Sustainable Animal Agricultural for Developing Countries (SAADC 2015). 27-31 October 2015, Pattaya, Thailand. 816-818.

Silva, M.A., Pessotti, B.M., Zanini, S.F., Colnago, G.L., Rodrigues, M.R.A., Nunes, L. 2009. Intestinal mucosa structure of broiler chickens infected experimentally with *Eimera tenella* and treated with essential oil of oregano. *Ciencia Rural, Santa Maria.* 39(5): 1471-1477.

PO-05-1

Combined actions of sodium diacetate with surfactant agents on pathogenic bacteria, isolated from pig carcasses, in meat medium

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ABSTRACT

The objective of this study was to investigate the combined actions of sodium diacetate (SD) with tween 80, triton x-100 and polyethylene glycol 400 (PEG) against *Staphylococcus aureus* TUS1 and *Escherichia coli* TUE1, isolated from pig carcasses, by determination of minimum inhibitory and bactericidal concentrations (MIC and MBC), fractional inhibitory and bactericidal concentration index (FICI and FBCI) and kill-time in meat medium. MIC and MBC of SD was 0.31 and 1.25% (w/v), respectively, for *S. aureus* TUS1 and 0.31 and 2.50% (w/v), respectively, for *E. coli* TUE1. For PEG, both MIC and MBC were 2.50% (w/v) for *S. aureus* TUS1. The effect of SD in combination with PEG was synergistic against this bacterium, exhibiting FICI and FBCIs of 0.51 and 0.31, respectively, for *S. aureus* and 0.51 and 0.51, respectively for *E. coli*. In kill-time study, SD + PEG combinations added at their MBC produced a bactericidal effect that was dependent on the type of bacteria and concentration of antimicrobial. This present investigation revealed that SD in combination with PEG is potentially anti-*S. aureus* and anti-*E. coli* agents, which can be applied to meat products.

INTRODUCTION

Since staphylococcal foodborne intoxication is established as one of the most common bacterial food-borne diseases causing problems in the food sector in many countries, strategies to control *Staphylococcus aureus* in foods are of particular interest. *Escherichia coli* is the important pathogenic foodborne bacterium. Meat and meat products are regarded as one of the leading vehicles for transmission of both pathogenic bacteria (Loir *et al*, 2003; Blaiotta et al., 2004).

The problem of safe preservation in the meat industry has grown to be more complex as today's products require more safety and greater assurance of protection from pathogens. Salts of organic acid are commonly used to control the growth of undesirable microorganisms. However, extensive research has examined the effect of diacetate on the viability of pathogens such as *S. aureus* and *E. coli* during refrigerated storage of vacuum-packaged meat products dipped or sprayed with chemicals (Pundir and Jain, 2011). Surfactants constitute the most important group of detergent products. Generally, these are water-soluble surface-active agents comprised of a hydrophobic portion, usually along alkyl chain, attached to hydrophilic or water solubility enhancing functional groups (McDonnell and Russell, 1999). Moreover, Vaamonde et al. (1982) showed that polyethylene glycol 400 (PEG) appeared to have a significant inhibitory effect on one strain of *S. aureus*.

The aim of this study was to compare the antibacterial activity of sodium diacetate (SD) and tween 80, triton x-100 and polyethylene glycol 400 (PEG) used either alone or in combination on the growth of *S. aureus* TUS1 and *E. coli* TUE1, isolated from pig carcasses.

MATERIALS AND METHODS

Bacterial strain. *S. aureus* TUS1 and *E. coli* TUE1 were previously isolated from pig carcasses in Southern Thailand abattoirs by the standard procedure (BAM, 2001 and 2010) and its identity was confirmed by the Department of Medical Sciences, Ministry of Public Health of Thailand. These organisms were maintained on Mueller Hinton agar (MHA) (Merck, Germany). The overnight cultures were prepared by inoculating approximately 10 ml 0.85%NaCl with 2-3 colonies taken from MHA. Inocula were prepared by diluting in saline to 10^8 CFU/ml (McFarland standard of 0.5). These suspensions were further diluted with saline as required. The initial concentration of approximately $5x10^5$ CFU/ml was adopted for susceptibility test, synergy and kill-time methods.

Susceptibility test methods. Susceptibility tests were performed by the agar well diffusion method of Bauer et al. (1966) with MHA. All the antimicrobials were dissolved in distilled water. Subsequent two-fold serial dilutions were performed in culture medium that final concentrations of the test samples in wells ranged from 0 – 2.5%(w/

v) for SD (Chemipan Corporation Co. Ltd., Bangkok, Thailand), 0 – 10%(v/v) tween 80, triton x-100 (Sigma-Aldrich Pte. Ltd., Singapore) and 0 – 2.5% (w/v) for PEG (Chemipan Corporation Co. Ltd., Thailand). The minimal inhibitory concentration (MIC) was determined by a broth microdilution method (CLSI, 2002) for each bacterium. Serial two-fold dilutions of the test substances were mixed with MHB in microtiter plates. The final concentrations of the inhibitors in the broth were the same as those used for the agar well diffusion method. The MIC was recorded as the lowest concentration that limited the turbidity of the broth to < 0.05 at absorbance of 600 nm by UVM 340 Microplate Reader (Biochrom Ltd., Cambridge, UK). The minimal bactericidal concentration (MBC) was determined by comparing the number of remaining viable bacteria with the initial number of bacteria. The MBC was then recorded as the lowest concentration that killed at least 99.9% of the initial number of bacteria.

Synergistic effects. To determine whether SD acted synergistically with tween 80, triton x-100 and PEG, the fractional inhibitory concentration index (FICI) and fractional bactericidal concentration index (FBCI) in MHB using checkerboard titration was estimated. The experiments were repeated three times and the mean MIC, MBC, FICI and FBCI were obtained. Synergy was indicated by an FICI and FBCI < 0.5; partial synergy/additive effect was apparent when the FICI and FBCI ranged from > 0.5 to 1.0; an FICI and FBCI of > 1 to < 2 suggested that there was no interaction, and antagonism was exhibited when the FICI and FBCI was > 2 (Tangwatcharin and Suksathit, 2015).

Kill-time methods. The effect of SD and PEG alone (1.25 and 2.5%(w/v), respectively) and in combinations of SD and PEG (0.31%(w/v) + 0.16%(w/v)) on the cell viability of *S. aureus* and *E. coli* over 12 h was evaluated by the viable cell count procedure. The resulting suspension was incubated at 35°C. At different time intervals (0, 5, 10, 15, 30, 60, 120, 180, 360 and 720 min), the cells that were capable of growth on solid media were enumerated using pour plate count in MHA (Tangwatcharin et al., 2007). The cell numbers (CFU) were determined following incubation at 35°C for 48 h.

Statistical analysis. Data of bacterial loading in kill time method were presented as means and standard deviations. All statistical computations were performed to determine significant differences ($p < 0.05$) by ANOVA followed by Duncan's new multiple range test.

RESULTS AND DISCUSSION

Susceptibility test. The results of the antimicrobial activity of SD, tween 80, triton x-100 and PEG tested by the agar well diffusion method against *S. aureus* TUS1 and *E. coli* TUE1 show that SD exhibited a favorable activity against both pathogenic bacteria. PEG exhibited a favorable activity against *S. aureus* only. It was inhibited at > 0.16 and 0.31%(w/v) of SD for *S. aureus* TUS1 and *E. coli* TUE1, respectively, and > 1.25%(w/v) of PEG for *S. aureus* TUS1. Salts of organic acid have also been suggested to have antimicrobial e?ects by causing hyper-acidi?cation via proton donation at the plasma membrane interface of the microorganism and intracellular cytosolic acidi?cation, an excess of which can disrupt the H^+-ATPase enzyme, which is required for ATP synthesis (Silva et al., 2012). For antimicrobial activity of PEG, this result according to Vaamonde et al. (1982) showed that polyethylene glycol 400 (PEG) appeared to have a significant inhibitory effect on one strain of *S. aureus*. On the other hand, tween 80 and triton x-100 non-exhibited activity against both pathogenic bacteria.

In addition, the MICs of SD for antibacterial against *S. aureus* TUS1 and *E. coli* TUE1 were determined to be 0.31%(w/v). However, the MBC of SD were four-fold and eight-fold for *S. aureus* TUS1 and *E. coli* TUE1, respectively, higher than the corresponding MIC. The MIC and MBC of PEG against *S. aureus* TU1 was 2.50%(w/v) (Table 1). Earlier study found MIC and MBC of SD against *S. aureus* were 0.78 and 6.25%(w/v) (Tangwatcharin and Suksathit, 2015). MIC and MBC of PEG for antibacterial against *S. aureus* TUS1 was 2.50%(w/v).

Synergistic effects. The FICI for the combined application of SD with surfactant agents on *S. aureus* TUS1 and *E. coli* TUE1 is shown in Table 2. FICI and FBCI indicate that application of SD in combination with PEG resulted in enhanced inhibition of both pathogenic bacteria. The enhancing effect of the combination was also evidenced by bactericidal responses produced at sub-MBC levels for each bacterium. FICI and FBCI of the combined action of SD + PEG for *S. aureus* TUS1 were 0.51 (0.16%(w/v) + 0.04%(w/v)) and 0.31 (0.31%(w/v) + 0.16%(w/v)) suggesting partial synergy and synergy of the assayed antimicrobials, respectively. Similarly, FBCI of the combined action of SD + PEG for *E. coli* TUE1 was 0.52 (1.25%(w/v) + 0.04%(w/v)) again suggesting partial synergy. The subsequent

calculation and analysis of FICI and FBCI (Table 2) indicate that application of SD with PEG resulted in synergistic inhibition of the pathogen, potentially resulting from SD, a weak organic acid salt was effective in inhibiting most tested bacteria.

Kill-time methods. To determine the rates at which bacterium was killed, *S. aureus* TUS1 and *E. coli* TUE1 was exposed to SD and PEG alone and in combination at MBC concentration in MHB (Fig. 1). Addition of SD (1.25%(w/v)) and LAE (2.50%(w/v)) to broth caused a sharp drop in bacterial counts after 360 min, and values under two log cycle were maintained for the remainder of the time studied. SD proved to be more effective against this bacterium in MHB than the PEG ($p < 0.05$). After an incubation period of 120 min the combination of SD with PEG at sub-bactericidal concentrations reduced *S. aureus* TUS1 count by greater than five log cycle compared with the initial bacterial load. The bacterial count found in MHB containing SD alone were significantly higher ($p < 0.05$) than the count obtained for the broth to which had been added the mixture of 0.31%(w/v) of SD and 0.16%(w/v) of PEG. Similarly, after an incubation period of 180 min the combination of SD with PEG reduced *E. coli* TUE1 count by greater than three log cycle compared with the initial bacterial load.

IMPLICATIONS

SD in combination with LAE is potentially anti-*S. aureus* and anti-*E. coli* agents, which can be applied to meat products.

Keywords: Sodium diacetate, Surfactant agents, Polyethylene glycol, Pathogenic bacteria, Meat medium

ACKNOWLEDGEMENTS

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Table 1. MIC and MBC[a] of SD and PEG to *S. aureus* TUS1 and *E. coli* TUE1

Strain	Antimicrobial[b, c]	MIC	MBC
S. aureus TUS1	SD	0.31	1.25
	PEG	2.50	2.50
E. coli TUE1	SD	0.31	2.50
	PEG	-	-

[a] MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.
[b] The units of antimicrobial are %(w/v) for SD and PEG.
[c] SD, sodium diacetate; PEG, polyethylene glycol 400.

Table 2. FICI and FBCI[a] of the combined action of SD with tween 80, triton x-100 or PEG to *S. aureus* TUS1 and *E. coli* TUE1

Strain	Antimicrobials[b, c]		Concentration	Value	Interpretation
S. aureus TUS1	SD + tween 80	FICI	0.31+0.04	1.02	no interaction
		FBCI	1.25+0.04	1.02	no interaction
	SD + triton x-100	FICI	0.04+5.00	0.62	partial synergy
		FBCI	0.63+0.31	0.53	partial synergy
	SD + PEG	FICI	0.16+0.04	0.51	partial synergy
		FBCI	0.31+0.16	0.31	synergy
E. coli TUE1	SD + tween 80	FICI	0.31+0.04	1.02	no interaction
		FBCI	2.50+0.04	1.02	no interaction
	SD + triton x-100	FICI	0.31+0.16	1.02	no interaction
		FBCI	2.50+0.16	1.02	no interaction
	SD + PEG	FICI	0.31+0.04	1.02	no interaction
		FBCI	1.25+0.04	0.52	partial synergy

[a] FICI, fractional inhibitory concentration index; FBCI, fractional bactericidal concentration index.

[b] The units of antimicrobial are %(w/v) for SD and PEG and %(v/v) for tween 80 and triton x-100.

[c] SD, sodium diacetate; LAE, PEG, polyethylene glycol 400.

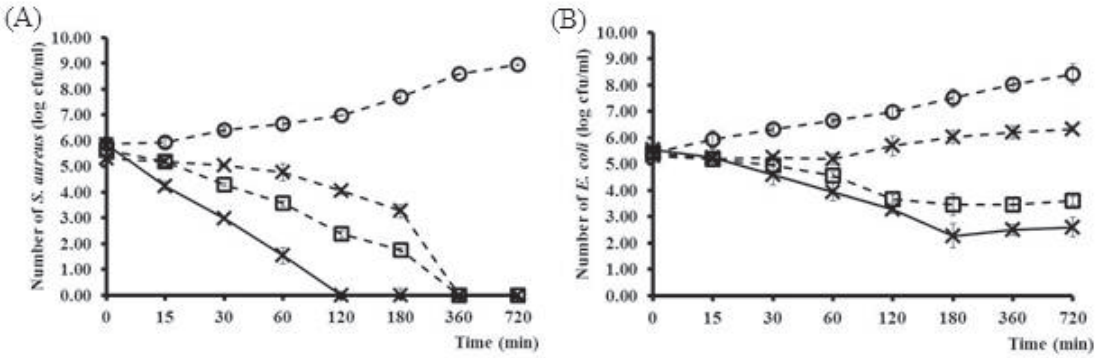

Fig. 1 Survivors curves of *S. aureus* TUS1 (A) and *E. coli* TUE1 (B) MHB at 35°C as a function of 1.25%(w/v) of SD and 2.50%(w/v) of PEG alone (dashed line) and in combination 0.31%(w/v) of SD + 0.16%(w/v) of PEG (solid line); control (circle), SD (square), and PEG (cross).

REFERENCES

Blaiotta, G., D. Ercolini, C. Pennacchia, V. Fusco, A. Casaburi, O. Pepe, and F. Villani. 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802. J. Appl. Microbiol. 97: 719–730.

Pundir, R. K. and P. Jain. 2011. Evaluation of five chemical food preservatives for their antibacterial activity against bacterial isolates from bakery products and mango pickles. J. Chem. Pharm. Res. 3: 24-31.

Bacteriological Analytical Manual (BAM) Online. 2001. *Staphylococcus aureus* U.S. Food and Drug Administration. Available from URL: http://www.cfsan.fda.gov/~ebam /bam-12.html. 9 December 2006.

Bacteriological Analytical Manual (BAM). 2010. *Escherichia coli*. U.S. Food and Drug Administration (Online). URL: http://www.fda.gov/Food/ScienceResearch/Laboratory Methods/BacteriologicalAnalytical Manual BAM/UCM064948. 30 July 2010.

Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45: 493-496.

CLSI. 2002. Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. Clinical Laboratory Standards Institute, Wayne, Pa.

Loir, Y. L., F. Baron and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. Genet. Mol. Res. 2: 63-76.

McDonnell, G. and A. D. Russell. 1999. Antiseptics and disinfectants: Activity, action, and resistance. Clin. Microbiol. Rev. 12: 147.

Vaamonde, G., J. Chirife and O. C. Scorza. 1982. An examination of the minimal water activity for *Staphylococcus aureus* ATCC 6538 P growth in laboratory media adjusted with less conventional solutes. J. Food Sci. 47: 1259-1262.

Tangwatcharin, P. and S. Suksathit. 2015. Synergistic effect of sodium diacetate with lauric arginate against *Staphylococcus aureus* in meat broth. In 5th International Conference on Engineering and Applied Sciences (ICEAS). 20-22 July 2015. Renaissance Sapporo Hotel, Hokkaido.

Tangwatcharin, P., S. Chanthachum, P. Khopaibool, and M. W. Griffiths. 2007. Morphological and physiological responses of *Campylobacter jejuni* to stress. J. Food Prot. 69: 2747-2753.

PO-05-2 Sequencing and Characterization of Divergent Marbling Levels in the Beef Cattle (Longissimus dorsi Muscle) Transcriptome

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Beef cattle (FLW) were humanely harvested at Snow dragon Beef Co. Ltd., (Dalian, Liaoning, China). The LD muscle samples were collected from animals immediately after exsanguinations. Based on the obtained marbling scores, two meat samples from the lowest marbling grade (labels Y1-1 and Y1-2) and two meat samples from the highest marbling grade (labels Y2-1 and Y2-2) were selected (Supplementary Figure 1D). RNA extraction and transcriptome sequencing using RNA sequencing technology, and the using bioinformatics to analysis the data.

RESULTS

Get a High-throughput sequencing of the bovine longissimus dorsi muscle transcriptome (see the paper) Got a large number of Differentially expressed genes between the high marbling and low marbling groups. The 16,020 total analyzed genes, 749 (366 up-regulated in the H group and 383 upregulated in the L group) showed fold changes of greater than 1.5 ($p<0.05$) between the two groups. from the Gene ontology analysis results, 25 specific GO terms related to lipid metabolism (noted in Table S6) were present in the middle of the list of all DEGs between the L and H groups, indicating that a substantial fraction of the genes identified were potentially associated with the traits under study. After analysised metabolic pathways and gene networks affected by the differentially expressed genes using IPA and David. A total of 9 canonical metabolic pathways and 8 signaling pathways were significantly represented in Supplementary Table 4(see the paper) by IPA. Twenty-three pathways were significantly represented among all of the DEGs by David (see the paper).

DISCUSSION

The transcriptome sequencing was performed to evaluate the divengent marbling phenotypes of FLW crossbreed cattle using the RNA-Seq method. This study revealed a large number of genes with known and unknown functions, thus greatly expanding a gene expression profile assoicated with the development of marbling in these cattle. This study also represents a first step toward an improved understanding of the functions of these genes and provides comprehensive and novel insights to facilitate for future research for beef quality improvement in beef cattle.

Figure 1

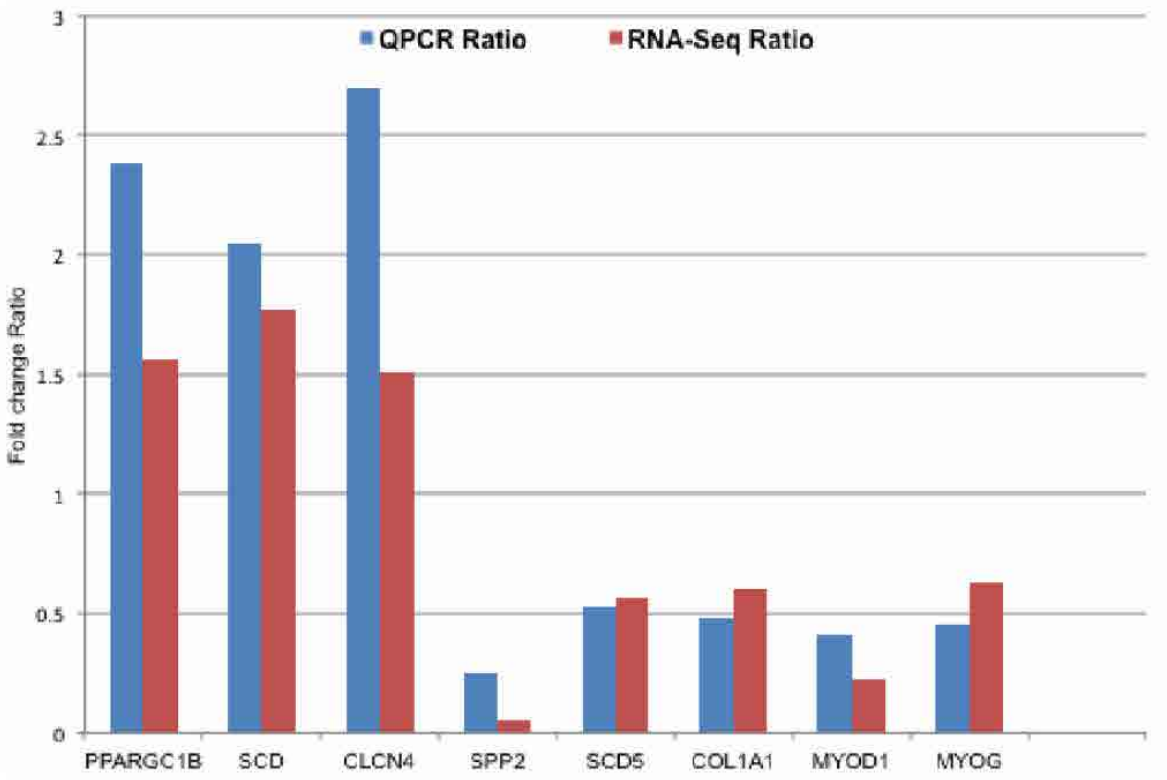


Figure 2

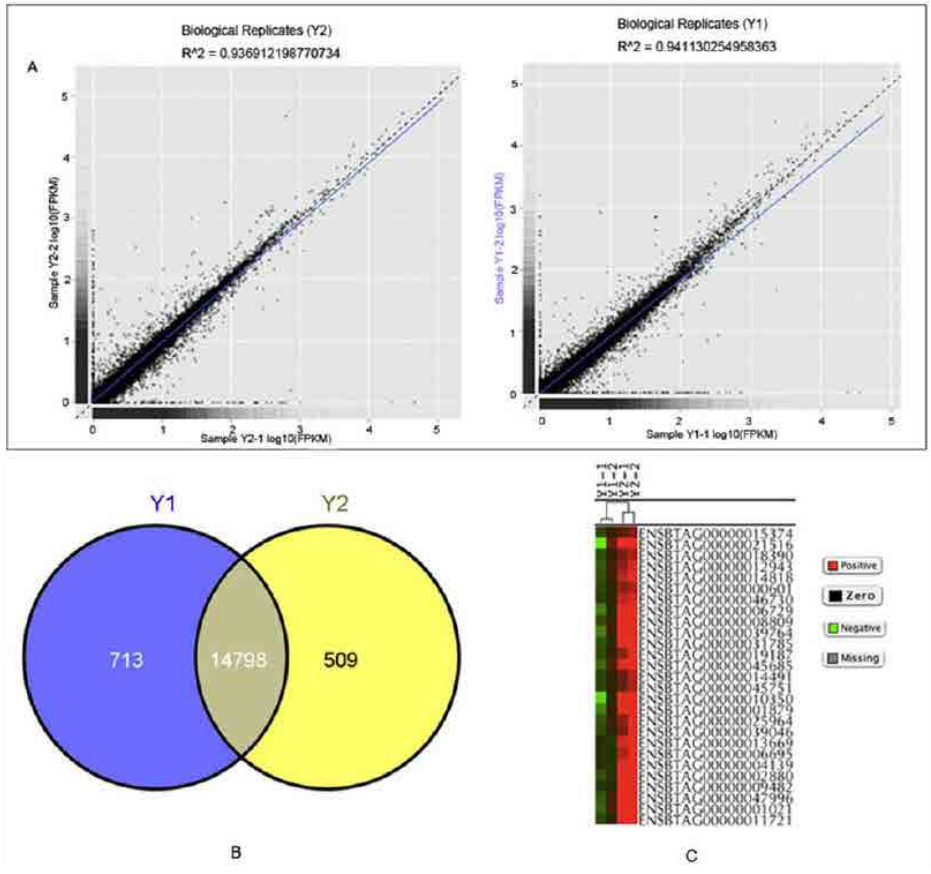
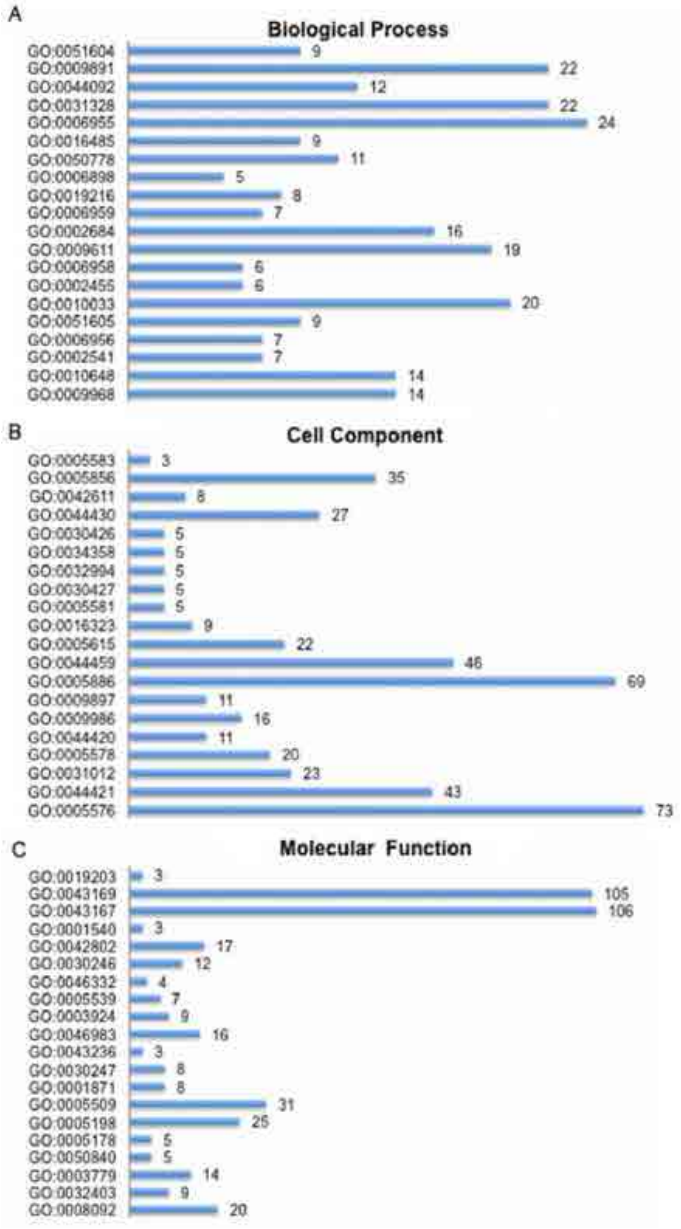


Figure 3


A
Biological Process
GO:0051604 9
GO:0009891 22
GO:0044092 12
GO:0031328 22
GO:0006955 24
GO:0016485 9
GO:0050778 11
GO:0006898 5
GO:0019216 8
GO:0006959 7
GO:0002684 16
GO:0009611 19
GO:0006958 6
GO:0002455 6
GO:0010033 20
GO:0051605 9
GO:0006956 7
GO:0002541 7
GO:0010648 14
GO:0009968 14
B
Cell Component
GO:0005583 3
GO:0005856 35
GO:0042611 8
GO:0044430 27
GO:0030426 5
GO:0034358 5
GO:0032994 5
GO:0030427 5
GO:0005581 5
GO:0016323 9
GO:0005615 22
GO:0044459 46
GO:0005886 69
GO:0009897 11
GO:0009986 16
GO:0044420 11
GO:0005578 20
GO:0031012 23
GO:0044421 43
GO:0005576 73
C
Molecular Function
GO:0019203 3
GO:0043169 105
GO:0043167 106
GO:0001540 3
GO:0042802 17
GO:0030246 12
GO:0046332 4
GO:0005539 7
GO:0003924 9
GO:0046983 16
GO:0043236 3
GO:0030247 8
GO:0001871 8
GO:0005509 31
GO:0005198 25
GO:0005178 5
GO:0050840 5
GO:0003779 14
GO:0032403 9
GO:0008092 20

REFERENCES

Baldwin, R. L. V., R. W. Li, C. Li, J. M. Thomson, and B. J. Bequette. 2012. Characterization of the longissimus lumborum transcriptome response to adding propionate to the diet of growing Angus beef steers. Physiol. Genomics 44:543-550.

Cloonan, N. and S. M. Grimmond. 2008. Transcriptome content and dynamics at single-nucleotide resolution. Genome Biol. 9: 234.

Cox, M. P., D. A. Peterson, and P. J. Biggs. 2010. SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. BMC Bioinformatics 11:485.

de Hoon, M. J. L., S. Imoto, J. Nolan, and S. Miyano. 2004. Open source clustering software. Bioinformatics 20:1453-1454.

Driver, A. M., F. Peñagaricano, W. Huang, K. R. Ahmad, K. S. Hackbart, M. C. Wiltbank, and H. Khatib. 2012. RNA-Seq analysis uncovers transcriptomic variations between morphologically similar *in vivo*- and *in vitro*-derived bovine blastocysts. BMC Genomics 13:118.

Grobet, L., L. J. R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset, and M. Georges. 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat. Genet. 17:71-74.

Hausman, G. J., M. V. Dodson, K. Ajuwon, M. Azain, K. M. Barnes, L. L. Guan, Z. Jiang, S. P. Poulos, R. D. Sainz, S. Smith et al. 2009. Board-invited review: the biology and regulation of preadipocytes and adipocytes in meat animals. J. Anim. Sci. 87:1218-1246.

He, H. and X. Liu. 2013. Characterization of transcriptional complexity during longissimus muscle development in bovines using high-throughput sequencing. PloS one 8(6):e64356.

Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4:44-57.

Kim, D., G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, and S. L. Salzberg. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14:R36.

Langmead, B. and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357-359.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402-408.

Marioni, J. C., C. E. Mason, S. M. Mane, M. Stephens, and Y. Gilad. 2008. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res. 18:1509-1517.

Nagalakshmi, U., Z. Wang, K. Waern, C. Shou, D. Raha, M. Gerstein, and M. Snyder. 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320:1344-1349.

Reecy, J., D. M. Spurlock, and C. H. Stahl. 2006. Gene expression profiling: insights into skeletal muscle growth and development. J. Anim. Sci. 84:E150-E154.

't Hoen, P. A. C., Y. Ariyurek, H. H. Thygesen, E. Vreugdenhil, R. H. A. M. Vossen, R. X. de Menezes, J. M. Boer, G-J. B. van Ommen, and J. T. den Dunnen. 2008. Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. Nucl. Acids Res. 36:e141.

Trapnell, C., A. Roberts, L. Goff, G. Pertea, D. Kim, D. R. Kelley, H. Pimentel, S. L. Salzberg, J. L. Rinn, and L. Pachter. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7: 562-578.

Trapnell, C., B. A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M. J. van Baren, S. L. Salzberg, B. J. Wold, and L. Pachter. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28:511-515.

PO-05-3 Decontamination of Staphylococcus aureus and Escherichia coli on the Chicken Drumstick Using Ozonated Water, Lactic Acid and Sodium Hypochlorite Solution

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INTRODUCTION

Thai traditional market is an open air place. Retailers display various parts of chicken on the stalls without temperature control. Because of the openness, pathogenic contamination cause more than 120,000 patients each year, due to consumption of food contamination with pathogenic microorganisms such as *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus* and *Vibrio* sp. (Thumdee and Pilasombut, 2013).

Ozone was used in microbial decontamination. Many reports have revealed that the potential uses of ozone in food industry include reduction of microorganisms on meat and poultry carcasses (Kaess and Weidemann, 1968; Sheldon and Brown, 1986; Restaino et al., 1995). Restaino et al. (1995) studied that ozonated water can effectively kill spoilage organisms (*Pseudomonas aeruginosa* and *Zygosaccharomyces bailii*), fecal contaminants (*Enterococcus faecalis* and *E. coli*) and food-borne pathogens (*Listeria monocytogenes, Bacillus cereus, Salmonella typhimurium, Yersinia enterocolitica* and *S. aureus*).

Antibacterial activity of chemical decontamination treatments on the surface of poultry carcass and parts mainly reduced the yield of contamination agents. The organic acid (lactic acid, acetic acid and citric acid) resulted in reductions of aerobic bacteria, coliforms, *Enterobacteiaceae*, *E. coli* and *Salmonella* (Loretz et al., 2010). Lactic acid was acceptable as natural organic acid and certified safety use by FAO/WHO. Increasing the lactic acid concentration in the immersion solution from 2% to 8% decreased aerobic bacteria by 0.7 log CFU g^{-1} (Ismail et al., 2001). Furthermore, *Campylobacter jejuni*, *E. coli* O157:H7 and *S. aureus* reduced by 0.2-1.7, 0.5-2.6 and 0.3-1.9 orders of magnitude, respectively (Loretz et al., 2010).

Sodium hypochlorite solution was another antimicrobial agent for reducing microbial population. Thumdee and Pilasombut (2013) found that sodium hypochlorite solution (50-200 ppm) could be used for microbial decontamination and extend the shelf-life of chicken breasts up to 3 days under refrigeration. The reduction of aerobic bacteria, coliforms and *E. coli* by 1.2, 0.6 and 0.6 log CFU/ml^{-1}, respectively was demonstrated by immersion in chilling 2% sodium hypochlorite for 45 min (Fabrizio et al., 2002).

Therefore, the purpose of this study was to perform the antimicrobial efficacy of ozonated water, lactic acid and sodium hypochlorite solution to decontaminate *S. aureus* and *E. coli* on chicken drumstick skin.

MATERIALS AND METHODS

Chicken drumsticks were transported immediately to laboratory in an ice box after being purchased from Thai traditional open market in Bangkok. They were randomly divided into 4 treatment groups, consisting of dipping with ozonated water, lactic acid or sodium hypochlorite solution and no dipping (control). Firstly, the chicken drumsticks were inoculated by dipping into tested strain suspension (*S. aureus* or *E. coli*) at 5 log CFU/g concentration. Then, the samples were immersed into 100 ppm ozonated water (ENALY model O_3 generator), 2% lactic acid or 200 ppm sodium hypochlorite solution for 5 min except the control. Subsequently, the samples were packed in foam trays, sealed with plastic film and stored in cold room at 4°C for 3 and 5 days. During experimental time interval, triplicate samples of chicken drumstick skin were taken for microbial contamination test as follow; before dipping, 10 min after dipping, 3 days and 5 days of storage. Pathogenic bacteria were analyzed according to Diliello (1982). Determination of *S. aureus* and *E. coli* counts were reported at log 10 colony forming unit (CFU).

RESULS AND DISCUSSION

The mean log reductions of the *S. aureus* and *E. coli* on chicken drumstick skin with treatments during storage

time are shown in Table 1 and Table 2, respectively. The results showed that the pathogenic bacteria on chicken drumstick skin treated with 100 ppm ozonated water, 2% lactic acid and 200 ppm sodium hypochlorite solution decreased more than control untreated group during storage time ($p<0.05$). The effective treatment showed in order was lactic acid, ozonated water and sodium hypochlorite, respectively. The drumsticks treated with lactic acid could reduce *S. aureus* and *E. coli* by 3.03 and logCFU/g, respectively at the first day.

The reduction in pathogenic organisms by lactic acid is due to the reduction in pH below the growth range and metabolic inhibition by the undissociated molecules which penetrate the bacterial cell membrane. The accumulation of the undissociated weak acid in the cytoplasm of the cell ultimately results in the acidification of the cytoplasm of the microorganism (Booth, 1985). Bolder (1997) reported that the use of lactic acid solutions at concentration of 1 – 2% reduced the bacterial counts on poultry carcasses immediately after slaughter and during storage, without affecting organoleptic characteristics such as colour and flavor.

There was a significant different ($p<0.05$) between the mean of microbial count on control and treatment groups on days 0, 3 and 5. When storage time increase up to 5 days, microbial reduction of chicken drumstick skin treated with ozonated water, lactic acid and sodium hypochlorite was better than control group. Lactic acid was the best treatment to decrease the number of *S. aureus* and *E. coli* throughout the 5 days of storage.

A study by Marcel et al. (1988) to decontamination of broiler carcass with 1 – 2% lactic acid before chilling revealed the improvement of bacterial safety and extends the refrigerated shelf life. Furthermore, Bolder (1997) informed that the combination of treating carcass with acid and packaging them in a modified atmosphere extends the shelf life for poultry and meat, primarily because it increased the lag phase of the microorganisms.

CONCLUSION

The reduction in prevalence of *S. aureus* and *E. coli* on chicken drumstick skin revealed that lactic acid, ozonated water and sodium hypochlorite solution could present a path to reduce the number of pathogenic bacteria. The microbial reductions resulting from the lactic acid treatment exhibited the most effective application to reduce the microbial load and increased shelf life of chicken drumsticks.

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This research was supported by Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang.

Table 1 Log reduction of *S. aureus* on chicken drumstick skin before and after treatments

Treatment	*Staphylococcus aureus* (log CFU/g)						
	Before	After	Log reduction Before-After	3 days	Log reduction Before-3 day	5 days	Log reduction Before-5 day
Control	$4.87^{a,x}$	$4.91^{c,x}$	Increased	$4.22^{b,x}$	0.65	$4.69^{b,x}$	0.18
Ozonated water	$4.95^{a,y}$	$3.63^{b,x}$	1.32	$3.44^{ab,x}$	1.51	$3.93^{b,x}$	1.02
Lactic acid	$5.00^{a,y}$	$1.97^{a,x}$	3.03	$2.56^{a,x}$	2.44	$2.31^{a,x}$	2.69
Sodium hypochlorite	$4.98^{a,y}$	$4.43^{bc,xy}$	0.55	$3.61^{b,x}$	1.37	$3.84^{b,x}$	1.14

^{a,b,c} Means with different superscript on the same column are significant ($p<0.05$)

^{x,y} Means with different superscript on the same row are significant ($p<0.05$)

Table 2 Log reduction of *E. coli* on chicken drumstick skin before and after treatments

Treatment	*Escherichia coli* (log CFU/g)						
	Before	After	Log reduction Before-After	3 days	Log reduction Before-3 day	5 days	Log reduction Before-5 day
Control	$4.16^{a,xy}$	$4.47^{b,y}$	Increased	$3.63^{b,x}$	0.53	$3.74^{b,x}$	0.42
Ozonated water	$4.71^{a,y}$	$4.39^{b,y}$	0.32	$3.12^{ab,x}$	1.59	$3.05^{b,x}$	1.66
Lactic acid	$4.78^{a,y}$	$1.63^{a,x}$	3.15	$2.45^{a,x}$	2.33	$2.28^{a,x}$	2.5
Sodium hypochlorite	$4.72^{a,y}$	$4.37^{b,y}$	0.35	$3.35^{b,x}$	1.37	$3.27^{b,x}$	1.45

^{a,b,c} Means with different superscript on the same column are significant ($p<0.05$)

^{x,y} Means with different superscript on the same row are significant ($p<0.05$)

REFERENCES

Bolder, N.M. 1997. Decontamination of meat and poultry carcass. Trends Food Sci. Technol. 8: 221 – 227.

Booth, I.R. 1985. Regulation of cytoplasmic pH of bacteria. Microbiol Rev. 49: 359 – 378.

Diliello, L.R. 1982. Methods in food and dairy microbiology. Connecticut: The AVI publishing company, Inc.

Fabrizio, K.A., R.R. Sharma, A. Demirci and C.N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. Poultry Sci. 81: 1598 – 1605.

Ismail, S.A., T. Deak, H.A. Abd El-Rahman, M.A. Tassien and L.R. Beuchat. 2001. Effectiveness of immersion treatments with acids, trisodium phosphate, and herb decoctions in reducing populations of *Yarrowia lipolytica* and naturally occurring aerobic microorganisms on raw chicken. Int. J. Food Microbiol. 64: 13 – 19.

Kaess, G. and J.F. Weidemann. 1968. Ozone treatment of chilled beef. I. Effect of low concentrations of ozone on microbial spoilage and surface colour of beef. J. Food Technol. 3: 325 – 334.

Loretz, M., R. Stephan and C. Zweifel. 2010. Antimicrobial activity of decontamination treatments for poultry carcass: A literature survey. Food Control. 21: 791 – 804.

Marcel, G.H., J.G. Logtestijn and D.A.A. Mossel. 1988. Bacteriological quality of broiler carcass as affected by in-plant lactic acid decontamination. Int. J. Food Microbiol. 6: 31 – 42.

Restaino, L., E.W. Frampton, J.B. Hemphill and P. Palnikar. 1995. Efficacy of ozonated water against various food-related microorganisms. Appl. Environ. Microbiol. 61: 3471 – 3475.

Sheldon, B.W. and A.L. Brown. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. J. Food Sci. 51: 305 – 309.

Thumdee, A. and K. Pilasombut. 2013. Efficacy of ozonated water and calcium hypochlorite to reduce microbial population on chicken breasts. 59th International Congress of Meat Science and Technology.

PO-05-5

Effect of Hibiscus sabdariffa L. (Roselle) Extract on Survival of Pathogenic Bacteria in Ground Pork

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INTRODUCTION

Emerging food borne pathogen is the main public health concern that becomes the major source of disease and mortality in human (Leroy et al., 2006). Various foods, particularly meat and meat products, can serve as source of food-borne illness such as *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* (Nørrung and Buncic, 2008; Verkade and Kluytmans, 2014). To overcome this problem, antibacterial activity of different decontamination treatments, such as chemical treatment were applied. Even though, the chemical treatments prove to be effective method to reduce microbial in meat, the consumer acceptance are concerned. Some countries in Europe ban the chemical use in meat (Loretz et al., 2010). On the other hand, natural antimicrobial substance such as herbal product is on demand. *Hibiscus sabdariffa* L. belongs to the family Malvaceae, commonly known in English as sorrel or roselle (Jung and Joo, 2013; Khalaphallah and Soliman, 2014). The benefit of roselle flower and calyx is the source of antioxidant as it contains phenolic compound, vitamin C, anthocyanins, minerals and bioactive compounds such as organic acid (Al-Hashimi, 2012; Awe et al., 2013; Fullerton et al., 2011; Jung and Joo, 2013). As our previous studied, ethanol extracts of roselle calyxes were evaluated for their antimicrobial activity against 16 tested strains of bacteria. At the concentration of 50 mg/ml, ethanol extract of roselle showed all antimicrobial activity against: *Lactobacillus sakei* TISTR 890, *Lactococcus cremoris* TISTR 1344, *Leuconostoc mesenteroides* subsp. *mesenteroides* TISTR 942, *Enterococcus faecalis* TISTR 888, *Streptococcus* sp. TISTR 1030, *Lb. plantarum* ATCC 14947, *Lactococcus lactis* 19435, *Enterococcus faecalis* TISTR 1344^{L}, *E. coli* TISTR 780, *Bacillus coagulans* TISTR 1447, *S. aureus* TISTR 118, *Bacillus subtilis* JCM 1465, *Listeria innocua* ATCC 33090^{T}, *Pseudomonas fluorescens* TISTR 358, *Salmonella* Typhimurium TISTR 292, and *Aeromonas hydrophila* TISTR 1321 (inhibition zone 11-23.33 mm) (Kongkarn et al., 2015). Therefore, the objective of this study was to investigate antibacterial activity of roselle extracts on survival of *S.* Typhimurium and *S. aureus* in ground pork.

MATERIALS AND METHODS

Plant materials

Dried red roselle calyxes were purchased from local market in Bangkok, Thailand. They were ground to a fine powder (100 mesh) in an electrical blender. One kilogram of powder was extracted with 95% ethanol at room temperature for 72 h. The extract was filtered through four layers of cheesecloth to remove any fiber debris and filtered through filter paper (Whatman No.1). Subsequently, the extract was evaporated by the rotary evaporator at 45 °C to give crude extract. This crude extract was stored at 5 °C for further studies. Total phenolic content of roselle extract was 675.80 mg GAE/100 g DW for ethanol extracts.

Effect of roselle extract on bacterial reduction in ground pork

Fresh pork from ham part and back fat were purchased from slaughterhouse in Thailand. Fresh pork ham (90%) and back fat (10%) were mixed and chopped using a chopper after all external fat and connective tissue were removed. The culture of *S*. Typhimurium or *S. aureus* were inoculated in ground pork. Subsequently, ground pork was divided into 2 groups which were control (no adding extract) and 50 mg of roselle extract per kilogram of meat added into ground pork. Then, ground pork were wrapped in plastic film and stored at 4 °C for 0, 2, 4, 6, 8 and 10 days. Survival of both pathogenic bacteria were counted and expressed as log 10 colony forming unit (CFU/g of meat).

Statistical analysis

All the experiments were carried out using Completely Randomized Design (CRD). Significant differences between

means were separated using Duncan's New Multiple Range test (DMRT) with SPSS (SPSS 16.0 for windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of roselle extract on survival of *S.* Typhimurium and *S. aureus*

The significant (P<0.05) decrease of *S. aureus* was detected on ground pork mixed with roselle extract (50 mg/ml). The initial inoculum was 3.20 log CFU/g during storage at 4 °C for 0 day. The log reduction figure was 0.81, 0.89, 1.13, 0.9 and 1.99 log CFU/g along storage time at 2, 4, 6, 8 and 10 days, respectively. Whereas, *S. aureus* in control treatment increased at 3.26, 4.16, 4.53, 4.86, 5.07 and 6.42 log CFU/g, respectively (P < 0.05) as shown in Table 1. On the contrary, the roselle extract had no effect on growth of *S*. Typhimurium in ground pork over storage period (Table 2).

As known, meat is highly perishable food product and become hazardous due to microbial growth (Turanta? et al., 2015). *Salmonella* spp. and *S. aureus* are the major problem of food-borne disease in many countries. *Salmonella* spp. was reported the highest number of case which mainly related to poultry and pig meat (Aymerich et al., 2008; Loretz et al., 2010). As the results displayed antibacterial activity against only *S. aureus* which was supported by Gupta et al. (2012). He reported that plant extracts are more active against gram positive bacteria than gram negative bacteria. This difference may be due to structural differences in cell wall of these bacteria. The gram negative cells wall is complex and multilayer structure. It has an outer phospholipids membrane carrying the structural lipopolysaccharide components, which makes a barrier to many environmental substances including synthetic and natural antibiotics. The gram positive bacteria contain a single outer peptidoglycan layer, which is not an effective permeability barrier.

CONCLUSION

The ethanol extract of roselle calyx showed antimicrobial activity against pathogenic bacteria, included *S. aureus*. The concentration of extract at 50 mg/ml was able to inhibit the growth of *S. aureus* in ground pork during storage at 4°C for 6 days. This research was beneficial for meat industrial to control pathogenic microorganism in meat.

Key words: Roselle extract, Pathogenic bacteria, Ground pork

Table 1 Effect of roselle extract on *Staphylococcus aureus* reduction in ground pork stored at 4 °C for 10 days

Storage time (days)	Number of *Staphylococcus aureus* (log CFU/g)		
	Control	Roselle extract (50 mg/ml)	Log reduction
0	$3.26 \pm 0.08^{a,A}$	$3.20 \pm 0.11^{a,A}$	0.06
2	$4.16 \pm 0.37^{a,B}$	$3.35 \pm 0.05^{b,A}$	0.81
4	$4.53 \pm 0.18^{a,C}$	$3.64 \pm 0.20^{b,AB}$	0.89
6	$4.86 \pm 0.03^{a,D}$	$3.73 \pm 0.23^{b,AB}$	1.13
8	$5.07 \pm 0.16^{a,D}$	$4.17 \pm 0.74^{b,B}$	0.90
10	$6.42 \pm 0.06^{a,E}$	$4.43 \pm 0.65^{b,B}$	1.99

[a-b]The means having different superscripts in a row are significantly different (P < 0.05)
[A-E] The means having different superscripts in a column are significantly different (P < 0.05)

Table 2 Effect of roselle extract on *Salmonella* Typhimurium reduction in ground pork stored at 4 °C for 10 days

Storage time (days)	Number of *Salmonella* Typhimurium (log CFU/g)		
	Control	Roselle extract (50 mg/ml)	Log reduction
0	$3.12 \pm 0.17^{a,A}$	$3.07 \pm 0.08^{a,A}$	0.05
2	$4.14 \pm 0.11^{a,B}$	$4.25 \pm 0.25^{a,B}$	Increased
4	$5.20 \pm 0.10^{a,C}$	$5.13 \pm 0.01^{a,C}$	0.07
6	$5.64 \pm 0.06^{a,C}$	$5.59 \pm 0.01^{a,C}$	0.05
8	$6.98 \pm 0.38^{a,D}$	$6.38 \pm 0.57^{a,D}$	0.60
10	$7.41 \pm 0.68^{a,D}$	$7.17 \pm 0.57^{a,E}$	0.24

[a] The means having different superscripts in a row are significantly different ($P < 0.05$)
[A-E] The means having different superscripts in a column are significantly different ($P < 0.05$)

REFERENCES

Al-Hashimi, A.G. 2012. Antioxidant and antimicrobial activity of *Hibiscus sabdariffa* L. extract. J. Food Sci. 6: 506-511.

Awe, F.B., Fagbemi, T.N., Ifesan, B.O.T. and Badejo, A.A. 2013. Antioxidant properties of cold and hot water extracts of cocoa, *Hibicus* flower extract, and ginger beverage blends. Food Res. Int. 52: 490-495.

Aymerich, T., Piconuet, P.A. and Monfort, J.M. 2008. Decontamination technologies for meat products. Meat Sci. 78: 114-129.

Fullerton, M., Khatiwada, J., Johnson, J.O., Davis, S., Williams, L.L. 2011. Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 isolated from food, veterinary, and clinical samples. J. Med. Food. 14: 950-956.

Gupta, P., Sharma, A. and Verma, A.K. 2012. GC/MS profiling and antimicrobial effect of six Indian tropical fruit residues against clinically pathogenic bacterial strain. Int. J. Adv. Pharm. Res. 3: 1229-1235.

Jung, E. and Joo, N. 2013. Roselle (*Hibiscus sabdariffa* L.) and soybean oil effects on quality characteristics of pork patties studied by response surface methodology. Meat Sci. 94: 391-401.

Khalaphallah, R. and W.S. Soliman. 2014. Effect of henna and roselle extracts on pathogenic bacteria. Asian Pac. J. Trop. Dis. 4: 292-296.

Kongkarn, N., Tangwatcharin, P., Teerarak, M., Hangsawake, K. and Pilasombut, K. 2015. Antibacterial activity of roselle (*Hibicus sabdariffa* Linn.) flower extract. 2nd International Symposium on Agricultural Technology. A-One The Royal Cruise Hotel Pattaya, Thailand. July 1-3 : 301-304.

Leroy, F., Verluyten, J., and Vuyst, L.D. 2006. Functional meat starter cultures for improved sausage fermentation. Int. J. Food Microbiol. 106: 270-285.

Loretz, M., R. Stephan and C. Zweifel. 2010. Antimicrobial activity of decontamination treatments for poultry carcass: a literature survey. Food control. 21: 791-804.

Nørrung, B. and Buncic, S. 2008. Microbial safety of meat in the European Union. Meat Sci. 78: 14-24.

Turantaş, F., Kilic, G.B., and Kilic, B. 2015. Ultrasound in the meat industry: general applications and decontamination efficiency. Int. J. Food Microbiol. 198: 59-69.

Verkade, E. and Kluytmans, J. 2014. Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. Infect. Gene. Evol. 21: 523-530.

PO-05-6

Effects of different fermentation methods on the quality characteristics of Yezo sika deer (Cervus nippon yesoensis) meat sauce products

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INTRODUCTION

It is well-known that Yezo sika deer (*Cervus nippon yesoenosis*) is one of the wild animals in Hokkaido, Japan. However, the number of individuals has explosively increased in eastern Hokkaido from 1980 to 1990. The explosive increase of Yezo sika deer (YSD) did severe damage to natural resources such as the natural forest, pasture and vegetable fields, and caused an increment of traffic accidents and changes to the ecosystem (Yezo Deer Association, 2006). The rate of growth later decreased through hunting, but after that, it has been on the rise again because YSD reproduce quietly and the hunting population was decreased due to an aging of the population, so its range-wide habitats spread in Hokkaido, Japan. The amount of damage to agriculture reached to about 6.3 billion yen in 2012 (Hokkaido Prefectural Environmental and Community Affairs Department, 2014). Due to these circumstances, in recent years, action is being taken through various approaches such as regional industry creation and promotion of regional development while considering individual adjustment and environmental preservation since the Hokkaido Prefecture regards YSD as an animal resource (Yezo Sika Deer Adaption Division, Department of Environment, Hokkaido Prefecture, 2014).

On the other hand, according to proximate component analysis of YSD meat, crude protein and lipid levels were about 22% and 1-5%, respectively, and the former level was almost same and the latter level was very small in comparison with the other species' meats although seasonal variation can be seen in YSD meat components (Sekikawa, 2003). The myoglobin level was considerably higher in YSD meat (6.0 mg/g) than in beef (1.9-4.1 mg/g), pork (1.1 mg/g) and chicken (1.4-1.6 mg/g). YSD meat is considered to be an excellent health food because it is rich not only iron but also in free carnitine (Yezo Deer Association, 2006). However, there are few applications in food processing of YSD meat due to problems such as its wild smell and the occurrence of a strong odour during storage (Watanabe *et al.*, 1998). For that reason, development of processing technology for food has been required in Japan.

In this study, the development of a popular meat sauce prepared using traditional soy sauce production technology for the purpose of efficient utilization of YSD was conducted and the differences in quality characteristics of the final products obtained by different fermentation methods were investigated.

MATERIALS AND METHODS

Materials

A commercial frozen YSD hind leg meat was used as the main materials in this study and the meat was frozen at -30 ºC until use. Soybean *koji* (SK), soy sauce *koji* (SSK) and rice *koji* (RK) were prepared by the standard method (Kitamoto, 2012). Preparation of minced meat *koji* (MMK) and cubed meat *koji* (CMK) was as follows. YSD meat was minced with a meat grinder. The MMK was prepared by incubating minced meat inoculated with *Aspergillus oryzae* (AOK 139, Akita Konno Co., Ltd., Daisen) at 25°C for 24 hours and then at 20°C for 24 hours after mixing. The CMK was prepared in the same manner as the MMK except for the use of cubed (1 cm) meat. Commercial halophilic lactic acid bacteria i.e. *Tetragenococcus halophilus* (Akita Konno Co., Ltd., Daisen) and soy sauce fermentation yeast i.e. *Zygosaccharomyces rouxii* (Akita Konno Co., Ltd., Daisen) were used in this study.

Preparation for meat sauce *moromis* and final products

The minced meat was prepared by a meat grinder after thawing at 4 ºC. According to soy sauce production technique (Itoh, 2014), fifteen kinds of meat sauce *moromis* (mashes) were prepared on a laboratory scale from the minced meat using salt, five kinds of *koji* molds (KM), *T. halophilus* and *Z. rouxii* and fermented at 30°C for 180 days (Table 1). The fermentation of the samples was conducted using a 1.5 L covered glass bottle and stirred

with a spoon at fixed intervals. 10^6 cfu/g level of *T. halophilus* and 10^6 cfu/g level of *Z. rouxii* were inoculated to the *moromis* at mixture time and after 2 weeks fermentation, respectively. After fermentation for 6 months, the *moromis* were centrifuged at 10,000 g for 30 min at 5 ºC. The supernatant after centrifugation was heated at about 85-90 ºC for 30 min and cooled at room temperature. After that, the sample was filtrated through a filter paper (No.5C, Advantec Co., Ltd., Tokyo) and the obtained filtrate (final product) was used as an assay sample in this study.

Physicochemical characteristics

The color of the sample was determined by the transition method. L*, a* and b* of the sample were measured using color-difference meter (TC-8600, Tokyo Densyoku Co., Ltd., Tokyo) with a glass cell (2 mm×40 mm×50 mm). The pH of the sample was measured using a glass electrode type of pH meter (HM-55, TOA Co., Ltd., Kobe). Total nitrogen level was determined by the Kjeldahl method (Yasui, 1982). Soluble solids excluding salt (SSES) were determined by subtracting from Brix to Salt content (Japanese Soy Sauce Research Institute, 1985). Brix was measured using a sugar refractometer (N-50E, ATAGO Co., Ltd., Tokyo) at 20 ºC. Salt content was determined by the Mohr's method (Japanese Soy Sauce Research Institute, 1985). The histamine level was determined by enzymatic method (Sato *et al.*, 2005) with histamine kit (Check Color Histamine, Kikoman Co., Ltd., Choshi).

Extractive components

The samples for free amino acid and organic acid analyses were prepared by deproteinization with 5% trichloroacetic acid solution and 50 fold dilution with distilled water, respectively. Free amino acid was analyzed using an amino acid automated analyzer (L-8500, Hitachi Co., Ltd., Tokyo). Organic acid was analyzed using HPLC (HPLC organic acid analysis system, Shimadu Co., Ltd., Kyoto).

Sensory evaluation

According to the soy sauce test method (Japanese Soy Sauce Research Institute, 1985), color, aroma, taste and overall quality of the final products were evaluated by the ranking method. Sensory evaluation was carried out by 14 trained sensory panelists (7 males: 7 females; age range, 22-30 years) from Rakuno Gakuen University. To begin with, the best sample was selected in each *koji* sample and then, the best choice sample was determined from the four kinds of the selected samples. The taste was evaluated using the diluted sample, which was adjusted to 1.5% salt content with distilled water.

Taste evaluation with multichannel taste sensor

Taste evaluation of the final product was conducted using a multichannel taste sensor (TS-5000Z, Insent Co., Ltd., Kanagawa). The assay sample was prepared using the sample, which was diluted to 1.5% salt content with distilled water. Thirteen kinds of tastes were determined by the multichannel taste sensor (MTS). Eight kinds of before tastes and five kinds of after tastes can be determined using the sensor membrane. Acid A, unfavorable taste concerning bittern and sweet were excluded from the thirteen kinds of tastes in this study because the former two did not contribute to soy sauce taste and the latter one possessed lower reliability of a numerical value related to the soy sauce taste etc.

Statistical analysis

A multivariate analysis of the data for multiple tastes obtained by MTS was conducted by software for the sensor accessory. The significant test of data for sensory score by ranking method was determined by Krammer's test (Kahaman *et al.*, 1973).

RESULTS AND DISCUSSION

Physicochemical characteristics and extractive components of YSD meat sauce products

Table 2 shows physicochemical properties of various kinds of meat sauce products. The yield was below 50% in MMK and CMK while it was over 50% in SBK, SSK and RK. The yield was higher in A sample than in B and C samples regardless of types of KM.

L* value was high in the following order: CMK >MMK >SSK ≧ RK >SK regardless of addition of *T. halophilus* and *Z. rouxii*. The a* value was low in SSK and RK. The b* value was high in the following order: SBK >RK ≧ SSK >MMK >CMK in all the samples. The b* value was lower in B and C samples than in A sample regardless of the types of

KM. Therefore, it was found that the color was deep in SBK, but it was pale in MMK and CMK, and its yellowish color was discolored by the addition of *T. halophilus and Z. rouxii*.

The range of pH value of the samples excluding SBK was 4.6-5.1. However, the pH values of A, B and C samples of SBK were 6.7, 5.4 and 6.3, respectively, and these pH values were higher than those of the other samples. Salt content was about 20% in all the samples.

Total nitrogen levels were 1.7-2.1 g/100mL in all the samples. These values were higher than special grade level (over 1.5 g/100mL) of regular soy sauce (Ministry of Agriculture, Forestry and Fisheries, 2014). However, SSES levels were lower than the special grade level (over 16%) (Ministry of Agriculture, Forestry and Fisheries, 2014).

It is considered that histamine accumulation might be caused by histamine producing bacteria (Satomi *et al.*, 2008) since the pH value of SBK increased during fermentation. It is conceivable that a rise of pH in the SBK was not caused by histamine accumulation because the histamine levels of all the samples were below 45 ppm. Nakazato *et al.* (2002) reported that histamine levels in commercial fish sauces were below 380 ppm. It was reported that the toxic dose level by histamine in the fish meat was 70 – 1,000 mg though there were individual differences (Hosogai, 2001). Moreover, it is said that non-volatile amines such as putrescine and cadaverine could affect the quantity generating histamine food poisoning (Hosogai, 2001). However, an outbreak of food poisoning might be very small and would be determined by how much condiments are used since the amount of intake is 38 mg if we eat the sample, which contains 380 ppm level of histamine (Nakazato *et al.*, 2002).

According to Yamajima *et al.* (2001), over 30 and 50 mg/100 mL of volatile basic nitrogen (VBN) levels in foods are judged as an initial stage of decomposition and absolute decomposition, respectively. However, in general, VBN levels in the fermentation foods are comparatively high (Nakazato *et al.*, 2002; Yamajima *et al.*, 2001). VBN levels of commercial fish sauces were 34-480 mg/100 mL, and among them, those of Chinese fish sauces (350-440 mg/100 mL) were considerably high (Nakazato *et al.*, 2002). High levels of VBN (156-234 mg/100 mL) were also detected in SBK in this study. Further study is needed to try to find the cause by investigating the effect of proteinase and microorganism in the main materials on the quality degradation of SBK due to VBN levels which increased during production process in spite of the presence or absence of fermentation microorganisms.

Table 3 shows organic acid compositions of various kinds of meat sauce products. Major organic acids in MSD meat sauce were lactic and pyroglutamic acids excluding SBK. On the other hand, it is estimated that acetic acid fermentation might be mainly progressed during YSD meat sauce production because the acetic acid level was higher than the lactic acid level in the SBK. This tendency was similar to that of *yeesui* made from Chinese samples and the flavor of *yeesui* was evaluated as unpreferred by Japanese people (Funatsu, 2000a). Lactic acid level was higher in B and C samples than in A sample excluding CMK. According to the relationship between organic acid compositions and pH of various kinds of meat sauce products, the percentage of acetic acid in the total of organic acids was about over 40% in the samples, which were shown at pH > 5.4, although it was about below 20% in the samples, which were shown at pH < 5.1.

Table 4 shows free amino acid compositions of various kinds of meat sauce products. Twenty one kinds of free amino acids were detected in the samples. Glutamic acid, lysine and leucine were rich in the samples. The contents of glutamic acid and aspartic acid, which are related to umami, were higher in SBK than in the other KM. Samples A and C in the SBK were not detected in arginine, but detected in ornithine. Therefore, it is conceivable that arginine might be converted to ornithine by enzymatic reaction such as arginine-deaminase during meat sauce fermentation (Uchida and Kobe, 1987).

Sensory evaluation and taste evaluation with MTS of YSD meat sauce products

Three kinds of SBK samples were excluded from sensory samples in this study because there was a serious problem with the samples which had a strong odor. To begin with, the best sample between MK was selected. Sample A was selected in SSK and RK while sample B and C were selected in MMK and CMK, respectively. Color, flavor and overall quality were significantly (P<0.05) better in SSK-A than in the other samples although aroma was significantly (P<0.05) better in RK-A than in the other samples. There were no significant differences in aroma, color, flavor and overall quality between MMK-C and the other samples. All four items were significantly (P<0.05) worse in CMK-C than in the other samples. Therefore, in overall quality, it has become clear that SSK-A was significantly (P<0.05) better than the other samples, but CMK-B had opposite results, and then, RK-B and MMK-C showed no significant difference (P>0.05) between the samples (Funatsu, 2016).

Figure 1 shows principal component analysis of twelve kinds of meat sauce products. PC1 indicates the strength of umami and weakness of sourness in initial taste. PC2 indicates the strength of peculiar flavor. Contribution

ratios of PC1 and PC2 were 97.0% and 2.4%, respectively. Umami was strongest in RK-A, and was weaker in RK-A than in SSK-A and MMK-A (PC1). The peculiar flavor was slightly stronger in SSK-A than in MMK-A (PC2). Umami in the sample A became weak by addition of *T. halophilus* except for the CMK samples (solid arrow). Not only umami but also peculiar flavor was stronger in the sample C than in the sample B by addition of *T. halophilus* and *Z. rouxii* except for CMK samples (dotted arrow). The reason why the addition effect of *T. halophilus* or *T. halophilus* + *Z. rouxii* was different CMK from the other KM might be caused by the difference in fermentation velocity due to uniform in the KM matrix. However, it is currently under consideration in detail.

According to the relationship between results of the sensory evaluation and taste evaluation with MTS, it was found that the best sample i.e. SSK-A had an adequate umami and peculiar flavor while the worst sample i.e. MMK-B was the weaker of the two.

In conclusion, it is necessary to take into account the differences in quality characteristics of meat sauce products which might be caused by different fermentation methods although it is possible to produce a popular Japanese meat sauce prepared from YSD meat using soy sauce production technology.

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Table 1 Compositions of the various kinds of meat sauce products

	Soybean *koji*			Soy sauce *koji*			Rice *koji*			Minced meat *koji*			Cubed meat *koji*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Ground meat (g)	450	450	450	450	450	450	450	450	450	450	450	450	450	450	450
Salt (g)	150	150	150	150	150	150	150	150	150	150	150	150	150	150	150
Water (mL)	300	295	290	300	295	290	300	295	290	300	295	290	300	295	290
Koji (g)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
T. halophilus (mL)	0	5	5	0	5	5	0	5	5	0	5	5	0	5	5
Z. rouxii (mL)	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5

A: *Koji*, B: *Koji* + halophilic lactic acid bacteria, C: *Koji* + halophilic lactic acid bacteria + soy sauce fermentation yeast.

Halophilic lactic acid bacteria: *Tetragenococcus halophilus* (Kita Konno Co., Ltd., Daisen), Soy sauce fermentation yeast: *Zygosaccharomyces rouxii* (Kita Konno Co., Ltd., Daisen).

Table 2 Physicochemical properties of various kinds of meat sauce products

	Soybean *koji*			Soy sauce *koji*			Rice *koji*			Minced meat *koji*			Cubed meat *koji*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Yield (%)	60.3	53.9	55.1	67.4	57.5	56.5	58.3	56.9	53.5	45.6	45.8	47.8	44.7	44.1	43.6
Color L*	80.70	87.00	84.09	89.56	90.29	92.14	89.50	91.26	91.86	91.73	93.70	94.18	94.10	94.58	94.24
a*	-0.36	-3.54	-2.75	-6.10	-6.20	-5.84	-5.81	-6.30	-6.22	-5.78	-5.98	-4.31	-4.42	-3.86	-6.22
b*	51.68	37.57	41.78	40.59	36.71	28.32	42.23	34.80	31.89	29.37	24.69	16.19	16.15	13.30	14.47
pH	6.7	5.4	6.3	4.9	4.6	4.7	5.1	4.6	4.7	4.8	4.6	4.7	4.8	4.8	5.1
Salt (g/100mL)	20.1	20.7	20.1	19.4	19.8	20.3	20.3	19.9	20.0	19.3	19.8	19.4	19.5	19.8	20.0
Total nitrogen (g/100mL)	2.1	2.1	2.1	1.8	1.8	1.8	1.8	1.8	1.8	1.9	1.9	1.9	1.7	1.7	1.7
Soluble solids excluding salt (%)	13.7	14.3	13.9	14.7	14.7	14.3	15.1	15.2	15.9	14.3	14.2	13.7	12.9	12.2	11.9
Histamine (ppm)	44.9	7.8	3.9	7.8	5.9	ND	21.5	7.8	ND	21.9	3.7	5.5	9.1	7.3	20.1

Fifteen kinds of meat sauce mashes were prepared in the same manner as Table 1.

See Table 1 for A, B and C. ND: not detected.

Table 3 Organic acid compositions of various kinds of meat sauce products (mg/100 mL)

	Soybean *koji*			Soy sauce *koji*			Rice *koji*			Minced meat *koji*			Cubic meat *koji*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Pyruvic acid	ND	25	23	ND	ND	11	ND	8	ND	ND	ND	14	ND	ND	6
Malic acid	15	6	10	ND	ND	ND	22	ND	ND	ND	ND	ND	ND	ND	ND
Succinic acid	97	29	34	44	46	58	47	54	55	7	ND	22	4	6	16
Lactic acid	120	319	282	537	895	805	239	827	799	829	1193	1170	1263	1213	1119
Formic acid	ND	ND	7	ND	ND	ND	4	3	4	4	21	ND	ND	ND	3
Acetic acid	624	577	650	20	14	11	186	46	37	250	67	14	10	ND	41
Pyroglutamic acid	385	487	429	398	399	382	351	343	340	384	374	406	324	327	335
Total	1241	1444	1435	1000	1355	1267	848	1283	1235	1475	1655	1626	1602	1546	1520

See Table 1 for A, B and C. ND: not detected.

Table 4 Free amino acid compositions of various kinds of meat sauce products (mg/100 mL)

	Soybean *koji*			Soy sauce *koji*			Rice *koji*			Minced meat *koji*			Cubed meat *koji*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Taurine	65	66	62	56	45	71	42	38	37	54	46	41	57	62	48
Aspartic acid	850	704	744	512	352	387	553	430	402	436	265	286	148	182	198
Threonine	415	456	478	395	371	382	323	308	291	389	313	337	253	259	281
Serine	12	105	22	348	320	329	198	265	251	239	275	297	197	225	210
Asparagine	55	59	58	240	251	255	91	157	142	238	209	215	208	213	234
Glutamic acid	1393	1213	1268	1057	1051	1113	874	935	879	1091	963	1022	833	847	882
Glycine	208	252	287	206	185	190	163	154	145	198	146	161	119	120	127
Alanine	574	681	766	582	642	605	511	536	496	653	590	612	526	507	551
Proline	335	231	259	230	208	215	195	184	173	222	159	170	149	136	152
Valine	640	590	619	524	514	542	457	461	435	542	464	487	409	406	439
Cystine	26	61	50	7	8	21	9	12	13	26	24	66	31	58	61
Methionine	277	279	277	245	253	266	218	229	216	273	235	255	221	226	243
Isoleucine	579	587	607	502	498	515	433	448	420	545	465	496	426	435	465
Leucine	909	956	971	856	857	919	775	822	772	933	824	877	757	773	822
Tyrosine	80	70	74	94	86	79	80	71	79	69	92	92	83	79	57
Phenylalanine	276	442	432	384	383	419	349	360	340	395	346	378	314	324	335
Tryptophan	106	97	103	10	19	ND	20	12	9	74	55	31	58	61	66
Ornithine	132	49	563	8	7	3	9	3	8	10	2	9	5	3	14
Lysine	1113	1041	1100	927	915	998	810	852	810	992	863	936	784	806	884
Histidine	217	254	258	199	196	202	152	163	151	221	184	204	162	166	181
Arginine	ND	464	ND	626	619	669	546	600	569	657	591	637	479	494	470
Total	8263	8658	9001	8008	7779	8178	6809	7039	6638	8256	7112	7608	6219	6382	6721

See Table 1 for A, B and C. ND: not detected.

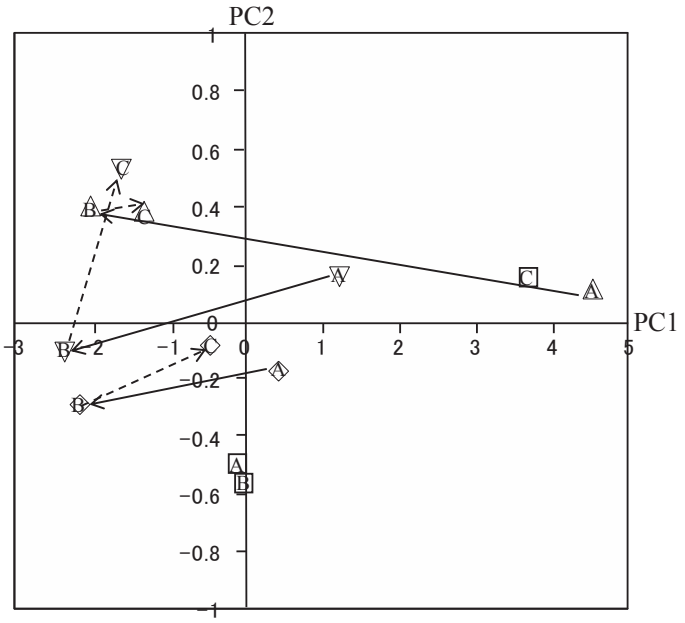

Figure 1 Principal component analysis of twelve kinds of meat sauce products ▽: Soy sauce *koji*, △: Rice *koji*, ◇: Minced meat *koji*,□: Cubed meat *koji*. See Table 1 for A, B and C. Solid arrow: Changes in taste characteristics between *koji* (A) and *koji*+*T. halophilus* (B). Dotted arrow: Changes in taste characteristics between *koji*+*T. halophilus* (B) and *koji*+*T. halophilus*+*Z. rouxii* (C).

REFERENCES

Funatsu, Y, Konagaya, S., Katoh, I, Takeshima, F, Kawasaki, K, Ino, S. 2000a. Extractive components of fish sauce from the waste of frigate mackerel surimi processing and a comparison with those of several Asian fish sauces. *Nippon Suisan Gakkaishi*, **66**, 1026-1035 (in Japanese with English abstract).

Funatsu, Y, Sunago, R, Konagaya, S, Imai, T, Kawasaki, K, Takeshima, F. 2000b. A comparison of extractive components of a fish sauce prepared from frigate mackerel using soy sauce with those of Japanese-made fish sauces and soy sauce. *Nippon Suisan Gakkaishi*, **66**, 1036-1045 (in Japanese with English abstract).

Funatsu. 2016. Quality characteristics of yezo sika deer *Cervus nippon yesoenisis* meat sauce products prepared using different fermentation methods. *Journal of the brewing society of Japan*, **111**, 150-159 (in Japanese).

Hokkaido Prefectural Environmental and Community Affairs Department. 2014. Promote for damage control of wild birds and animals such as a yezo sika deer, a Steller's sea lion and an earless seal. [homepage on the Internet]. Hokkaido Prefectural Environmental and Community Affairs Department, Hokkaido; [cited 20 August 2014]. Available from URL: http ://www.pref.hokkaido.lg.jp/file.jsp?id =742120

Hosogai, R. 2001. Food Safety Seminer (1) Food poisoning, Chuohoki Publishing Co., Ltd, Tokyo (in Japanese).

Iiyama, S, Ikeda, T, Toko, K, Yahiro, M. 1997. Measurements of shoyu (soy sauce) with multichannel taste sensor. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **44**, 615-622 (in Japanese with English abstract).

Itoh, H. 2014. Soy sauce. In : Kikuo, N (ed), *A Revision of The Zymurgy*, pp.151-181. Kodansha Scientific Co., Ltd., Tokyo (in Japanese).

Japanese Soy Sauce Research Institute. 1985. Soy sauce test method. Japanese Soy Sauce Research Institute, Tokyo (in Japanese).

Kahaman G, Cooper D, Papavasiliou A, Kramer A. 1973. Expanded tables for determing significance of differences for ranked data. *Food Technology* May 64-68.

Kitamoto, K. 2012. *A Revision of Molecular Biology of the Koji Molds*, The Brewing Society of Japan, Tokyo (in Japanese).

Mikami, M, Nguyen HT, Shimada, K, Sekikawa, M, Fukushima, M, Ono, T. 2007. Properties of "shishibishio" fermented pork meat sauce. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **54**, 152-159 (in Japanese with English abstract).

Ministry of Agriculture, Forestry and Fisheries. 2014. Japanese agricultural standard of soy sauce. [homepage on the Internet]. Ministry of Agriculture, Forestry and Fisheries, Tokyo; [cited 21 March 2014]. Available from URL: http://www.maff.go.jp/j/jas/jas_kikaku/pdf/kikaku_syouyu_h210831.pdf

Nakazato, M, Kobayashi, C, Yamajima, Y, Takeishi, Y, Kawai, Y, Yasuda, K. 2002. Determination of volatile basic nitrogen (VBN) and non-volatile amines in fish sauce. *Ann. Rep. Tokyo Metr. Res. Lab. P.H.*, **53**, 95-100 (in Japanese).

Nguyen HT, Shimada K, Sekikawa M, Ono T, Mikami M. 2005. Fermentation of meat with koji and commercial enzymes, and properties of its extract. *Journal of Science Food Agriculture*, **85**, 1829-1837.

Okada, Y, Muramoto, T. 2013. Physical and chemical properties of three muscles of wild yezo sika deer. *Nihon Chikusan Gakkaiho*, **84**, 169-174 (in Japanese with English abstract).

Sato T, Horouchi T, Nishimura I. 2005. Simple and rapid determination of histamine in food using a new histamine dehydrogenase from *Rhizobium* sp. *Analytical Biochemistry*, **346**, 320–326.

Satomi M, Fushita M, Oikawa H, Yoshikawa-Takahashi M, Yano Y. 2008. Analysis of 30 kbp plasmid encoding decarboxylase gene in *Tetragenococcus halophilus* isolated from fish sauce. *International Journal of Food Microbiology*, 126, 202-209.

Sekikawa, M. 2003. Proximate composition and processing characteristics of Yezo sika deer meat. *Animal Science and Agriculture Hokkaido*, **59**, 19-20 (in Japanese with English abstract).

Sekikawa A, Ham KH, Shimada K, Fukushima M, Ishikawa T, Lee CH, Mikami M. 2003. Color and its stability in venison from *Cervus nippon yesoensis* (Japanese Yezo Deer). *Korean Journal for Food Science Animal Resources*, **23**, 309-314.

Taira W, Funatsu Y, Satomi M, Takano T, Abe H. 2007. Changes in extractive components and microbial proliferation during fermentation of fish sauce from underutilized fish species and quality of final products. *Fisheries Science*, **73**, 913-923.

Uchida, K, Kobe, K. 1987. Study on functional diversity of soy sauce lactic acid bacteria and its application. *Journal of Japan Soy Sauce Research Institute*, **13**, 251-258 (in Japanese).

Watanebe, A, Sato, H, Matsumoto, M, Hodate, K. 1998. Off-flavor and lipid oxidation in raw venison during storage. *Nihon Chikusan Gakkaiho*, **69**, 489- 492 (in Japanese with English abstract).

Yamajima, Y, Kobayashi, C, Ohno, I, Miyakawa, H, Taguchi, N, Nakawato, M, Saito, K. 2001. Hygienic studies on oyster sauce. *Ann. Rep. Tokyo Metr. Res. Lab. P.H.*, **52**, 73-77 (in Japanese).

Yasui, K. 1982. Kjeldahl method. In: Editorial Committee of Japanese Society for Food Science and Technology concerning Analysis method for foods (eds), *The Analysis method for foods*, pp.111-113, Korin, Tokyo (in Japanese).

Yezo Sika Deer Adaption Division, Department of Environment, Hokkaido Prefecture. 2014. Dealing with efficient utilization of Yezo sika deer. [homepage on the Internet]. Yezo sika deer Adaption Division, Department of Environment, Hokkaido Prefecture; [cited 5 March 2014]. Available from URL: http://www.pref.hokkaido.lg.jp/ks/est/H25yuukoukatuyoutorikumi.htm

Yezo Deer Association. 2006. Report for Yezo sika deer forum. [homepage on the Internet]. Yezo Deer Association, Hokkaido; [cited 15 May 2014]. Available from URL: http://www.yezodeer.com/shirou/forums/2006forum/2006forumreport.pdf

Yoshikawa, S, Tanaka, A, Nishikiori, T, Ohta, T. Development of fish sauce from chum salmon by fermentation with barley koji and halo-tolerant microorganism. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **53**, 281-286 (in Japanese with English abstract).

PO-05-8

Relationship between cattle breeds and characteristics of soup stock prepared from bovine bone

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Objectives

Animal bones have used widely as material of soup stock. There are some reports about the characteristics of the soup stocks prepared from chicken bone, and porcine bone [1-3]. But, little is known about the characteristics of chemical composition of these soup stocks among the animal breeds. In this study, we used bovine bones of Japanese black cattle (JB), Holstein-friesian cattle (HF), the crossbreeding beef cattle (CB) and investigate the characteristics of the soup stocks prepared from these bones.

Materials & Methods

Sample preparation

Bovine femurs were get from a meat packer and these were stored at -20°C p to extract the soup stock. The periosteum and the red meat removed from the bone. The bone was boiled as pretreatment to remove the blood, cut into three parts by the slide compound saw (model 2400B, Makita Co., Anjo, Japan). And then, the bones were placed into a pot with just enough deionized water to cover them and extracted with water at 80~90°C by the IH cooking heater. During the heating, the pot was not cover and scum was removed by a ladle as much as possible. The extract was cooled in the refrigerator and the fat layer was filtered out from it with 40-50 μm pore diameter. Resultant bovine soup stock was kept at three volume of the weight of the bovine bone by using distilled water. This bovine soup stock was used to analyze the proximate composition (moisture, crude protein, crude fat, ash), mineral (magnesium, phosphorus, calcium, iron), dry extract, pH, salt concentration and color values (CIE L*a*b*), and were stored at -20°C until analysis.

Analysis of the bovine soup stock composition

The amounts of moisture and dry extract were determined by desiccation to constant weight at 105 °C. We used the dry extract for analysis of crude protein, crude fat and ash. Analysis of crude protein content and crude fat content have done according to Kjeldahl method and Soxhlet extraction method. To determine crude ash, the sample was heated over a gas burner and then in an electric muffle furnace at 550°C until constant weight was reached. The carbohydrate level (%) was calculated as 100 – (moisture + crude protein + crude fat + crude ash).

To determine mineral (Ca, P, Mg, Fe), dry extract was used. Samples for mineral analysis were prepared by the digestion at 550°C, and then dissolved with 2M HCl at 60°C. For analyzed sample, the resultants were diluted to 10 times by deionized water. The analysis was performed by the ICP emission spectrometry (ICPS-8100, Shimadzu Co., Kyoto, Japan).

Analysis of pH, salt concentration and color values (CIE L*a*b*) have done by the bovine soup stock sample directly. In order to determine pH and salt concentration, the pH meter (F-51, Horiba, Ltd., Kyoto, Japan) and the food salt meter (TS-999i, Tokokagaku Co., Tokyo, Japan) were used. Color values measurements of the bovine soup stock were made using a Minolta CM-5 spectrophotometer (Konica Minolta Inc., Tokyo, Japan) in the CIEL*a*b*space under D65, 10°.

Statistical analysis

All data are represented as means ± standard deviation (SD). Significant difference among treatment groups were determined by ANOVA with Turkey's test and significant difference between means were analyzed with student's t-test. Analyses were performed using Enterprise 3.0 software (SAS Institute, Cary, NC, USA).

Results and Discussions

Optimal pretreatment time of bovine bone

The boiling as pretreatment before extraction of soup stock have effect that remove bloods which was responsible

for turbidity in the soup stock. The bovine bone was suspended in a water and boiled for pretreatment time. After pretreatment, the water became cloudy by issue impurities such as blood from the bovine bone. The turbidity gradually increased and became constant. Thus, turbidity (absorbance at 660 nm) was set as criterion of pretreatment time. It shows the result of experiment to determine the optimal pretreatment time in Figure 1. When the turbidity becomes maximum and constant, we determined that the impurities such as bloods ware flow out to some extent. Turbidity has increased with pretreatment time and showed the maximum at 25 minutes. After 25 minutes, it showed constant. We decided the pretreatment time of bovine bone for 30 minutes in consideration of samples variation.

Decision of extraction time of soup stock

Major taste components in the soup stock are nitrogen compounds such as amino acids and peptides. Thus, total nitrogen content in the bovine soup stock in each extraction time was shown in Figure 2. The amount of total nitrogen reached a maximum at 36 hours. And then, there was no difference between 36 and 48 hours. Therefore, we decided that the extraction time of bovine bone was 36 hours.

Proximate composition

Proximate composition of the bovine soup stocks were shown in Table 1. The bovine soup stock was composed of moisture and dry extract. Moisture amounts was the highest in proximate composition, and was in much order, 98.2 ± 0.3 g (CB), 98.1 ± 0.4 g (HF) and 97.6 ± 0.2 g (JB)/100 g wet weight amount of the bovine soup stock. Conversely, the amounts of dry extract was 2.4 ± 0.2 g (JB), 1.9 ± 0.4 g (HF) and 1.8 ± 0.3 g (CB)/100 g soup stock in much order. The dry extract in JB was the heaviest among three groups. Crude protein was secondly an ingredient of much quantity, and occupied 91.7- 94.4% of dry extract. Their amounts were 2.2 ± 0.2 g (JB), 1.7 ± 0.3 g (HF) and 1.7 ± 0.7 g (CB)/100 g soup stock in much order and it in JB was the highest among three groups ($p<0.05$). Ash amount was 0.09 ± 0.02 g (JB), 0.08 ± 0.01 g (CB) and 0.07 ± 0.01 g (HF)/100 g bovine soup stock. In ash of the bovine soup stock, the difference was not observed among three groups. Carbohydrate was 0.11 ± 0.09 g (HF), 0.05 ± 0.02 g (CB) and 0.01 ± 0.01 g/100 g soup stock. Crude fat content of soup stock was the lowest in proximate composition, and was 0.026 ± 0.007 g (JB), 0.021 ± 0.012 g (HF) and 0.004 ± 0.001 g (CB)/100 g bovine soup stock. Although the difference was observed in carbohydrate and crude fat, these were very small amount. Thus, it suggest that the crude protein amount greatly contributes to the difference in the weight of the dry extract.

Mineral component, pH, and salt concentrations

Mineral component of the bovine soup stock were shown in Table 2. Magnesium amount was the most highest in four kinds of mineral (Ca, P, Mg, Fe) and was in much order, 0.4 ± 0.1 μg (JB), 0.3 ± 0.1 μg (HF) and 0.3 ± 0.0 μg (CB)/ml bovine soup stock. The second most content of mineral was phosphorus, and was 0.3 ± 0.0 μg (JB), 0.2 ± 0.1 μg (HF) and 0.2 ± 0.1 μg (CB)/ml soup stock. In four kinds of mineral of bovine soup stock, the difference was not observed among three groups.

The pH in three kinds of the bovine soup stocks was 8.4 ± 0.1 (JB), 8.2 ± 0.1 (HF) and 8.2 ± 0.1 (CB). Salt concentrations was 0.04 ± 0.01 % (JB), 0.04 ± 0.01 % (HF) and 0.05 ± 0.01 % (CB). In the pH and salt concentrations, there was no significant difference observed among three groups. The color values (CIE L*a*b*) of the bovine soup stock shown in Table 3. L* value in each soup stock was 84.4 ± 5.2 (JB), 93.3 ± 1.2 (HF), 95.5 ± 1.5 (CB). In a* value and b* value, a* value was 1.6 ± 0.7 (JB), 0.4 ± 0.3 (HF) and 0.1 ± 0.1 (CB), b* value was 19.2 ± 2.2 (JB), 12.1 ± 1.6 (HF), 9.7 ± 1.1 (CB), respectively.

The color values in JB were significantly differences among three groups ($p<0.05$). L* value in JB was lower than HF and CB. On the other hand, a* and b* value in JB was the highest among three group. We accepted that JB bone soup stock was more cloudy and yellowish than other soup stocks.

Conclusions

It became clear that JB soup stock has more crude protein (dry extract) than HF and CB. Major taste components of the bovine soup stock are nitrogen compounds such as amino acids and peptides. We have not investigated amino acids and peptides yet. If there is a difference in these contents, it has been suggested that quality of soup stock prepared from the cattle bone is different, depending on the breed.

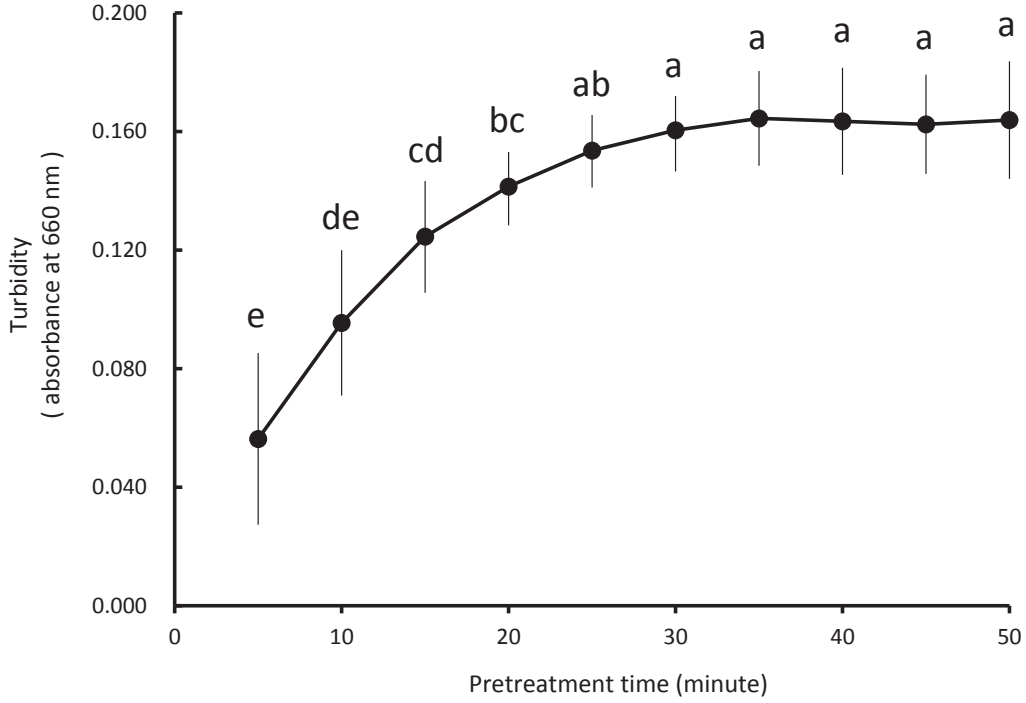

Figure 1. Relationship of pretreatment time and turbidity of the bovine bone soup. Data are expressed as Means ± standard deviation. Statistical processing using the Tukey-Kramer test. Same superscription letters within the figure are not significant difference at $p < 0.05$.

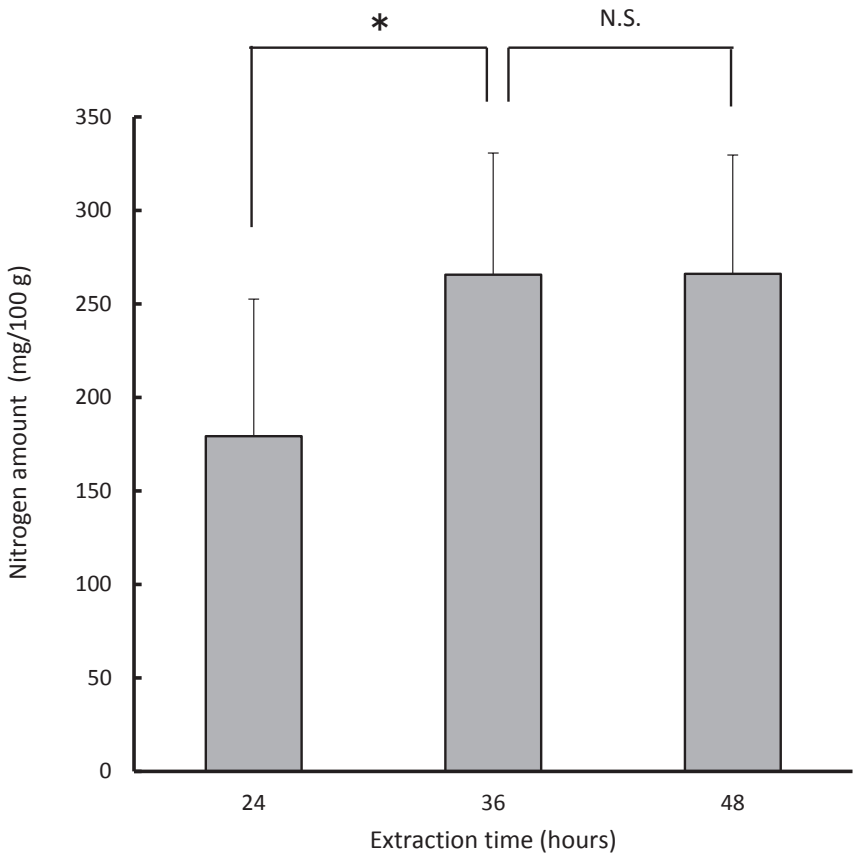

Figure 2. Influence of extraction time on nitrogen content in the bovine bone soup. Data are expressed as Means ± standard deviation. Statistical processing using the Student`s t-test. ✻ showed significant difference at $p < 0.01$ and N.S. indicated not significant difference.

Table 1. Proximate composition of soup stock

	JB [1]	HF [1]	CB [1]
Moisture (%)	97.6±0.2 [b]	98.1±0.4 [ab]	98.2±0.3 [a]
Dried extract (%)	2.4±0.2 [a]	1.9±0.4 [ab]	1.8±0.3 [b]
Crude protein (%)	2.2±0.2 [a]	1.7±0.3 [b]	1.7±0.7 [b]
Ash (%)	0.09±0.02 [a]	0.07±0.01 [a]	0.08±0.01 [a]
Carbohydrates (%)	0.01±0.01 [b]	0.11±0.09 [a]	0.05±0.02 [ab]
Crude fat (%)	0.026±0.007 [a]	0.021±0.012 [a]	0.004±0.001 [b]

Date are expressed as Means ± standard deviation. Statistical processing using the Tukey-Kramer test. Same superscription letters within the figure are not significant difference at $p < 0.05$.

[1] JB, HF and CB represent, Japanese Black Cattle, Holstein-friesian cattle and the crossbreeding beef cattle, respectively.

Table 2. Mineral component of soup stock

	JB [1]	HF [1]	CB [1]
Magnesium (μg/ml)	0.4±0.1 [a]	0.3±0.1 [a]	0.3±0.0 [a]
Phosphorus (μg/ml)	0.3±0.0 [a]	0.2±0.1 [a]	0.2±0.1 [a]
Calcium (μg/ml)	0.1±0.1 [a]	0.1±0.0 [a]	0.1±0.0 [a]
Iron (μg/ml)	0.0±0.0 [a]	0.0±0.0 [a]	0.0±0.0 [a]

Date are expressed as Means ± standard deviation. Statistical processing using the Tukey-Kramer test. Same superscription letters within the figure are not significant difference at $p < 0.05$.

[1] JB, HF and CB represent, Japanese Black Cattle, Holstein-friesian cattle and the crossbreeding beef cattle, respectively.

Table 3. The color value of soup stock

	JB [1]	HF [1]	CB [1]
L*	84.4±5.2 [b]	93.3±1.2 [a]	95.5±1.5 [a]
a*	1.6±0.7 [a]	0.4±0.3 [b]	0.1±0.1 [b]
b*	19.2±2.2 [a]	12.1±1.6 [b]	9.7±1.1 [b]

Date are expressed as Means ± standard deviation. Statistical processing using the Tukey-Kramer test. Same superscription letters within the figure are not significant difference at $p < 0.05$.

[1] JB, HF and CB represent, Japanese Black Cattle, Holstein-friesian cattle and the crossbreeding beef cattle, respectively.

Reference

1. Ota, M., Kuroda, M. and Kawaguchi, H. (2007) Analysis of Porcine Bone Extract soup. Food science technology research (Jpn.), 13, 261-264.

2. Shibata, K., Watanabe, Y. and Yasuhara, Y. (2007) Effect of the Variety and Quantity of Extract Samples (Beef Shank, Chicken Bone and Vegetables) on the Taste and Taste Components of Soup Stock. The Japan Society of Cookery Science, 40, 6, 411-419.

3. Adachi, M. (1994) The Effects of the Preliminary Treatment of Chicken Bones on the Taste and Odor of Soup Stock. The Japan Society of Cookery Science, 27, 4, 282-286.

PO-05-9

Characteristics of restructured steaks made with beef trimming derived from round and neck portions

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INTRODUCTION

Much of the growing demand for beef consumption worldwide estimated 0.37% per year during 2010-2014 (Office of Agricultural Economic, 2015). Additionally, the increasing costs of beef production have prompted the industry to develop strategies to utilize the carcasses and value-added of low-value meat cuts and trimming by-product generates additional revenue. Restructured meat offer many advantages for consumer and meat industry. It prepared from small cuts of meat in an effort to increase the yield of marketable products. Due to without any added sodium chloride or phosphates, the use of commercial microbial transglutaminase (MTGase) as a binding agent in restructured meat products yielding 'healthy' meat products has been reported by Kuraishi et al. (1997). MTGase is an enzyme promoting protein binding in muscle foods through covalent cross-linking between glutamine and lysine residues, resulting in the formation of high molecular weight polymer (Lee and Lanier, 1995). MTGase is active at the pH range 5-8 and temperature range 2-6°C. There are some data of MTGase has been used in the production of restructured beef as influenced by salt and phosphate (Kuraishi et al., 1997; Nielsen et al., 1995), added walnuts (Serrano et al., 2004) muscle-fiber/fiber-bundle alignment (Farouk et al., 2005) and post-mortem time of meat (Farouk et al., 2005), but the quality of products containing MTGase as impacted by various types of beef Trimming by-product has not been determined. Therefore, the aim of the present experiment was to evaluate the quality characteristics in term of physico-chemical, microbiological and sensorial properties in restructured beef related to beef trimming derived from round and neck portions.

MATERIALS AND METHODS

Trimmed beef scraps from round and neck portions portion were obtained from a commercial butcher, Thailand and then their proximate composition were determined. Beef trimming samples were striped into about 20×10×50 cm and a 2-kg restructured beef was manufactured for each treatment. The samples (2-kg) were manually mixed with 1% (w/w) MTGase (ACTIVA®TG-B Powder Sprinkle QS-Type, Ajinomoto (Thailand) Co., Ltd.). The resulting mixture was stuffed into a stainless steel ham mould/press. The stuffed moulds were held for 2-4°C for 4 h to allow the binder to bind the restructured meat pieces, then frozen at -20°C for 24 h. The reformed meats were removed from the mould and then packed in vacuum bags and stored at -20°C until analysis. Frozen samples were semi-thawed to the temperature of -2°C to 0°C and then sliced into a 20-mm thick steaks for further analysis. For determination of cross-linking among protein, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed. After grilling steak samples in a pan until the core temperature (CT) reached 71°C, cooking loss determination, color measurement as CIE L*a* b*, texture profile analysis, microbiological test and sensory evaluation as 7-point hedonic scales using 30 semi-trained panelists were carried out. Mean comparison among samples was analyzed by independent sample t-test with the SPSS package.

RESULTS AND DISCUSSION

Proximate composition of beef trimming

Beef trimming that obtained from round portion showed higher moisture, protein and ash contents with a lower fat content compared to those obtained from neck portion ($P<0.05$) (Table 1). As a result of beef trimming obtained from round portion and its containing 75.65% moisture, 21.16% protein, 1.65% fat and 1.07% ash, this quality was classified as class RI according to Sethakul and Sivapirunthep (2012) (distinguishes 5 categories of beef into RI– RV based on their composition). While beef trimming originated from neck portion (with partially removed tendons, connective tissue and fat) had 61.52% moisture, 17.59% protein, 16.03% fat and 0.87% ash, categorizing as class RIII.

Protein pattern

Regardless the effect of types of beef trimming, under non-reducing conditions (absence bME), a loss of myosin heavy chain (MHC) concomitant with the formation of high molecular weight protein above 200 kDa and the newly formed protein on the top of resolving gel as well as a loss of actin, was largely observed in all samples (Fig. 1). Under reducing conditions (presence bME), the density of MHC among MTGase treated samples cloud partially reproduced under reducing condition with some loss of high molecular weight protein above 200 kDa. However, there was remaining the aggregated protein on the top of resolving gel. Concerning actin, it seems to be completely reproduced under reducing condition. These results suggested that protein cross-link reaction among restructured beef adding MTGase supported by both disulfide and non-disulfide bond of MHC, and also disulfide bond of actin in beef samples. Thus, both beef trimming samples could be tightly bound together stabilizing by cross-links of MHC and actin, leading to satisfactory obtained restructured products as shown in Fig. 2.

Physical characteristics

Restructured products processed from beef trimming from neck portion showed higher cooking loss value than those from round portion ($P<0.05$) (Table 2). Furthermore, lightness value of cooked steak was significantly influenced by the type of beef trimming, which round portion showed increased lightness value than neck portion ($P<0.05$). A more intense discoloration (ΔE) upon cooking was found in neck portion(containing high-fat content) than in round portion ($P<0.05$). Similar results have been reported in beef patties with higher fat content had more intense color changes (Utera et al., 2014). Lower haem pigments and the formation of protein oxidation induced by higher lipid content during cooking could have affected light reflection and yellowness, leading to color deterioration of beef patties (Utera et al., 2014). In terms of texture profile analysis, lower hardness was found in steaks from neck portion than round ones ($P<0.05$).

Microbial count

Although there were no significant differences among raw samples, restructured steaks showed viable counts determined on Plate Count Agar ranging from 4.73 to 5.87 log CFU/gfor psychrotrophic bacteria and from 3.68 to 3.99 log CFU/g for mesophilic bacteria depending on treatments, while thermophilic bacterial counts showed a below the detection level (Table 2).The number of psychrotrophic bacteria was, on average, 1 log cycle higher than mesophilic bacteria in each sample. These results are in agreement with Ercolini et al. (2009) reported in refrigerated beef. They stated that bacteria developing on meat at chill temperatures are regarded as psychrotrophic population. Chilled beef belongs to microbial genera of both gram-positive, such as lactic acid bacteria, and gram-negative bacteria, such as Pseudomonas spp. and Enterobacteriaceae (Holzapfel, 1998). Species of Pseudomonas are particularly involved in the spoilage of meat stored at chill temperatures (Ercolini et al., 2007; Jay et al., 2003). After grill steaks until the CT reached 71°C, psychrotrophic and mesophilic bacteria counts of all samples were reduced to an undetectable level.

Sensory evaluation

Restructured steak from neck portion had a higher tenderness score than those from round portion. Moreover, higher sensory scores including color, appearance, flavor, juiciness and overall quality were tended to be found in restructured beef made from beef trimming from neck portion as compared to the other one (Table2). An increase in fat content increased the tenderness, juiciness and fattiness scores were also found in cooked beef as reported by Iida et al. (2015) and they found that an increasing the fat content up to a certain point greatly enhanced the umami intensity and beef flavor intensity in the meat quality evaluation and raised the overall evaluation score.

Table 1. Proximate composition of beef trimming from round and neck portion

Parameters	Round portion	Neck portion
pH	5.70 ± 0.20^{b,†}	6.00 ± 0.10^{a}
Moisture (%)	75.65 ± 0.08^{a}	61.52 ± 2.09^{b}
Protein (%)	21.16 ± 0.02^{a}	17.59 ± 0.34^{b}
Fat (%)	1.65 ± 0.30^{b}	16.03 ± 0.26^{a}
Ash (%)	1.07 ± 0.02^{a}	0.87 ± 0.01^{b}

Values are given as means ± SD of each meat batch (n=3).
† Different superscripts in the same row indicate significant differences among treatments ($p<0.05$).

Table 2. Characteristics of restructured beef containing MTGase

Characteristics	Round portion	Neck portion
Physical characteristics of cooked samples		
Cooking loss (%)	32.11 ± 2.00^{a,†}	37.8 ± 2.00^{b}
Lightness (CIE L*)	34.22 ± 2.32^{a}	28.11 ± 2.06^{b}
Redness (CIE a*)	8.77 ± 0.45^{a}	9.97 ± 0.79^{a}
Yellowness (CIE b*)	23.20 ± 1.34^{a}	21.28 ± 1.57^{a}
Color deference (ΔE)‡	12.43 ± 0.19^{b}	20.63 ± 0.96^{a}
Hardness (N)	22.27 ± 0.03^{a}	19.63 ± 0.05^{b}
Cohesiveness (ratio)	0.61 ± 0.03^{a}	0.62 ± 0.05^{a}
Gumminess (N)	11.38 ± 1.55^{a}	13.82 ± 2.28^{a}
Springiness (ratio)	0.9 ± 0.02^{a}	0.91 ± 0.02^{a}
Chewiness (N)	12.82 ± 1.92^{a}	12.60 ±2.09^{a}
Microbial counts of raw samples (log CFU/g)		
Psychrotrophic	4.73 ± 0.38^{a}	5.87 ± 0.03^{a}
Mesophile	3.99 ± 0.06^{a}	3.68 ± 0.35^{a}
Thermophile	ND§	ND
Microbial counts of cooked samples		
Psychrotrophic	ND	ND
Mesophile	ND	ND
Thermophile	ND	ND
Sensorial scores of cooked samples		
Color	4.20 ± 1.20^{a}	4.50 ± 1.30^{a}
Appearance	4.40 ± 0.93^{a}	4.60 ± 1.16^{a}
Flavor	4.10 ± 1.32^{a}	4.50 ± 0.82^{a}
Tenderness	3.80 ± 1.49^{b}	4.63 ± 0.93^{a}
Juiciness	3.80 ± 1.56^{a}	4.27 ± 0.93^{a}
Overall quality	4.08 ± 1.26^{a}	4.50 ± 0.86^{a}

Values are given as means ± SD of each meat batch (n=3).
† Different superscripts in the same row indicate significant differences among treatments ($P<0.05$).
‡ Color differences among raw and cooked steak.
§ Counts below the detection level.

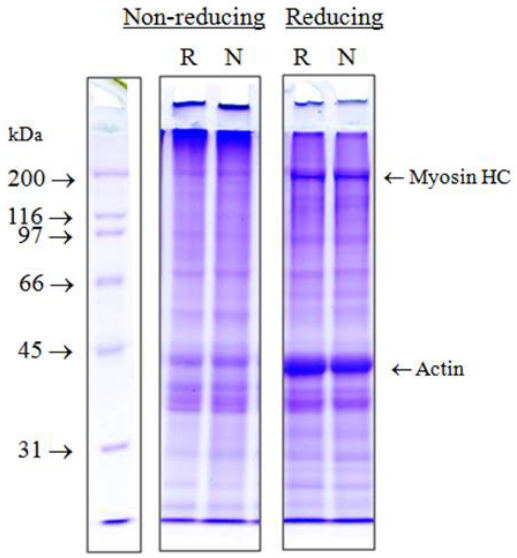

Figure 1. Electrophoretic patterns of muscle protein from raw restructured beef steaks containing MTGase separated by 5% running gel. R: beef trimming from round portion; N: beef trimming from neck portion

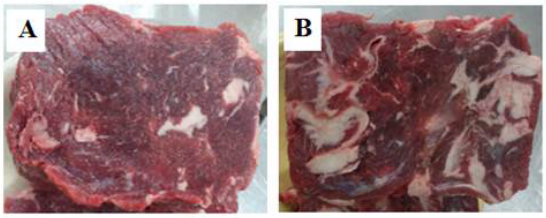

Figure 2. Raw restructured steaks of beef trimming from round (A) and neck (B) portions.

REFFERENCES

Ercolini, D., F.Russo, A.Nasi, P. Ferranti and F. Villani.2009.Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Appl. Environ. Microbiol. 75: 1990–2001.

Farouk, M. M., W. K.Hall, K. J.Wieliczko and J. E.Swan.2005. Processing time and binder effect on the quality of restructured rolls from hot-boned beef. J. Muscle Food. 16: 318–329.

Holzapfel, W. H. 1998. The gram-positive bacteria associated with meat and meat products. In: The microbiology of meat and poultry. Davies, A. and Board, R. (ed) Blackie Academic & Professional, London, England, pp. 35–74.

Iida, F., K. Saitou, T.Kawamura, S. Yamaguchi and T. Nishimura. 2015. Effect of fat content on sensory characteristics of marbled beef from Japanese Black steers. AnimSci J. 86: 707–715.

Jay, J. M., J. P.Vilai and M. E. Hughes.2003. Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5-7°C. Int. J. Food Microbiol. 81: 105–111.

Kuraishi, C., J.Sakamoto, K. Yamazaki, Y.Susa, C.Kuharaand T.Soeda.1997. Production of restructured meat using microbial transglutaminase without salt or cooking. J. Food Sci. 62: 488–490.

Lee, H. G. and T. C. Lanier.1995. The role of covalent cross-linking in the texturizing of muscle protein sols. J. Muscle Foods. 6: 125–138.

Nielsen, G. S., B. R. Petersen and A. J. Moller. 1995. Impact of salt, phosphate and temperature on the effect of a transglutaminase (F XIIIa) on the texture of restructured meat. Meat Sci. 41: 293–299

Office of Agricultural Economic.Situation of agricultural products and trends in Thailand. Available from: http://www.oae.go.th/download/document_tendency/ journalofecon2558.pdf. Accessed Jun. 20, 2015. (in Thai)

Serrano A., S. Cofrades and C. Jiménez. 2004. Transglutaminase as binding agent in fresh restructured beef steak with added walnuts. *Food Chem.* 85: 423–429.

Sethakul, J. and P.Sivapirunthep.2012. Meat processing. Mean Service Supply Ltd., Part, Bangkok. pp, 29-34. (in Thai).

Utrera, M., D.Morcuende and M. Estévez.2014. Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. LWT– Food Sci. Technol. 56: 62–68.

PO-05-11

CANINE PET FOOD RATION BY USING MECHANICALLY SEPARATED CHICKEN MEAT BYPRODUCT

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OBJECTIVE

The dog food plays a major role among the pet food industry. Developed countries have rapidly expanding the market than developing countries. This scenario is arising due to lack of locally available formulated pet food under lower price. Therefore this experimental study was designed to formulate a palatable canine food using locally available ingredients for low cost budget which confirm the major nutritional requirements and determine its influence on growth rate of local breeds.

METHODOLOGY

Six healthy puppies from two separate litters belonging to the age range of 5 to 6 weeks were selected. Puppies were divided into two groups based on sex and individual body weight. The study was conducted as an experimental study, during the period of March to April, 2014.

Materials:

All ingredients which were required to prepare a canine ration (mechanically separated chicken meat byproduct, mechanically separated chicken meat, fish meal, maize, bread crumble) were purchased from commercial stores and chicken meat byproducts were gathered from the poultry processing plant. Maize and bread crumble were ground into powder form. Chicken meat byproduct was ground twice by meat mincer.

Preparation of canine ration :The ingredients were weighted using a digital scale.

Feeding schedule:

Daily feed requirements of puppies were calculated separately by measuring individual body weights on a daily basis. It was 10% of the puppy's body weight. Feeding was done at morning and evening sessions. Each puppy received 150ml of milk every morning at 9.00a.m. and 5.00p.m. Puppies received their own feed separately.

Data collection

Chemical analysis of feed :

Determination of moisture content - method specified in AOAC (2000).

Determination of Fat content - method specified in AOAC (2000) and the Soxtherm manual.

Determination of crude protein content - kjeldahl method.

Physical analysis of puppies

Daily body weight, heart girth, body length, belly girth were measured as growth parameters.

Absolute growth rate

Actual increase of weight or size of an individual per unit time. Body weight were measured daily and body parameters were weekly. Absolute growth rate was calculated for weight gain and size incensement.

Relative growth rate

How much puppies gain their body weight comparatively to the initial body weight.

Statistical analysis procedure of data

Statistical analysis was conducted using SAS two sample t-test computer analysis program. (test is significant at $P < 0.05$)

RESULTS

A total of 6 puppies were recruited to the study of canine ration formulation of using mechanically separated meat products. The rate of weight gaining, heart girth, belly girth, increasing rate of body length and cost condition were used in analysis in this report. The total sample obtained, consisted of 04 females and 02 males aged 05-06 weeks.

Growth performances

Puppies in group-A were fed using new formulated canine ration and group-B were fed mechanically separated

meat byproducts which was introducing as local pet food item in Sri Lanka.

Weight gain (Figure 03)

The figure 02 exhibits the average weight gain rate in both groups. It pointed out the initial average weight in both groups and after the feeding trial it has changed.

The P value was 0.046(significant at P< 0.05). The daily weight gain of group A was 0.078kg/day and group B was 0.032kg/day. By considering weight gain values of each individual in both groups, significantly higher in group A. When considering lowest value in Group A which is code no.03, the weight gain rate was 0.389kg/week. In group B, the highest value is $B_{3.}$ This was 0.275kg/week. When comparing those two values, it exhibits the group A has higher weight gain rate than group B.

When considering the numerical values, the initial average weight in group A was 10391kg and group B was 1.250kg. After four weeks of feeding trial it was deviate as the group A was 3.180kg and group B was 2.100kg.

Heart girth (Figure 04)

According to the t-test, heart girth gaining rate of group A was significantly differ from group B values (P<0.05). The mean heart girth gain values of group A was 3.0cm/week and group B was 1.7cm/week.

The initial heart girth values were nearly same in both groups. After a month, the rate of heart girth gaining in group A was greatly higher than group B. The P value was 0.018 (significant at P<0.05).

Belly girth

During the feeding trial the mean belly girth gain of group A was 4.3cm/week and group B it was 2.6cm/week. By the end of the feeding trial, group A shown a higher growth rate. The P value was 0.018 (significant at P< 0.05). The mean belly girth gain of group A was 4.3cm/week and group B was 2.6cm/week.

Body length

The P value was 0.017(significant at P< 0.05). The mean value of body length gain in group A was 4.1cm/week and group B was 2.1cm/week. The final average value of rate of length increasing in group A was 27.67cm and group B was 23.33cm.

When considering regression analysis between weight and feed intake, group A has positive relationship and the R-square value was 0.9944. In group B, there also a positive relationship and the R-square value was 0.9825.

Nutrient standards:

Table 01 represents the standard denoted by AAFOC for manufacture who engaged in canine food industry.

CONCLUSION

Finally it can be conclude that formulated canine ration has significant effect on growth performance of puppies. This low cost product can be replace local pet food which is contains mechanically separated byproduct meal in Sri Lankan market.This also a great substitute for imported canine feed due to comparatively standard nutrient value and easiness for application or usage. The new formulated canine ration is pre cooked feed item. It cause to easily prepare within two minutes. Itcan make more palatable by wetting with milk. This product highly accepted by puppies. Due to semisolid product this can be preserved by blast freezing without adding any perseverates. The product producing method save more energy due to no need for drying.

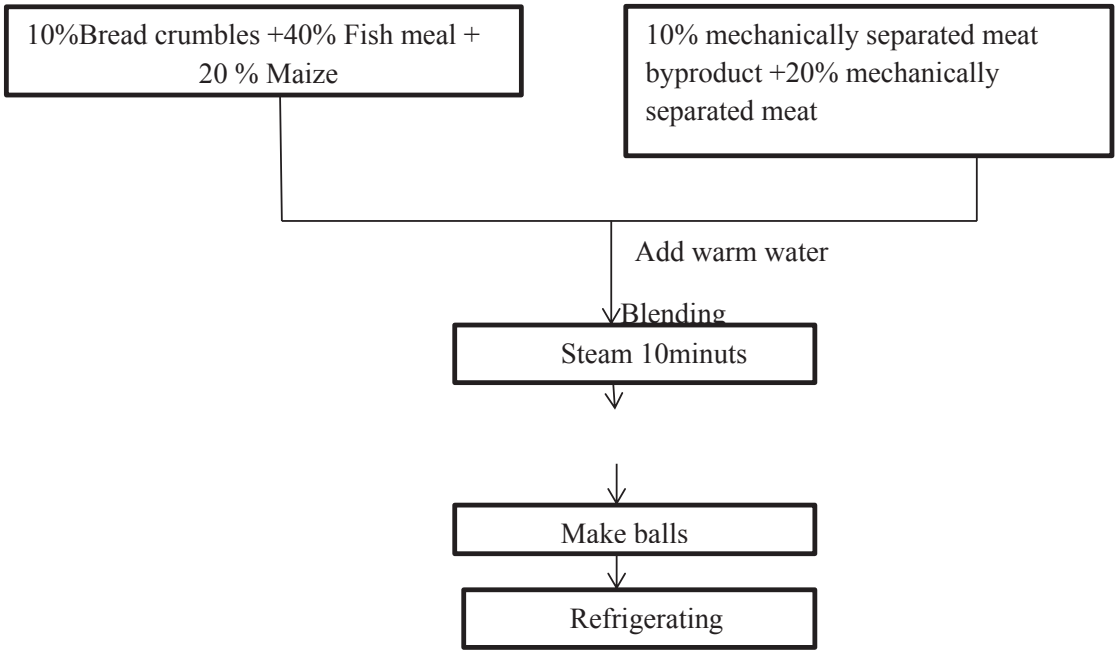

Figure 01: Preparation procedure for canine ration

Weight gain

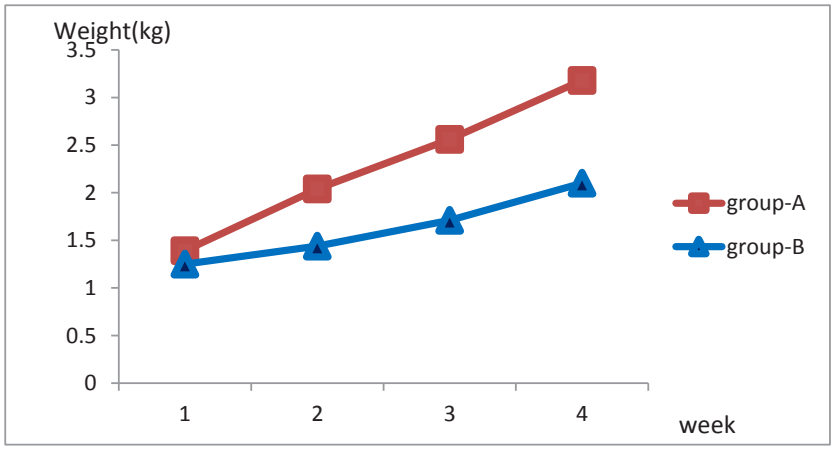

Figure 02: Average weight gain for group A and B

Heart girth

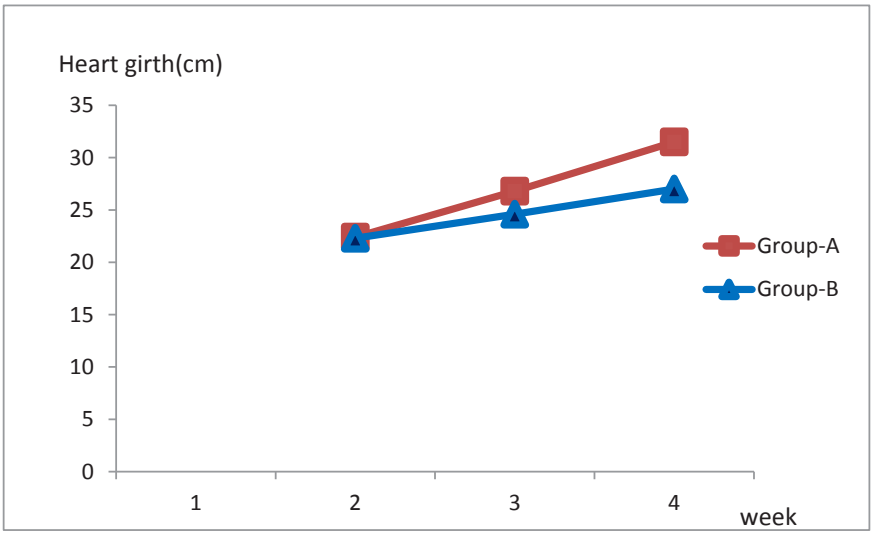

Figure 03: Average heart girth gain rate for group A and B

Table 01: AAFOC standards, nutrient measurements in formulated feed.

Nutrient	Standard nutrient requirement for puppies*	New formulated feed - nutrient profile (Group- A feed)
Protein	31%	31.7%
Fat	7%-10%	9.41%
Moisture	30%-40%	42%
Energy	3.5kcal/g	3.1kcal/g

*NRC (AAFCO) dog nutrient profiles published in 2008.

REFERENCES

1. Carpenter, J. A.1956. Species differences between taste preferences. J. Comp. Physiol. Phychol. 49:139-144.
2. Dog food project. (2004-2012) (Dog food project, 2007)http://www.dogfoodproject.com (04 May 2007)
3. FDA tips for preventing food borne illness associated with pet food and pet treats. Electronic reference. Retrieved May 24, 2014 fromhttp://paradiseamerica.hypermart.net
4. EdneyA.T.B.Dog and cat nutrition.Hand book for veterinarians, breeders, owners and students., Animal studies center. Chapter 7.
5. Headhammer A.A. 1994.Dogs and cats nutrient.
6. How to measure your dog, Electronic reference. Retrieved March 04, 2014 from http://paradiseamerica.hypermart.net
7. Janowitz, H. D., and M. I. Grossman. 1949. Effect on variations in nutritive density on intake of food of dogs and cats. Physiol. Behave. 15:611-618.
8. Jewell, D.E., and P.W. Toll. 1996. Effects of fiber on food intake in dogs Vet. Clin. Nutr. 3:115-118.
9. Kane, E. 1989. Feeding behavior of the cat. Pp. 147-158 in Walthar Symposium 7 Nutrition of the dog and cat, I.H. Burger, and J.P.W. Revers. Eds. Cambridge: Cambrigeuniversity Press.
10. Kane, E., I. G. Moriss, and Q. R. Rogers. 1981a. Acceptability and digestibility by adult cats of diets made with various source and levels of fat. J. Ani. Sci. 53:1516-1523.
11. Kumazawa, T. , and K. Kurihara. 1990. Large synergism between monosodium glutamate and 5-nucleiotides in canine taste nerve responses. Am. J. Physiol. 253:R420-R426.
12. Lewis L.D., Morrise M.L., Michael S.H., Diet characteristics recommended for dog foods, Feeding dogs and cats.pp: 23-25
13. National academy of sciences. (1974). Nutrient requirement of Dogs, Wasington,D.C.
14. National research council of the national academies.(2006). *Your dog's nutritional needs.* National academic press.
15. Pet food industry, (2013) Electronic reference. Retrieved May 24, 2014 from http://www.petfoodindustry.com
16. Prasad, D., Guide for pet owners and practitioners. CBS Veterinary sciences and Agriculture text. CBS Publisher and distributers. pp: 24-65.
17. The poultry site news desk, Electronic reference. Retrieved March 21, 2014 from http://www.thepoultrysite.com/poultrynews/26172/sri-lanka-chicken-demand-up-in-2011
18. World poultry, Electronic reference. Retrieved March 21, 2014 from http://www.worldpoultry.net

PO-05-14

Effects of "HEBESU" lees as supplemental feed on the meat productivity and meat quality of pigs

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Introduction

The use of natural materials such as citrus depressa squeezed lees as supplemental feed may prove to be ideal. Recently, the use of by-products in feed is a concern of many researchers throughout the world, particularly in Japan. In generally, Miyazaki prefecture, located on the island of Kyushu in the southern part of Japan, "HEBESU" is produced in at Hyuga city, many by-products and remnants of citruses are discarded without benefit during dressing and drink production. In previous studies, we have determined the effectiveness of introducing the litters and the leaves of sweet potatoes to the diet of egg-laying hens. We found that when the amount of sweet potato litters in their diet was increased, the feed intake of the birds increased (Takenoyama et al., 2007). Furthermore, we found that the nutritional quality of the meat of pigs fed sweet potato litters improved, particularly the level of vitamin E content of the meat, which increased significantly in the fat tissues (Takenoyama et al., 2008). These data suggested that this kind of forage may also have significant effects on the productivity of meat animals such as pigs and beef cattle. The purpose of this study was to determine whether "HEBESU" lees could be used as functional forage for pigs. This study investigated the effects of these litters on the growth condition of the pigs, meat productivity, the nutritional quality and nutritional properties of their meat. Meat quality, in this context, included lipid content, vitamin E content and fatty acid compositions, among others.

Materials and Methods

The subjects of this experiment were pigs of a new generation from ternary cross- breeding (Large White × Landrace × Duroc). The pigs were divided into two groups based on diet type. The control group was fed an ordinary diet, while the experimental group was fed an ordinary diet supplemented by the citrus(HEBESU) depressa squeezed dried lees (5%). All of the seeds, skins, fruits and seeds of the citrus, which had been left behind after the first pressing of juice were added to the diet when the animals weighed 70 kg each and the feeding diet was completed when those animals had gained 120kg. The animals were raised in normal environmental conditions at a pigsty in the Kurogi Farm. The animals were subjected to weight gain tests and body size determination before being slaughtered. We examined the growth performance of the animals (including fattening days, daily weight gain, feed intake and feed demand rate, etc.). Then, the animals were slaughtered in a local slaughter house and were measured for their meat productivity. Thereafter, all of the postmortem pigs were subjected to further tests such as carcass traits (carcass weight, carcass yield and back fat thickness, carcass length, loin length, etc.) . The meat quality was determined and nutrient contents were measured. Vitamin E content was determined by the method of Yamauchi et al., (1980). Lipid content and fatty acid compositions were also examined as we described in a previous publication (Takenoyama et al., 1999). In addition, heating loss and toughness of the meat were also measured.

Results and Discussion

The animals found this diet to be highly palatable, as demonstrated by observing the food consumption. As a result, this diet positively affected the growth of the pigs. The number of fatting days and the daily gain weight in the experimental sample were consistent with the control samples. Feed intake in the control group was less than the feed intake of the test animals (Table 1).

Table 1. Effects of "HEBESU" lees as supplemental feed on growth condition of pigs.

Sample	Start Weight	Last Weight	Fattening Days	Feed Intake (kg)	Daily Gain (kg/day)	Feed Demand
Control (n=6)	77.0	118.5	69.2	639.0	0.69	3.85 (kg/kg)
Test (n=7)	71.5	125.5	74.0	799.0	0.73	3.68 (kg/kg)

The diet containing "HEBESU" lees had no impact on the carcass weight, carcass yield or back fat thickness, and so there was no significant difference in meat productivity compared with the control group (data not shown). Feeding with the diet containing "HEBESU" lees result in good growth condition and meat productivity of fattening pigs which were equivalent to those fed the basal diet. Thus, a diet containing "HEBESU" lees was found to be useful as a fattening diet for pigs. From the economic point of view, this reduction in the feed demand could considerably contribute to the financial condition of those who concern about meat production.

From the data of the nutrient composition, the small increase in the lipid content in the loin muscles was insignificant (Figure 1). These data means that the fattening pigs ate feed well, lipid was accumulated to the meat, Therefore, the diet containing "HEBESU" lees was effective in improving meat quality, suggesting the possibility of producing characteristically high quality meat. Fatty acid compositions remained at a normal level without any change (Figure 2). When measuring the effects of the "HEBESU" lees feeding on free amino acid contents in loin were insignificantly increased in the test group (Figure 3). Especially, alanine with the sweetness showed a high price tendency. These data suggested that production of good taste meat. Vitamin E content in the loin meat slightly increased in the test group (Figure 4). The results suggest that the "HEBESU" lees diet is a good tool in improving some nutritional profiles, especially vitamin E. In this regard, the results of this research encourage the use of agricultural industry by-products in the fields of animal nutrition. We examined the sensory evaluation (Figure 5). The level of flavor and juicy were higher test group than control. Furthermore, the level of total assessment was slightly higher test group than control. These data suggested that "HEBESU" lees may also have effects on the productivity of meat animals such as pigs and beef cattle. Therefore this kind of lees can be used instead of any other expensive ingredients that come from overseas.

Conclusions

In this study, we investigated the effects of "HEBESU" lees on the growth of pigs, meat productivity and nutritional quality of the meat to determine whether the citrus depressa squeezed lees can be utilized as functional forage. The pigs were fed an ordinary diet supplemented by HEBESU lees (5%). We examined the growth of the experimental animals within fattening days (daily weight gain, feed intake, feed demand rate) and meat productivity, as well as meat quality (lipid content, vitamin E content, fatty acid compositions, free amino acid contents, sensory evaluation, etc.). The meat of the pigs fed the "HEBESU" lees infused diet was nutritionally improved, particularly in the level of vitamin E content of the meat, which was increased tendency. The small increase in the lipid content in the loin muscles was insignificant. Fatty acid compositions remained at a normal level without any change. When these pigs were fed these lees, their feed demand was decreased. From the economic point of view, this reduction in the feed intake could considerably contribute to the financial condition of those who concern about meat production. Most of the ingredients in the diets that used by these companies are imported from overseas. Therefore this kind of lees can be used instead of any other expensive ingredients that come from overseas.

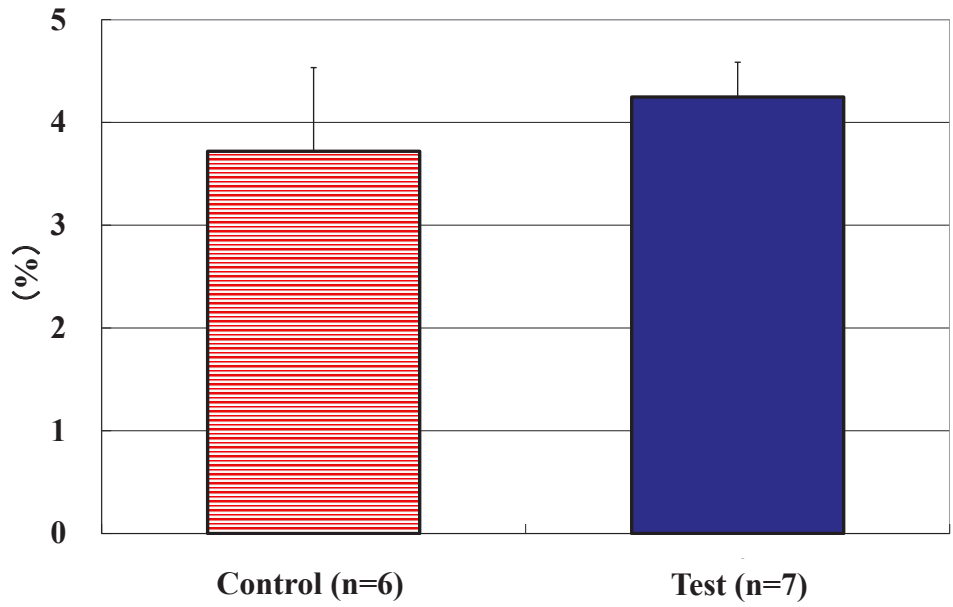

Figure 1. Effects of "HEBESU" lees on lipid contents of loin from fattening pigs.

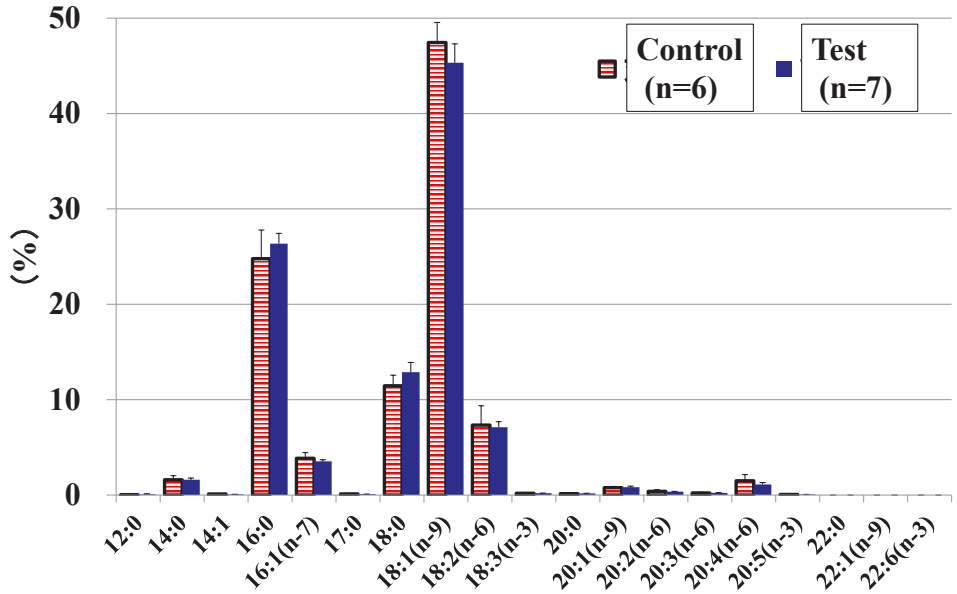

Figure 2. Effects of "HEBESU" lees on fatty acid compositions of loin from fattening pigs.

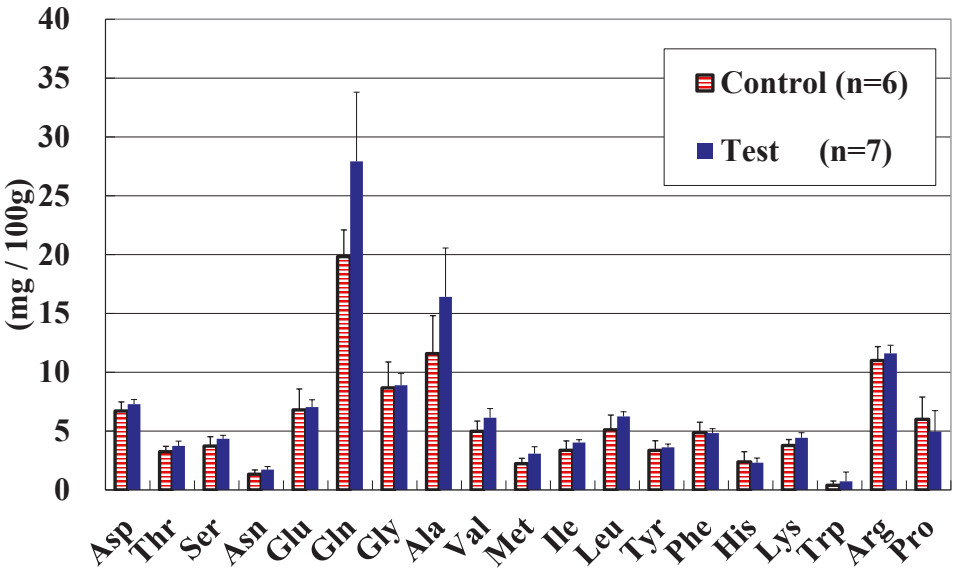

Figure 3. Effects of "HEBESU" lees on free amino acid contents of loin from fattening pigs.

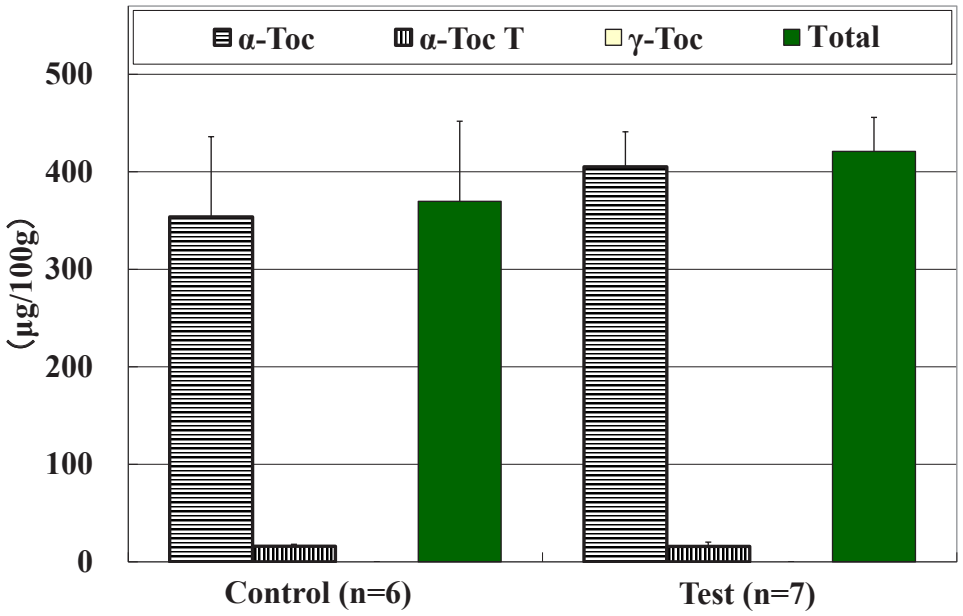

Figure 4. Effects of "HEBESU" lees on vitamin E contents of loin from fattening pigs.

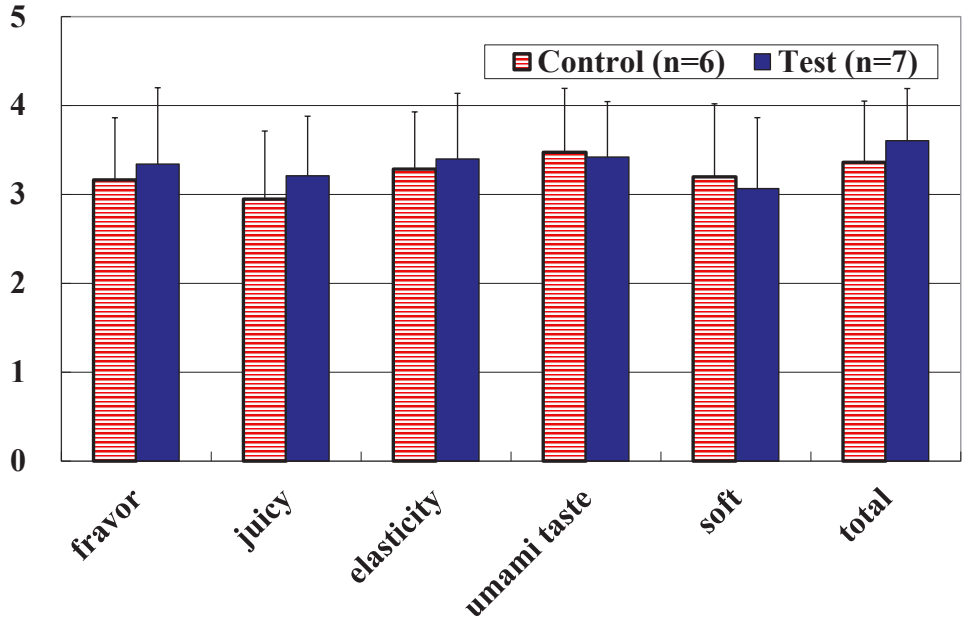

Figure 5. Effects of "HEBESU" lees on sensory evaluations of loin from fattening pigs.

References

1. Takenoyama, S., Kawahara, S., Murata, S., and Yamauchi, K. (1999). Investigation of some preparation procedures of fatty acid methyl esters for capillary gas-liquid chromatographic analysis of conjugated linoleic acid in meat. *Animal Science Journal, 70,* 336-342.

2. Takenoyama, S., Kawahara, S., Hayashi, H., Imoto, T., Nagase, T., Ando, T., Ishiguro, K., Yoshimoto, M., and Muguruma, M. (2007). Effect of sweet potato litters as supplemental feed on egg-laying of hens. *Bulletin of the Faculty of Agriculture, University of Miyazaki, 53,* 93-99.

3. Takenoyama, S., Hayashi, H., Imoto, T,. Kawahara, S., Iwakiri, M,. Kurima, F,. Yoshimoto, M., and Muguruma, M. (2008). Effect of unused parts of sweet potato as supplemental feed on the growth situation, meat productivity and meat quality of pigs. *Nihon Chikusan Gakkaiho, 79,* 491-496.

4. Yamauchi, K., Nagai, Y., and Ohashi, T. (1980). Quantitative relationship between alpha-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in porcine skeletal muscle. *Agricultural and Biological Chemistry, 44,* 1061-1067.

PO-05-15

Carcass Characteristics of Hanwoo Steers by Age at Slaughter and Beef Quality Grades

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Introduction

The end-point at slaughter is causing remarkably conflict between productivity and beef qualities such as tenderness, juiciness and flavor on beef industry in Korea. Therefore, this study was conducted to find the appropriate countermeasures to make the use of the improvement of productivity and the investigation of carcass characteristics on age at slaughter and quality grade of Hanwoo steer.

Materials and Methods

Sample preparation

Hanwoo steers (n=142) of Heongseong Chukhyup that had been slaughtered from November, 2012 to December, 2013 were analyzed for their carcass traits. Carcass records used were cold carcass weight, backfat thickness, longissimus muscle and marbling score. Half carcasses were chilled at 0℃ and 5℃ . After a 24 h chill, carcasses were weighted and cut between the 13th rib and the 1st lumbar vertebrae on the left side to evaluate ribeye muscle area, backfat thickness, marbling score according to the guidelines of the beef grading system of the ministry of agriculture, food and rural affairs, Korea. The quality grade was classified from 5 possible values (1 + + , 1 + , 1, 2, 3) based on marbling score, lean meat color, fat color, firmness, texture of lean meat, and maturity of the exposed longissimus dorsi (LD) muscle at the 13th rib interface(National Livestock Cooperatives Federation, 1998)

Chemical compositions

Moisture content, crude fat and crude ash content were determined using an oven drying method (AOAC, 1995). Crude protein content was obtained through the Kjeldahl method (Cundiff, 1998).

Fatty acid compositions

Total lipids of beef samples were extracted by using chloroform-methanol (2:1, v/v) according to the procedure of Folch et al.(1957). An aliquot of total lipid extract was methylated as described by Morrison and Smith (1964). Fatty acid methyl esters were analyzed by a gas chromatography-FID (Agilent6890, USA) fitted with a fused silica capillary column (100m x 0.25mm x 0.2μm, SP-2560, supelco). The injection port was at 225℃ and the detector was maintained at 260℃ . Results were expressed as percentages based on the total peak area.

Statistical methods

An analysis of variance (ANOVA) was performed on all of the variables measured by the General Linear Model (GLM) procedure using SAS statistical package (SAS, 2002). The Duncan's multiple range tests ($p < 0.05$) were used to determine the statistical significance among the means.

Results

Effect of carcass weight, ribeye area, backfat thickness and marbling by different slaughtering age are shown in Table 1. Within this age interval, age was not a significant source of variation for cold carcass weight, eye muscle area, backfat thickness, or marbling score, with ranges of which were 429.3~462.2kg, 86.0~93.1cm^2, 9.6~13.9mm, and 4.8~6.4point, respectively. The moisture, protein, fat and ash contents of the longissimus muscles from Hanwoo steers by different slaughtering age and by quality grades are presented in Table 2 and Table 3. The moisture content was significantly higher for 28-mon-old (65.21%) steers than that for 34-mon-old (60.20%) steers. There were no significant differences in the protein content between the 7 slaughtering ages ($p>0.05$). Fat content was lower in 28-mon group (12.72%) then that in from 31-mon group (17.13%) to 34-mon group(19.01%) ($p < 0.05$). The ash content of 28-mon-old (0.87%) was highest. In quality grades, the moisture, protein and ash content of the 3 grade was higher ($p < 0.05$) and the fat content lower ($p < 0.05$) compared to the quality grades. The fatty acid mainly in charge for soft fat in Japanese and Korean cattle is oleic acid (18:1n-9)(Smith et al., 2009). The fatty acid compositions (%) of longissimus muscles by slaughtering age and by quality grads are provided in Table 4 and Table 5. There were only oleic acid (C18:1n-9) significantly differed between slaughtering ages

while no significant differences on the other individual fatty acid component of Hanwoo steer. In quality grades of Hanwoo steer, there were no significant difference from oleic acid (C18:1n-9), SFA, UFA and MUFA. However PUFA was significantly higher for the 2 grade than that for the other groups ($p < 0.05$)

Table 1. Effect of carcass traits by different slaughtering age

Slaughtering age (mon)	Carcass weight (kg)	Ribeye area (cm^2)	Backfat thickness (mm)	Marbling (score)
28	433.50±7.15	87.00±0.71	12.75±0.85	4.80±0.37
29	429.32±10.29	87.88±1.83	10.88±0.96	5.63±0.41
30	441.96±6.41	89.34±1.29	10.93±0.71	5.41±0.34
31	436.74±6.43	89.15±1.53	10.28±0.53	6.00±0.29
32	447.73±8.77	86.00±1.72	11.77±0.70	5.81±0.40
33	462.21±12.38	92.43±2.17	13.92±0.61	6.00±0.51
34	434.14±34.29	93.14±5.27	9.57±1.69	6.42±0.90

* Mean±SE

Table 2. Chemical compositions (%) of longissimus muscles of Hanwoo steer beef by different slaughtering age

Slaughtering age (mon)	Crude moisture	Crude protein	Crude fat	Crude ash
28	$65.21±1.15^{a}$	20.01±0.37	$12.72±1.33^{b}$	$0.87±0.01^{a}$
29	$63.07±0.89^{abc}$	20.04±0.31	$15.36±1.13^{ab}$	$0.86±0.01^{ab}$
30	$63.52±0.51^{ab}$	20.37±0.25	$14.54±0.76^{ab}$	$0.86±0.01^{ab}$
31	$61.70±0.60^{bc}$	19.67±0.20	$17.13±0.80^{a}$	$0.83±0.01^{abc}$
32	$61.52±0.73^{bc}$	19.62±0.24	$17.34±0.99^{a}$	$0.84±0.01^{abc}$
33	$60.92±0.97^{bc}$	19.45±0.33	$18.07±1.32^{a}$	$0.81±0.02^{bc}$
34	$60.20±1.92^{c}$	19.17±0.75	$19.01±2.75^{a}$	$0.79±0.03^{c}$

* Mean±SE in the same row with different superscript different significantly ($p<0.05$)

Table 3. Chemical compositions (%) of longissimus muscles of Hanwoo steer by quality grades

Quality grades	Crude moisture	Crude protein	Crude fat	Crude ash
1++	$57.49±0.50^{e}$	$18.19±0.20^{d}$	$23.09±0.68^{a}$	$0.76±0.01^{d}$
1+	$61.47±0.37^{d}$	$19.42±0.13^{c}$	$17.54±0.48^{b}$	$0.83±0.01^{c}$
1	$63.94±0.33^{c}$	$20.51±0.12^{b}$	$13.85±0.39^{c}$	$0.87±0.01^{bc}$
2	$66.50±0.59^{b}$	$21.37±0.20^{b}$	$10.43±0.76^{d}$	$0.89±0.01^{ab}$
3	$69.30±0.62^{a}$	$22.55±0.72^{a}$	$5.87±0.01^{e}$	$0.89±0.04^{a}$

* Mean±SE in the same row with different superscript different significantly ($p<0.05$)

Table 4. Fatty acid compositions (%) of longissimus muscles of Hanwoo steer by different age at slaughter

Slaughtering age (mon)	Oleic acid (C18:1n-9)	SFA	UFA	MUFA	PUFA
28	50.86±1.44 [a]	41.88±1.39	58.12±1.39	56.11±1.41	2.01±0.23
29	48.17±0.54[b]	43.48±0.81	56.52±0.81	54.40±0.77	2.12±0.14
30	51.07±0.41 [a]	41.82±0.45	58.18±0.45	55.99±0.45	2.16±0.13
31	50.90±0.50 [a]	41.67±0.50	58.33±0.50	56.19±0.47	2.14±0.08
32	49.44±0.54 [ab]	43.25±0.55	56.75±0.55	54.60±0.57	2.16±0.10
33	51.39±0.63[a]	41.43±0.74	58.57±0.74	56.56±0.68	2.02±0.13 [ab]
34	51.61±0.77[a]	40.88±0.82	59.12±0.82	57.17±0.83	1.95±0.22

* Mean±SE in the same row with different superscript different significantly ($p<0.05$)

Table 5. Fatty acid compositions (%) of longissimus muscles of Hanwoo steer by quality grades.

Quality grade	Oleic acid (C18:1n-9)	SFA	UFA	MUFA	PUFA
1++	50.35±0.61	42.25±0.63	57.74±0.63	55.81±0.65	1.92±0.09[ab]
1+	50.57±0.38	42.24±0.38	57.76±0.38	55.81±0.37	1.92±0.06[b]
1	50.51±0.40	41.98±0.43	58.02±0.43	55.78±0.42	2.23±0.08[ab]
2	49.28±0.95	42.96±1.00	57.04±1.00	54.44±0.94	2.60±0.25[a]
3	49.28±1.13	41.20±0.27	58.81±0.27	56.37±0.39	2.43±0.66[ab]

* Mean±SE in the same row with different superscript different significantly ($p<0.05$)

References

1. **AOAC. 1995. Official methods of analysis of AOAC. 17th ed. Association of Official Analytical Chemists. Washington D.C.**
2. **Folch, J., Lees, M., and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-500**
3. **Morrison, W. R. and Smith, L. M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5. 600-608.**
4. **SAS. 2002. SAS/STAT User's Guide. V. 9.1 SAS Institute Inc., Cary, NC, USA**
5. **Cundiff, P. A. 1998. Official Methods of Analysis of AOAC International (6th ed.). Association of official Analytical Chemists, Arlington.**

PO-05-19

Diversity of microbiota in vegetable-based and meat-based fermented foods produced in Hue, the middle of Vietnam

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Introduction

Most East-Asian fermented foods are non-dairy products featuring various raw materials such as cereals, soybeans, fruits, and vegetables, as well as fish and other marine products. These foods are produced largely in the household or small scale using local indigenous microbiota, which is different from western countries where fermented foods are industrially produced on a large scale using selected starter cultures.

Many vegetables such as cabbage, eggplant, beansprouts, carrot, bamboo shoot, scallion, and cauliflower can be used as a material for fermentation. Common dishes from the north to south of Vietnam are *dua gia*, *dua cai,* and *mang chua*, which are fermented beansprouts, cabbage, and bamboo shoot respectively. Fermented meat is also a daily dish and *nem chua* is consumed all over Vietnam. The main ingredients include ?nely ground pork lean (55-60%), boiled and sliced pork rind (30-35%), and other ingredients such as ground roasted rice, sugar, salt, and spices. *Tre* is another kind of fermented meat produced and consumed in the middle of Vietnam. In addition to pork lean and rind, ear and nose of the pig are often included in *tre*. Likewise, the meat mixture is subjected to heat treatment (grilling

In this study, three vegetable-based and two meat-based fermented foods were obtained from local markets and households in Hue, a city in the middle of Vietnam, and the LAB diversity was assessed by quantitative real-time PCR and qualitative denaturing gradient gel electrophoresis. Differences in relation to retailers and households were examined.

Materials and methods

Samples of fermented foods

A total of 25 samples with five commodities each of *dua gia* (bean sprouts)*, dua cai* (cabbage)*, mang chua* (bamboo shoot), *nem chua* (uncooked pork), and *tre* (cooked pork) were purchased from 12 shops at six local markets in Hue.

Chemical and microbiota analyses

Dry matter content was determined by drying the materials in an oven at 60°C for 48 h. The lactic acid, short chain fatty acid, and alcohol contents were determined from water extracts using an ion-exclusion polymeric high-performance liquid chromatography method with refractive index detection.

DGGE was performed on a variable (V3) region of the bacterial 16S rRNA gene using the primers and PCR protocols as previously described (Wu and Nishino, 2016). The PCR products were separated according to their sequences by using a DCode Universal Mutation Detection System (Bio-Rad Ltd., Tokyo, Japan). Select bands were excised and extracted DNA was purified by cloning into the pTAC-1 vector. The DNA base was sequenced and BLAST searches were performed against the GenBank database.

Quantification of total bacteria and *Lactobacillus* spp. was performed using Mini Opticon real-time PCR system. Plasmid DNA prepared with 16 S rRNA genes of *Lactobacillus brevis* (JCM 1059^T) was used as standards for qPCR analyses.

Quantitative data were subjected to one-way analysis of variance and Tukey's multiple range test was used to examine the differences between food items. Qualitative DGGE band profiles were converted to a list of binary numbers and principal coordinate (PCoA) analysis was made on the Bray-Curtis similarities matrix.

Results and discussion

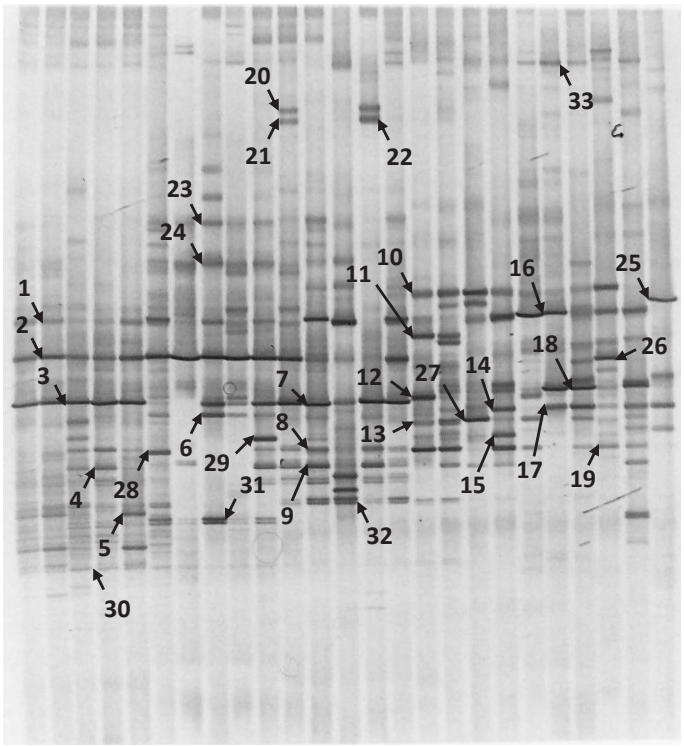
All samples were evaluated as being good based on acidity and fermentation products composition. Dry matter contents of vegetable-based fermented products were about 7 g kg^{-1} and those of meat-based ones were 34-50 g kg^{-1}. *Dua gia* (bean sprouts), *dua cai* (cabbage), and *nem chua* (uncooked pork) had greater contents of lactic

acid than acetic acid, whereas *mang chua* (bamboo shoot) and *tre* (cooked pork) showed the opposite trend. Total bacterial populations were from 1.9 ´ 10^9 to 9.1 ´ 10^9 copies g^{-1} and *Lactobacillus* spp. populations were from 1.5 ´ 10^9 to 7.6 ´ 10^9 copies g^{-1}. *Tre* (cooked pork) had greater LAB numbers than *dua cai* (cabbage) and *mang chua* (bamboo shoot).

The DGGE analysis demonstrated distinctive diversity of microbiota within and between vegetable-based and meat-based fermented foods. In vegetable-based products, bands corresponding to *L. plantarum*, *L. fermentum* and *L. helveticus* were shared by all three food items. Bands for *L. delbrueckii* were clearly seen for *dua cai* (cabbage) and band for *L. pontis* was distinctive for *mang chua* (bamboo shoot). *L. acidophilus*, *L. casei*, and *L. acetotolerans* were occasionally detected in vegetable-based fermented foods.

Band patterns of *nem chua* (uncooked pork) and *tre* (cooked pork) were largely different from those of *dua gia* (bean sprouts), *dua cai* (cabbage), and *mang chua* (bamboo shoot). Common bands for two meat-based products were *P. pentosaceus*, *W. cibaria*, and *L. lactis*, whereas *P. pentosaceus* was more often seen in *nem chua* (uncooked pork) and *W. cibaria* was more distinctive in *tre*. Other LAB species occasionally detected in *nem chua* (uncooked pork) and *tre* (cooked pork) were *L. fallax* and *W. paramensenteroides*. In addition, several non-LAB species, i.e. *Staphylococcus sciuri*, *Acinetobacter gandensis*, and *A. parvus*, were detected exclusively in *nem chua* (uncooked pork).

These results can be good additions to previous findings, because association of *L. helveticus*, *W. cibaria*, and *L. lactis* was newly depicted in this study. Several indigenous LAB species may be used as good starters to assure hygienic quality of *nem chua* (uncooked pork) fermentation, which is regarded unstable and occasionally annoyed with development of undesirable microorganisms.



1 *Lactobacillus plantarum*
2 *Lactobacillus fermentum*
3 *Lactobacillus helveticus*
4 *Lactobacillus acidophilus*
5 *Lactobacillus casei*
6 *Lactobacillus delbrueckii*
7 *Lactobacillus helveticus*
8 *Lactobacillus plantarum*
9 *Lactobacillus pontis*
10 *Pediococcus pentosaceus*
11 *Leuconostoc fallax*
12 *Acinetobacter gandensis*
13 *Lactococcus lactis*
14 *Staphylococcus sciuri*
15 *Acinetobacter parvus*
16 *Weissella cibaria*
17 *Weissella cibaria*
18 *Weissella paramesenteroides*
19 *Lactococcus lactis*
20 *Lactobacillus pontis*
21 *Lactobacillus acetolerans*
22 *Lactobacillus coleohominis/*
23 Uncultured bacterium
24 *Lactobacillus fermentum*
25 *Weissella paramesenteroides*
26 *Weissella cibaria*
27 *Pediococcus pentosaceus*
28 *Lactobacillus fermentum*
29 *Lactobacillus pontis*
30 *Lactobacillus fermentum*
31 *Lactobacillus delbrueckii*
32 *Lactobacillus delbrueckii*
33 *Lactobacillus plantarum*
34 *Lactobacillus plantarum*

Figure 1. Denaturing gradient gel electrophoresis analysis of the microbiota of vegetable-based and meat-based fermented foods produced in the middle of Vietnam.

Table 1. Acidity, concentrations of dry matter, lactic acid, and acetic acid, and real time PCR assessment of total bacteria number, *Lactobacillus* spp. number, and their relative proportion of vegetable-based and meat-based fermented foods produced in the middle of Vietnam.

	Dua gia (bean sprouts)	*Cai chua* (cabbage)	*Mang chua* (bamboo shoot)	*Nem chua* (pork)	*Tre* (cooked pork)
pH	4.33 ± 0.10^{bc}	4.45 ± 0.16^{b}	4.10 ± 0.06^{c}	4.91 ± 0.13^{a}	5.09 ± 0.33^{a}
Dry matter (g kg^{-1})	7.58 ± 0.37^{c}	7.32 ± 1.26^{c}	7.26 ± 1.96^{c}	35.4 ± 1.26^{b}	48.0 ± 6.73^{a}
Lactic acid (g kg^{-1})	4.20 ± 1.18^{bc}	3.02 ± 1.13^{c}	3.92 ± 1.00^{bc}	13.1 ± 2.84^{a}	8.37 ± 4.11^{b}
Acetic acid (g kg^{-1})	0.68 ± 0.40^{c}	2.22 ± 0.77^{bc}	4.46 ± 2.51^{abc}	5.86 ± 3.22^{ab}	9.25 ± 3.85^{a}
Total bacteria (log copies g^{-1})	9.67 ± 0.18^{ab}	9.31 ± 0.17^{b}	9.28 ± 0.36^{b}	9.61 ± 0.17^{ab}	9.96 ± 0.15^{a}
Lactobacillus spp. (log copies g^{-1})	9.63 ± 0.25^{ab}	9.19 ± 0.19^{b}	9.19 ± 0.43^{b}	9.58 ± 0.22^{ab}	9.88 ± 0.19^{a}

Data are means ± standard deviations for five samples of each fermented food. Values in the same row with different superscript letters are significantly different ($p < 0.05$).

PO-05-24

The visiting pattern of cows to an automatic milking unit with a different cow traffic systems

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Rakuno Gakuen University

Introduction

The automatic milking system (AMS) is based on the voluntary visit of cows to an automatic milking unit (AM unit). When cows do not visit the AM unit by themselves (less than two times per day), the manager must fetch the cows and bring them to the AM unit for milking. Fetching operations defeat the purpose of the introduction of AMS, which is to reduce labor.

Kageyama et al. (2003) concluded that the free cow traffic resulted in less visits to the AM unit rather than the forced cow traffic. Other studies also reported that forced cow traffic encouraged more visits to the AM unit (Bach et al. (2009); Ketelaar-de Lauwere et al. (1998)). On the other hands, the daily milking times did not differ between the free and forced cow traffic methods. For frequent milking and efficient use of the AMS, an equalization of hourly use is need.

The objective of this study was to examine the visiting pattern of the cows to the AM unit in an automatic milking system with different cow traffic systems.

Material and Methods

The data of the total milking times and milk production were collected for seven days from 35 commercial AMS dairy farms. And, the data of visiting and milking were collected for seven days from three commercial dairy farms with an automatic milking system, one farm with free cow traffic (FC type, 54 cows, 31 kg milk/day, 2.6 milking times/day), and two farms with forced cow traffic system.

The order of MF type was lying area, selection unit, AM unit, then feeding basic ration area (45 cows, 28 kg milk/day, 2.1 milking times/day). The order of the FF type was lying area, feeding basic ration area, selection unit, then AM unit (51 cows, 38 kg milk/day, 2.6 milking times/day).

For the evaluation of individual difference and equalization of use (milking and refusal) of the AM unit, coefficient of variation (CV) was used. The average of the use of the AM unit were compared using the Kruskal-Wallis test.

Results and discussion

There was no difference in the relationship between the number of visits and the amount of milk production per one AM unit with the different cow traffic system. [Figure 1]

The averages of the visits to the AM unit in the forced cow traffics (MF and FF types) were significantly ($P<0.05$) higher than that in the FC type. The average of the milking of FC type was significantly ($P<0.05$) higher than that in the FF and MF type. [Figure 2]

The individual difference in the FF type was the lowest and was the highest in the FC type. Fifteen percent of cows who visited the AM unit equal to and less than two times per day in the FC type of traffic. There were no cows who visited the AM unit equal to and less than two times in the FF and MF types. When the manager planned for the milking to take place at least twice a day, some cows had to be fetched in the free cow traffic system. [Figure 3]

The average of the hourly times of the visiting to the AM unit was the highest in the FF type and the smallest in the FC type of traffic. The hourly variation of the visiting through 24 hours of the FC type was smaller than that of the forced cow traffic (FF and MF) type. This equalization of the use of the AMS kept the frequent milking times in the FC type, even though the number of visits was the smallest.[Figure 4]

It was concluded that the number of individual visits and visiting patterns of the cows kept in the different traffic system differed. The increasing number of those visiting the AM unit and the decreasing of the individual difference of visiting in the free cow traffic (the motivation for visit), and the equalization of hourly visiting in the forced cow traffic, are need for the improvement of the efficiency of the use of the automatic milking system.

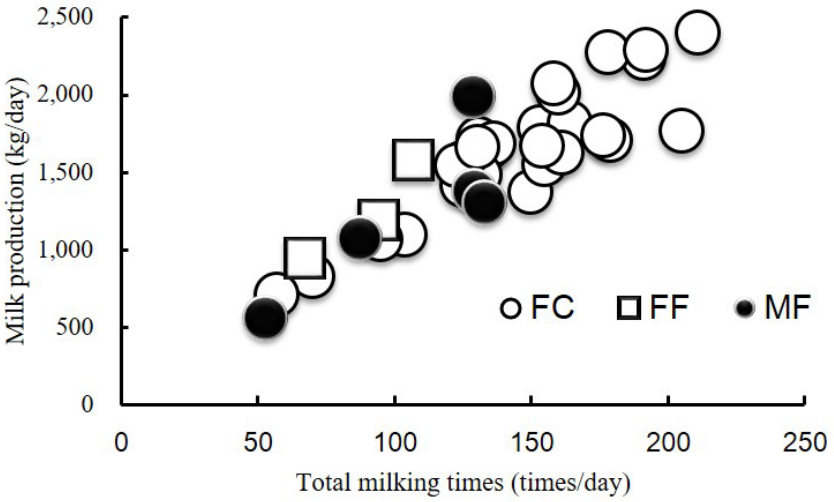


Figure 1. The relationship between the number of total visit and the amount of milk production per AM unit with three cow traffic system.

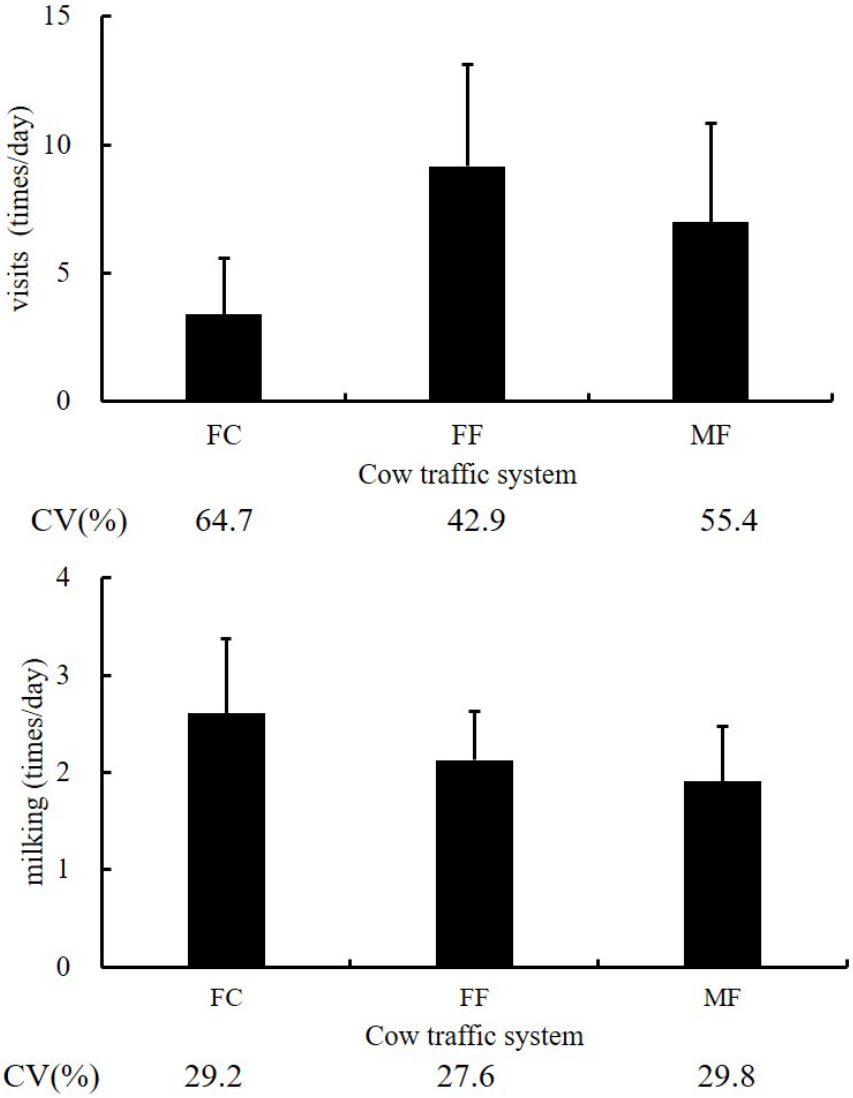


Figure 2. The frequency of visits and milkings with three cow traffic system and individual differences (CV).

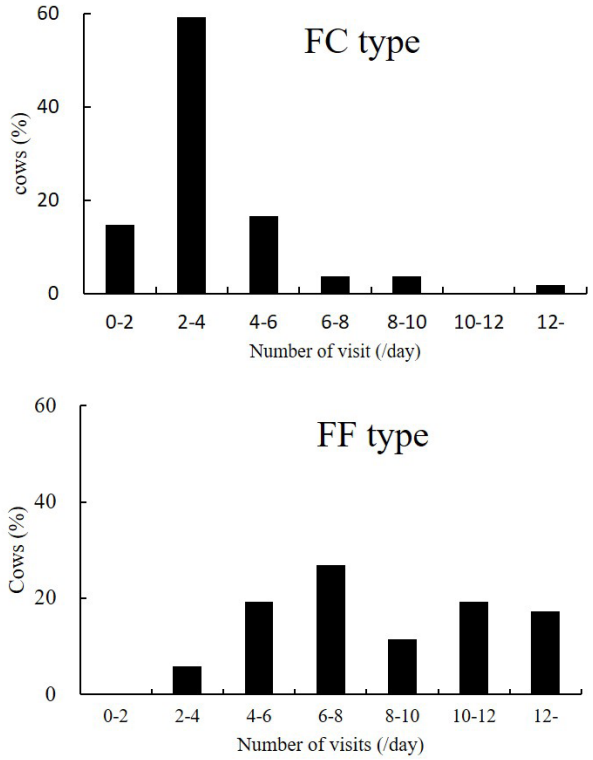
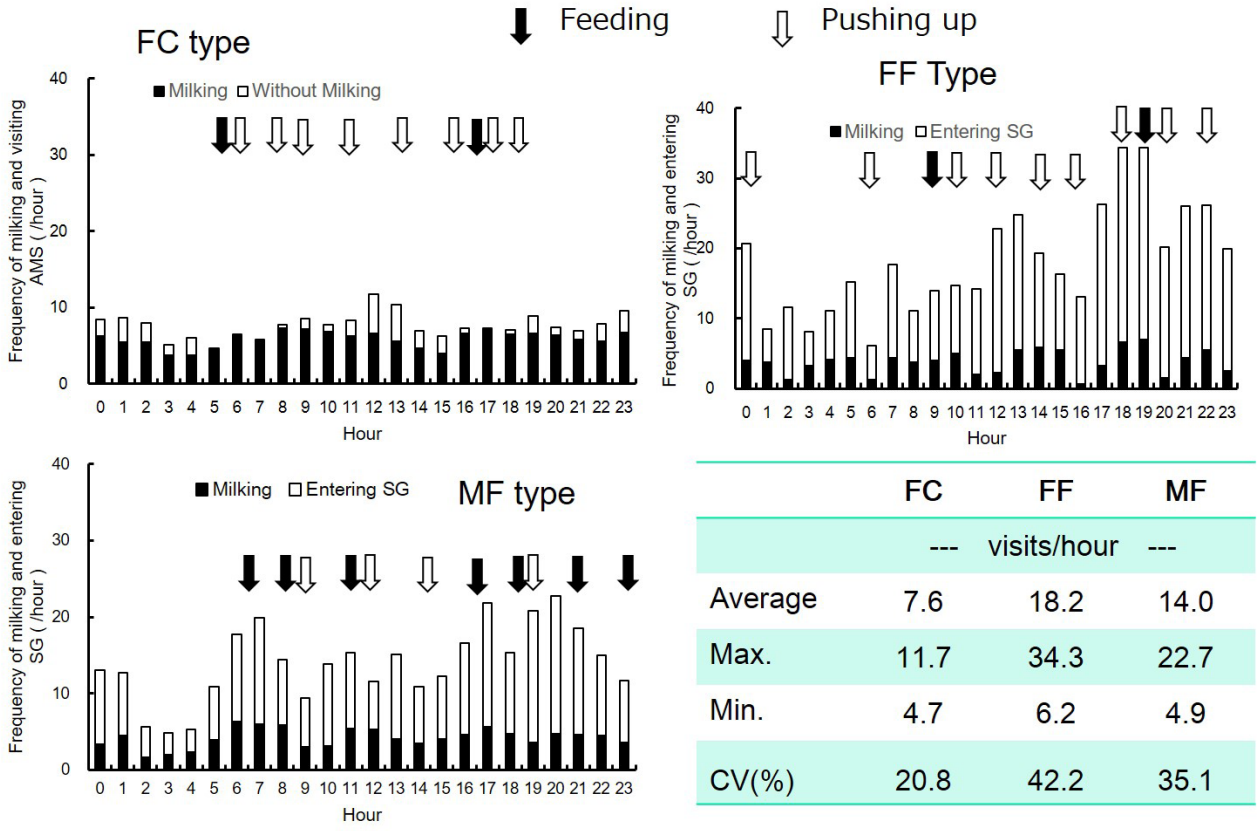


Figure 3. The distribution of the frequency of visits with three cow traffic systems.



	FC	FF	MF
	---	visits/hour	---
Average	7.6	18.2	14.0
Max.	11.7	34.3	22.7
Min.	4.7	6.2	4.9
CV(%)	20.8	42.2	35.1

Figure 4. Diurnal pattern of visiting and milking of cows kept in three cow traffic system

References

Bach A, Devant M, Igleasis C, Ferrer A. 2009. Forced traffic in automatic milking system effectively reduces the need to get cows, but alters eating behavior and does not improve milk yield of dairy cattle. Journal of Dairy Science 92:1272-1280.

Kageyama A, Morita S, Murakami A, Saitou T, Kawakami H, Komiya M, Hoshiba S. 2003. The effect of cow traffic of free-stall housing with an automatic milking system on eating behavior of milking cows. Journal of Rakuno Gakuen University 28:67-72.

Ketelaar-de Lauwere CC, Hendriks, MMWD, Metz JHM, Schouten WGP. 1998. Behaviour of dairy cows under free and forced cow traffic in a simulated automatic milking system environment. Applied Animal Behavioral Science 56:13-28.

PO-05-27

Methane production from rumen and feces of Madura cattle receiving different level feeding

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ABSTRACT

Most of studies aimed to reducing methane production from enteric fermentation by modifying feeds and feeding. However, the methane production from feces resulting that modified feeding has never been evaluated. This study was done by allowing 12 heads of 1.5-2.0 y.o. Madura cattle with averaged body weight (BW) of 154 + /- 11.6 kg to three levels feeding, i.e. 2.5, 3.0, and 3.5%BW, respectively. The feeding was formulated to give crude protein 12.9% and total digestible nutrients 58.6%. Parameter measured was dry matter intake (DMI), BW gain, and methane production from rumen (feed) and feces. Methane production from ruminal digestion was measured by facemask method for 3x24 h, while methane production from feces was measured on 10 kg fresh wt from 3-d collection using methane analyzer and lasted when methane was similar to the level of methane at open air. The results showed that level feeding of 2.5, 3.0, 3.5%BW increased DMI ($P<0.05$), but failed to significantly increased BW gain. Increasing feeding level tended to increase daily methane production from ruminal fermentation ($P=0.09$) and from feces. Increasing feeding level at 3.5%BW reduced ($P<0.05$) methane production per unit of feed, but increased methane production per unit feces. This study concluded that increasing feeding level may increase total methane production from feed and feces, but could reduce methane production per kg DMI.

INTRODUCTION

Madura cattle is known for custom events such as karapansapi (bull-races). Up to now, most data reported that Madura cattle raised in traditional farm was low, ranged from 0.23 to 0.47 kg per day (Harmadji, 1993). This is caused by poor feeding given by the farmers either in quantity and quality. However, the study by Umar et al. (2015) showed that Madura cattle has high productivity as proven by average daily gain can reached 0.8 kg per day.

The effort to improve productivity could be done by increasing feed intake, by means of feeding level. Different levels diet will affect daily intake, because the availability of diet will suggest the cattle to eat. The higher feed intake will increase ruminal outflow rate. The higher of ruminal outflow rate can decrease the existence of feed in rumen. Increasing feeding level will increase methane production from ruminal fermentation, but may reduce the digestibility and resulted to higher organic matter in feces. The higher organic matter in feces will result the higher methane production from feces (or manure). Up to now, most studies merely evaluated methane production from enteric fermentation by modifying feeds and feeding (Hammond et al., 2014; Lima et al., 2011; Cao et al., 2010), but methane production from feces is limited. The purpose of this study was aimed to evaluate the methane production from ruminal fermentation and from feces in Madura cattle at different levels diet.

MATERIALS AND METHODS

Materials

Twelve male Madura cattle weighing 154 ± 11.61 kg (CV = 7.54%), aged 1.5-2 years old were used in this study. The cattle were divided into three groups each group contained four cattle and fed different feeding levels. First group of cattle was given 2.5% of body weight (2.5BW), second group was given 3% of BW (3.0BW) and the third group was given 3.5% of BW (3.5BW). The feed given was contained 12.87% of crude protein (CP) and 58.63% of total digestible nutrients (TDN) which was given twice a day at 08.00 and 16.00.

Parameters measurement

Methane emission from rumen fermentation was measured using facemask method (Kawashima et al., 2001) for two days (2x24 h) using a mask which is connected to a methane analyzer (Horiba Ltd., Jepang) and airflow meter. Methane emissions were recorded automatically by IBM PC for 10 minute at 3 hours intervals. The results obtained are converted into weight units (1 liter CH_4 = 0.714 g).

Methane production from feces was measured on 10 kg fresh wt from 3-days fecal collection using methane

analyzer. Feces were placed in container which is veiled by transparent cover so that sunlight can penetrate the feces. The cover is not completely veiled the feces in order to give a aerobic condition (the condition when the feces was thrown away in daily operation). Daily methane measurement was carried out at 3 hours interval, until the end when methane was similar to the level of methane at open air. An overview of equipment is illustrated at Figure 1.

Data analysis

The data were analyzed by analysis of variance (F-test). If there was a significant difference among treatments, the further test by Duncan multiple range test was carried out.

RESULT AND DISCUSSION

Data of the effects of different feeding levels on chewing activity are presented in Table 1. The results showed that the dry matter intake (DMI) was significantly different among treatments ($P < 0.01$). The observed differences in dry matter intake were attributed to the treatments. However, the daily body weight gain was found similar, being ranged at 0.67-0.80 kg per d.

Methane production from rumen fermentation was tend to different among the treatment ($P<0.096$), while from feces were not different among the treatments. Methane from feces was ranged at 1.39 – 2.17 ng/d, much lower than from rumen and only ranged at 0.00015- 0.00023% of methane from rumen. This value was much lower than methane production from dairy manure reported by (Amon et al., 2006), being 125 - 166 nano liter (nL) CH4 per kg Volatile Solid or equal to 90-120 ng. This lower was considered as an aerobic condition in this study, while in Amon et al. (2006) was carried out in anaerobic conditions. Methanogenic bacteria is anaerobic bacteria involves the degradation and stabilization of organic materials under anaerobic conditions and leads to the formation of biogas (a mixture of carbon dioxide and methane) (Kelleher et al., 2000).

CONCLUSION

This study concluded that methane production from feces under open-air (aerobic condition) was found very low and seems could be ignored if compared to methane production from ruminal fermentation.

keywords: methane production, feces, feedinglevel, cattle.

REFERENCES

Amon, Th., B. Amon, V. Kryvoruchko, V. Bodiroza, E. Potsch, and W. Zollitsch. 2006. Optimising methane yield from anaerobic digestion of manure: Effects of dairy systems and of glycerine supplementation. International Congress Series 1293, 217 – 220

Cao, Y., T. Takahashi, K. Horiguchi, N. Yoshida, Y. Cai. 2010. Methane emissions from sheep fed fermented or non-fermented total mixed ration containing whole-crop rice and rice bran. Animal Feed Science and Technology 157: 72–78

Hammond, K.J., D. Pacheco, J.L. Burke, J.P. Koolaard, S. Muetzel, G.C. Waghorn. 2014. The effects of fresh forages and feed intake level on digesta kinetics and enteric methane emissions from sheep. Animal Feed Science and Technology 193: 32–43

Kawashima, T., W. Sumamal, F. Terada, and M. Shibata. 2001. Respiration trial system using ventilated flow-through method with facemask. JJIRCAS Journal 9: 53-74.

Kelleher, B.P., J.J. Leahy, A.M. Henihan, T.F. O'Dwyer, D. Sutton, M.J. Leahy. 2000. Advances in poultry litter disposal technology – a review. Bioresour. Technol. 83, 27–36.

Lima, R., R.F. Díaz, A. Castro, V. Fievez. 2011. Digestibility, methane production and nitrogen balance in sheep fed ensiled or fresh mixtures of sorghum–soybean forage. Livestock Science 141: 36–46

Umar, M., B. Kurnadi, E. Rianto, E. Pangestu. 2015. The Effect of Energy Level of Feeding on Daily Gain, Bood Glucose, and Urea on Madura cattle. J. Indonesian Trop. Anim. Agric. 40(3):159-166

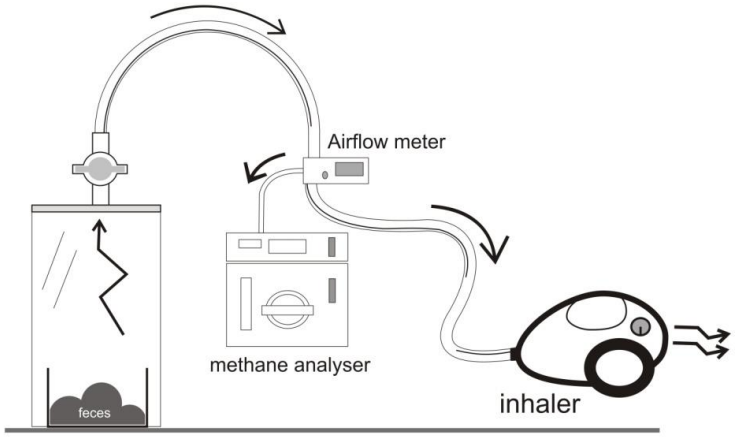

Figure 1. Illustration of methane measurement from feces

Table 1.Methane production from ruminal fermentation and from feces.

Parameters	Treatments			P value
	2.5BW	3.0BW	3.5BW	
Dry Matter Intake, kg/day	4.36 C	5.44 B	6.24 A	0.007
Body weight gain, kg/d	0.67	0.75	0.80	0.542
Dry matter digestibility, %	56.78	54.10	53.00	0.552
Feces excretion, kg/d	2.11	2.84	3.26	0.402
Methane production				
Ruminal methane, g/d	93.74	121.01	94.26	0.096
Fecal methane, ng/d*	1.39	2.17	2.07	0.861

*) ng= nano gram

PO-05-28

Greenhouse gas emissions and manure management from dairy production in Thailand

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ABSTRACT

Greenhouse gas emissions inventories provide a baseline to develop mitigation projects for reducing emission. However, a detailed inventory of livestock gas emission is not suitable for Thailand. This study attempts to fill this gap. The methodology selected comes from the 2006 intergovernmental Panelon Climate Change (IPCC) guidelines to quantify emission from dairy production. Tier2 methodology was implemented using dairy population in 2015, analyzed comparing between manure management ratio of year 2006 and 2015. First of all, EF for manure management were analyzed by tier 2 method. The manure management from dairy production by biogas, solid, pasture and daily spread were 5 , 10 , 75, and 10 % in 2006 respectively, as well as were changed to 0, 65, 25, and 10% in 2015 respectively. The total GHG emissions (enteric methane, nitrous oxide, and manure methane) of 2006 and 2015 were reducing from 0.644 to 0.584 mt.CO_{2eq}/year. Manure management can reduce GHG emissions by reducing nitrous oxide and manure methane. Finally, manure management can be improve to sustainable production system. In part of biogas, it can substitute methane for light and cooking and generate electricity. It is necessary to extend this technology in dairy farm and other animal production for mitigation option of GHG emission in Thailand.

INTRODUCTION

The FAO report *"Livestock long shadow: environmental issues and options"* (FAO, 2006) claims that livestock production is a major contributor to the world's environmental problems. In consequence of these trends, increasing volumes of livestock manure are produced, which are a source of greenhouse gases (GHGs) contributing to global anthropogenic GHG emission, although highly variable across the world (Adrian et al.,2010). Using a life cycle approach, the relative contribution of global livestock production to anthropogenic GHG emissions was estimated to be 18% (Steinfeld et al., 2006) Recycling and management of organic residues, especially manure is critical for the sustainability of agricultural production. To avoid the exchange of environmental as well as economic issues of waste management must be considered from the perspective of integrated farming. Individual manure management is linked to assess the environmental impact and economic effects of mitigation measures. In this study, it is focused on the calculation of greenhouse gas (GHG) from dairy cattle production in Thailand, comparing between manure management from dairy production by biogas, solid, pasture and daily spread were 5, 10, 75, and 10 % in 2006 respectively, as well as were changed to 0, 65, 25, and 10% in 2015 respectively.

MATERIALS AND METHODS

This study focuses on greenhouse gas (GHG) emissions from dairy cattle production in Thailand. The data was implemented using dairy population in 2015, analyzed comparing between manure management ratio of year 2006 and 2015. The population size of dairy in Thailand was 509,524 heads. (DLD,2015) There are two systems in operation, farm management were 35% intensive farm and 65 % semi-intensive farm

Data collection were separated into 2 parts:

Primary data was collected by interviewingSecondary data as relevant information from research papers, book, etc.The data were analyzed for greenhouse gas emissions according to the Intergovernmental Panel on Climate Change (IPCC, 2006) using Trier 2 method. In Tire 2 estimation, the population is used as annual average population(AAP). The manure management from dairy production by biogas, solid, pasture and daily spread were 5 , 10 , 75, and 10 % in 2006 respectively, as well as were changed to 0, 65, 25, and 10% in 2015 respectively. Manure management of dairy cattle production Thailand is presented in Table 1.

Table 1 Manure management of dairy cattle production in Thailand

Year	Production system	Manure management			
		Biogas	Solid	Pasture	Daily spread
2006	Semi-intensive 65%	0%	65%	25%	10%
	Intensive 35 %				
2015	Semi-intensive 65%	5%	10%	75%	10%
	Intensive 35 %				

RESULTS AND DISCUSSION

Dairy cattle sub category in Thailand and production system

Total dairy cattle were two systems in operation, farm management were intensive farm and semi-intensive farm. Most of the dairy in Thailand, are reared on smallholder and medium farms. The farmers produced organic compost from dairy cattle manure to replace chemical fertilizers in their crop land. (Table 2)

Table 2 Dairy cattle sub category in Thailand

Production system	Manure management	Age group
Semi-intensive (65%)	Biogas	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Solid	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Pasture	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Daily spread	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
Intensive (35 %)	Biogas	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Solid	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Pasture	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow
	Daily spread	Male 0-12 m, Female 0-12 m,12-27m, Milking cow, Dry cow

Country-specific Tier 2

The number of dairy cattle was 509,524 heads, the population is used as annual average population(AAP). In Tier 2 estimation, sub categories were separated by age group production system. Average body weight gain, total energy intake and estimated methane emission factor of dairy cattle in Thailand is presented in Table 3. Estimated enteric methane emission factors of dairy cattle is a wide range from 17.21 to 109.97($kgCH_4$/head/year).

Table 3 Estimated enteric methane emission factors of dairy cattle

Production system	Age group		Average weight (kg)	Estimated emission factor ($kgCH_4$/head/year)
Semi-intensive (65%)	Male	0-12 m.	87.50	24.82
	Female	0-12 m.	87.50	24.82
	Female	12-27 m.	225.00	66.10
	Female	Milking Cow	350.00	109.97
	Female	Dry Cow	300.00	66.94
Intensive (35 %)	Male	0-12 m.	96.25	17.21
	Female	0-12 m.	96.25	17.21
	Female	12-27 m.	247.50	45.80
	Female	Milking Cow	385.00	80.27
	Female	Dry Cow	330.00	45.75

CH_4 and N_2O emission from manure : Trier 2 emission estimates

Dairy cattle sub categories were separated by age, average body weight, weight gain, total energy intake, farming

system and manure management. Estimated of emission factor is considered to feed quality and production system. Greenhouse gases emission of dairy cattle production, main sources of emissions are enteric CH_4 and manure CH_4. The result show that manure management can reduce greenhouse gases emissions from 0.644 to 0.584 Mt.CO2eq/year in 2006 and 2015 respectively. Greenhouse gases emissions were reduced by biogas and pasture. In 2006, manure methane and nitrous oxide were 0.09 and 0.02 respectively as well as were changed to 0.05 and 0.004 $MtCO_2eq$/year in 2015 respectively. (Table 4)

Table 4 Greenhouse gases emission of dairy cattle production in Thailand

	Greenhouse gases ($MtCO_2eq$/year)			
Year	Enteric CH_4	Manure CH_4	N_2O	Total
2006	0.53 (82.57%)	0.09 (13.76%)	0.024 (3.67%)	0.644
2015	0.53 (90.91%)	0.05 (8.42%)	0.004 (0.67%)	0.584

CH_4 emissions may occur from all manure environments, but is mainly associated with liquid or compacted manure (Osada et al., 2000). Methanogenes is occurs only under strictly anaerobic conditions where it is coupled to other processes involved in the breakdown of manure organic matter (Valentine, 2007). The microbial ecology of manure environments is critically important for proper estimation of greenhouse gases emissions from manure management, and for efforts to predict effects of management changes and develop greenhouse gases mitigation strategies. Greenhouse gases emissions tend to be higher for solid manure based systems. At all stages, management has an impact on greenhouse gases emissions.

There were evident of manure management from dairy production by biogas, solid, pasture and daily spread ratio that importance contributing to greenhouse gases emission. The analyzed show that greatest reductions in greenhouse gases emissions would be achieved by manure management to reduce manure CH_4 and N_2O. Finally, manure management can be improve to sustainable production system.

CONCLUSION

The opportunities to reduce Greenhouse gases emissions would be achieved by manure management. In part of biogas, it can substitute methane for light and cooking and generate electricity. It is necessary to extend this technology in dairy farm and other animal production for mitigation option of Greenhouse gases emission in Thailand.

Keywords :Dairy,Manure management,Greenhouse gas

REFERENCES

Adrian Leipl, Franz Weissl, Tom Wassenaar2,4, Ignacio Perez3,5, Thomas Fellmann3, Phillipp Loudjani2, Francesco Tubiello2, David Grandgiarad2,6, Suvi Monni1,7, Katarzyna Bial1,8. 2010. Evaluation of the livestock sector's contribution to the EU greenhouse gas emissions(GGELS)-final report. European Commission, Joint Research Centre.

DLD. 2015. Livestock Statistic. Available :http://ict.dld.go.th/th2/index.php/th/report/13-statistic.

FAO. 2006. Livestock long shadow: environmental issues and options. Food and Agriculture Organization of the United Nations, Rome, Italy.

IPCC. 2006. 2006. IPCC Guidelines for National Greenhouse Gas Inventories. Available :http://www.ipcc-nggip.iges.or.jp/public/2006gl/, July 15, 2015.

Osada T, K. Kuroda, and M. Yonaga. 2000. Determination of nitrous oxide, methane, and ammonia emissions from a swine waste composting process. Journal of Material Cycles and Waste Management 2, 51–56.

Steinfield H.P. Gerber, T. Wassenaar, V. Castel, M. Rosales, and C. de Haan. 2006. Livestock's long shadow : environmental issues and options. Food and Agriculture Organization, Rome, Italy.

Valentine D,L 2007. Adaptations to energy stress dictate the ecology of the archaea. Nature Reviews – Microbiology 5, 316–323.

PO-05-30 Chemical Composition of Litter of Thai Wild Boar Kept In A Deep — Litter Pig Production System.

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Objective

In Thailand, Thai wild boars (*Sus scrofa jubatus*) have unique characteristics with a wide range of body weight, elongated tough head and tough snout used for digging and a smaller rear part with thin legs and hooves, narrow face, small ears and big black eyes. Their piglets are light brown with lighter stripes across bodies and the bristly hair and the color will become black or brown in the adult (Tanomtong et al., 2007). By a large, Wild boars, usually rooting around national parks and wildlife sanctuaries, and often expand their boundaries to human neighborhood's gardens to find their own favors on food and plants (Chhanggani & Mohnot, 2004) Whereas, Prapruetdee (2013) reported that crossbred pigs (Largewhite-Landrace x Duroc) fed only 50% commercial diet and 50% grasses/native vegetables, kept in a deep - litter pig production as a low cost pig production system, using indigenous microbial mixture in drinking water and sprayed on rice hull as bedding material, had the same growth performance as conventional concrete based house that fed 100% commercial diet. But, Prapruetdee (2014) that used bedding materials with 10 parts of rice hulls and 1 part of soil, then topping by salts about 500 g and addition of a bacterial mixture as 30 cm height layer and repeated 2 times to completed 90 cm. height, the results of chemical composition of litter bedding, using rice hull as a main content, demonstrated that litter from the deep – litter production at totally 90 cm depth could not be defined as organic fertilizer because some of its contents (organic matter and total nitrogen) were not concordant with the specification of Thai Department of Agriculture.
The objective of this study was to investigate decayed litter after keeping Thai wild boars (*Sus scrofa jubatus*) in a deep - litter pig production system, with different bedding practice from the original practice (Prapruetdee, 2014) for its chemical composition.

Methodology

Ten wild boar piglets (6 males and 4 females) were transported from their natural free range habitationsat 4 months of age to adjust to a deep litter pig house. At the beginning of the study at 5 months of age, at 11.51 kg of average body weight, were placed into a deep - litter house, filled bedding materials with 5 parts of coconut coir, 5 parts of coffee hulls and 1 part of soil and topping by salts about 500 g and addition of a bacterial mixture as 30 cm height layer and repeated 2 times to completed 60 cm. and 90 cm. height, during the latter stage of completed decayed litter. Wild boars received commercial diet and local roughage (20/80). Body weights were measured every 21 days and feed intakes were observed daily through the study. The observation collected from the beginning to the end of the first phase of the experiment at 365 days of age (Yearling). Decayed litter after finishing pigs in the deep - litter house were randomly collected for 12 points (1 kg/point) from every single layer (a layer depth is 30 cm). The chemical composition of litter were obtained from the percentage of organic matter, total N, total P_2O_5, total K_2O, total CaO, total MgO, pH, C/N Ratio and EC, using the standard analysis.

Results and discussions

The results of chemical composition of litter bedding, using filled bedding materials with 5 parts of coconut coir, 5 parts of coffee hulls and 1 part of soil and topping by salts about 500 g and addition of a bacterial mixture as 30 cm height layer and repeated 2 times to completed 60 cm. and 90 cm. height, during the latter stage of completed decayed litter, showed that all pH, EC, Summation of total N, P_2O_5, and K_2O, and C/N Ratio (**Table 1**) could be concordant with the specification of the department of agriculture of Thailand, except organic matter (less than 30%). According to the ratio of commercial diet and local roughage (20/80), wild boarsconsumingvery low concentrate meal, resulting in avery low organic matter and low total N from collected litter samples. Therefore, It is concluded that decayed litter after keeping Thai wild boarsin a deep - litter pig production system, with different bedding practices from the original practices (Prapruetdee, 2014), could not be defined as organic fertilizer.

Table 1. Chemical composition of litter of Thai wild boars kept in a deep – litter pig production system.

Source	Chemical composition								
	pH (%)	EC dS/m	Organic Matter (%)	total N (%)	total P_2O_5 (%)	total K_2O (%)	total CaO (%)	total MgO (%)	C/N Ratio (%)
Deep – litter pig system	8.92	3.29	12.88	0.54	0.86	1.70	1.02	0.40	13.93 : 1
				Summation of these three compositions = 3.10					
Department of agriculture of Thailand (standard)	5.5 – 8.5	≤ 6	≥ 30	≥ 1.0	≥ 0.5	≥ 0.5	Not specified	Not specified	< 20 : 1
				Or summation of these three compositions ≥ 2.0					

References

Chhanggani, A. K and Mohnot. 2004 Crop rapiding by wild boars (Sus scrofa) in and around aravilli, and its management in Rajastan, India. Tigerpaper. 31(2): 385 - 389

Prapruetdee, P. 2013. Conventional and deep-litter pig production system: the effects on growth performance. Proceedings of the 4th international conference on sustainable animal agriculture for developing countries. 27 - 31 July, 2013, Lanzhou, China. 396 – 397.

Prapruetdee, P. 2014. Chemical composition of litter in a deep – litter pig production system. Proceeding of the 16th AAAP Congress. 10 - 14 November, 2014. Yogyakarta, Indonesia. 2682 – 2683.

Tanomtong, A., Supanuam, P., Siripiyasing, P. and Bunjonrat, R. 2007. A comparative chromosome analysis of Thai wild boar (Sus scrofa jubatus) and relationship to domestic pig (*S. s. domestica*) by conventional staining, G-banding and high-resolution technique Songklanakarin J. Sci. Technol. 29(1) : 1-13

PO-05-35

Evaluation of the efficacy of image processing to detect mounting estrus in cattle

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ABSTRACT

Mounting movement of the cattle is one of the typical sign to find the estrous cow. It is well known that proper detection of the estrous cow is the key factor to implement artificial insemination efficiently. Although observations of the animals have been carried out to detect estrous cow, demanding observation of the animals became difficult because it requires too much cost, time and cause variable accuracy with the increasing of animals in number. Thus, establishing automated activity monitoring system in the farm is an important issue for the intensive farm management. The aim of this study was to evaluate the efficacy of image processing technology to detect mounting movement of the cattle. There are some reports focusing on the automated monitoring system to detect estrous in cattle. Most of the methods developed need wearable tools such as pedometer or activity sensor which is invasive to the animals. In this study, mounting behavior was monitored by video recorder, and data obtained were analyzed by the image processing method. Cattle behavior was recorded using time lapse technique. The behaviors of the animals were quantified and analyzed as follows. Input image data were converted into a gray image, and background image was estimated by using updated background images to calculate background difference. Background difference was obtained by subtracting uploaded background images from current image. Image region of the cow were detected by subtracting updated background after noise was removed by using image labeling and morphology processing. Twenty-eight out of 35 cases were detected correctly, and accuracy rate of detecting mounting movement of the animals were 80 % in this study. In conclusion, new automatic detection system of mounting movement of the animals was developed by using image processing technology. Further studies are needed to improve the accuracy of detection.

INTRODUCTION

Proper detection of estrus to optimize appropriate timing of artificial insemination is one of the important point in attaining high conception rate in cattle. Inefficient detection of estrus leads to failure of conception, damages on the reproductive performance and causes economic loss for the livestock farming. As the cattle on heat show many types of estrus behavior such as walking around, separation from the herd, frequent urination, bawling, chin resting, licking, sniffing, flehmen's reaction and so on, mounting movement is one of the typical sign to find the estrous cow. Cows in the early stage of estrus show mounting movement to the other cows and cows in the late stage of estrus show immobile standing, which indicates that the cow is definitely on heat, for the other cows mounting to her. Detecting mounting movement and standing estrus, which is traditionally done by observing changes in cow behavior, is labor intensive and time consuming method, and result of fertility depends on the skill or experience of the observation. It requires too much cost for a large scale livestock farm which is increasing in number in Japan. Although many techniques for heat detection such as measurement of vaginal pH, progesterone in the milk, hormonal synchronization, monitoring of ovarian status by ultrasonography, etc. have been developed (Rao et al., 2013), more accurate, cost-effective and laborsaving methods are still needed. Thus, methods for automating the process of monitoring the animal behavior have become increasingly important recently. Although there are some reports focusing on the automated monitoring system to detect estrous in cattle, most of the methods developed need wearable tools such as pedometer or activity sensor which may be invasive to the animals (Brehme et al., 2008, Okada et al., 2012). As the method with video sensor is non-invasive and avoids handling of the sensors, image processing technologies have been implemented for livestock management such as body weight estimation, lameness detection (Okada et al., 2012) and identification in cattle (Hyeon et al., 2005). The aim of this study was to evaluate the efficacy of image processing technology to detect mounting movement of the cattle.

MATERIALS AND METHODS

A total of 27 Japanese Black cows (7.2 ± 0.6 parity, mean ± SEM) raised at Sumiyoshi livestock station (an experimental farm of University of Miyazaki) were enrolled in the experiment in 2015. All of the protocols were approved by the Institutional Review Board for animal experiments of the University of Miyazaki. Animals were pastured from 09:30 to 15:30 daytime and kept in a freestall barn from 15:30 to 09:30 during the experiment. Concentrates that meet the demands of cows were also provided. The observed field was 26×23m^2 in a freestall barn, and mounting behavior was monitored by a video recorder (CS-W80HD, PLANEX COMMUNICATIONS Inc.). The recorder was installed horizontally at the heights of 1.8 meters above the ground. Cattle behavior was recorded using time lapse technique.

Data obtained were analyzed by the image processing method. The flow diagram was shown in Figure 1. One of the major procedures in a background subtraction algorithm consisted from preprocessing, background modeling, foreground detection and data validation were used in this study (Sen-Ching S. 2004). Features of the animals in each video frame were detected by comparing current image to the calculated background image. Five image data per second were recorded, and calculated values from previous 200 image data were used for background modeling in this study. Background image was uploaded in every 5minutes to adjust changes in illumination, to reduce the noise, and to avoid detecting non-stationary background objects such as swinging leaves, rain, birds and shadow cast in the environment. Background difference was obtained by subtracting uploaded background images from current image. Pixels in the current frame that deviate significantly from the background are considered to be moving objects. Input image data were converted into a gray scale image to minimize the time for calculations of the image. Smoothing and recursive approximated median filter method were used to reduce the noise in the frame. Labeling methods were used to extract candidate foreground pixels from the input frame to identify the position and feature of the animals.

Mounting movements of the estrus cows were detected when the foreground moving objects were extracted over the threshold height line in each video frame. Each frame was divided into 4 regions; near, middle, far and sky regions depending on the area in the frame, as the indicator for estimating distance of the object from the video recorder. Distance of the foreground object from the video recorder was decided depending on the position and size of the foreground moving object in the frame. Three threshold height lines corresponding to the distance of the object were set in each frame. The procedure was conducted using a PC and Matlab R2015a software. To evaluate the performance of this algorithm, all the mounting movements of the cows were checked by observing recorded video data.

RESULTS AND DISCUSSION

The rate of detection of mounting movement of the cows are shown in Table 1. Twenty-eight out of 35 cases of mounting movement confirmed by visual observation were detected by this algorithm correctly, and accuracy rate of detecting mounting movement of the animals were 80 % (near; 67%, middle; 89%, far; 73%) in this study. Although there was no false positive detection, there were some false negative cases especially near and far distance areas from the recorder. In the cases of false negative, there were some mounting cows moved out from the frame could not be detected as mounting cows. Some mounting behavior could not be detected at near and far areas because of reflection or shadow cast of the sunshine in the morning or evening time. Some cases were not detected because of an occlusion caused by some trees. As only a single video recorder was installed in this study, multiple recorder system will be useful to reduce the false negative cases. Some researchers have been developed methods such as using radiotelemetry (Xu et al., 1998), electronic heat-mount detector, electronic activity tag (At-Taras et al., 2001), pedometer (Brehme et al., 2008, Okada et al., 2012), activity meter (Jonsson et al., 2011), video recording and automated analysis (Firk et al., 2002, Choi et al., 2014) to detect estrous cows automatically. However, we need to develop another accurate and economic solution to detect the activity of the animals as all these methods have some disadvantages for the livestock animals and farmers. Also, as the efficiency and accuracy is critical in the automated monitoring systems, improvement of detection of foreground object and estrous cows are strongly needed. In conclusion, we proposed an automatic detection system for mounting movement of the animals in a relatively wide space by using image data from a single video camera. Further studies and refinements of automatic detection system are needed to improve the accuracy and cost of detection.

Keywords: Cattle, Image processing, Video recorder, Background difference

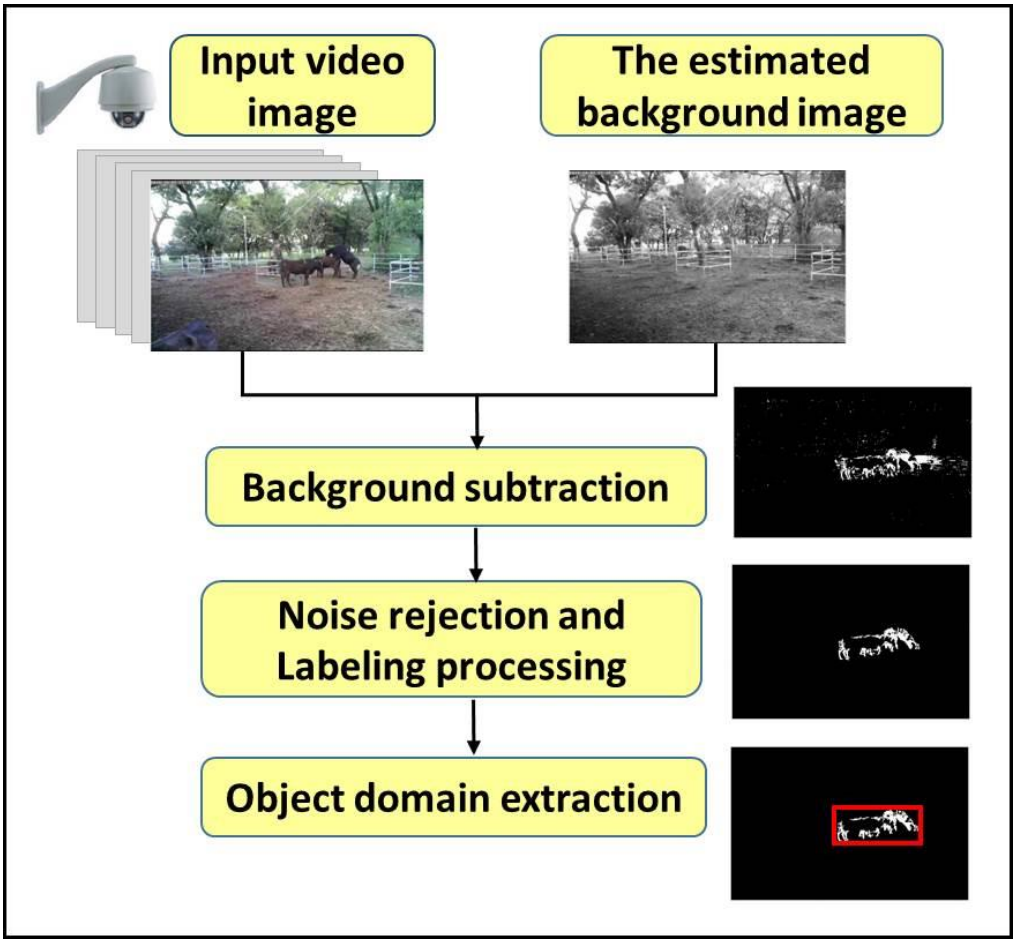

Figure 1. Flow diagram of a foreground object extraction algorithm.

Position	True	False	Total	Rate (%)
Near	4	2	6	66.7
Middle	16	2	18	88.9
Far	8	3	11	72.7
Total	28	7	35	80.0

Table 1. The rate of detection of mounting movement of the cows.

REFERENCES

At-Taras E. E. and S. L. Spahr, 2001. Detection and characterization of estrus I dairy cattle with an electronic heatmount detector and an electronic activity tag. J. Dairy Sci. 84: 792-798.

Brehme U., U. Stollberg, R. Holz and T. Schleusener, 2008. ALT Pedometer –New Sensor-aided measurement system for improvement in oestrus detection. Comput. Electron. Agric. 62: 73-80.

Choi D., H. Kim, Y. Chung and D. Park, 2014. Automatic detection of cattle mounting using motion and color information. Proceedings of 3rd International Conference on Computational Techniques and Artificial Intelligence (ICCTA): 22-25.

Firk R., E. Stamer, W. Junge and J. Krieter, 2002. Automation of oestrus detection in dairy cows: a review. Livest. Prod. Sci. 75: 219-232.

Hyeon T., H. Kim, D. Choi, W. Lee and c. Yong, 2005. Recognition of individual Holstein cattle by imaging body patterns. Asian-Aus. J. Anim. Sci. 18: 1194-1198.

Jonsson R., M. Blanke, N. K. Poulsen, F. Caponetti and S. Hojsgaard, 2011. Oestrus detection in dairy cows from activity and lying data using on-line individual models. Comput. Electron. Agric. 76: 6-15.

Okada K., H. Kobayashi, N. Hanada, H. Hiranuma, N. Hayashi, Y. Arashi, H. Chida, Y. Deguchi and S. Sato, 2011. Detection of hoof diseases in cattle using a triaxial accelerometer. Sangyo-Doubutsu Rinshou Igaku Zasshi (in Japanese), 2: 183-188.

Rao T. K. S., N. Kumar, P. Kumar, S. Chaurasia and N. B. Patel, 2013. Heat detection techniques in cattle and buffalo. Vet. World 6: 363-369.

Sen-Ching S. C. and K. Chandrika, 2004. Robust techniques for background subtraction in urban traffic video. Proceedings of the SPIE, Volume 5308, p. 881-892.

Xu Z. Z., D. J. McKnight, R. Vishwanath, C. J. Pitt and L. J. Burton, 1998. Estrus detection using radiotelemetry or visual observation and tail painting for dairy cows on pasture. J. Dairy Sci. 81: 2890-2896.

PO-05-38

Residual impact of stress factors that newborn calves suffer just after birth

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INTRODUCTION

Calves suffer from physical stress during passage through the birth canal (Chan *et al.*, 2015). In particular, dystocia is a stressful and traumatic event not only for cows but also for calves (Murray and Leslie, 2013). After birth, calves have to adapt to the extra-uterine environment including thermal conditions (Kirovski, 2015). Additionally, in most dairy production systems, calves are separated from their dams immediately after birth. Early breaking of the maternal bond between a dam and her calf may cause distress to calves (Kälber and Barth, 2014). Therefore, the degree of impact of these stress factors, i.e., difficulty of birth, high air temperature at birth, and early dam-calf separation, on newborn calves was compared. Their residual impact on the first three weeks of the life of calves was also detected.

MATERIALS AND METHODS

Six Holstein calves born in July and October and ranging between 36.5 and 52.3 kg body weight were used. The time required for birth (TB), maximum temperature on the birth day (MT), and time until dam-calf separation (TS) were measured. Calves were separated from their dam and penned (1.2 m × 2.6 m) individually afterward. Colostrum of dams was collected after separation and given to the calves using a teat feeder.

In consideration of the effect of heat stress becoming biggest 2–3 d later (Ueno, 1997), blood was collected from the jugular vein of calves 4 d after birth. Blood samples were placed in a cold box and then centrifuged at 0°C to 5°C after collection. The plasma was frozen at -25°C until analysis for cortisol. Hair samples were collected 1 and 2 wks after birth from the tail by using an electric razor. Hair samples were washed, dried, and ground with a bead crusher (μT-01; Taitec Co. Ltd., Saitama, Japan). Then hair cortisol was extracted according to the method as described by Peric *et al.* (2013). To measure the plasma and hair concentrations of cortisol, an enzyme immunoassay kit (ADV900-071; Cosmo Bio Co. Ltd, Tokyo, Japan) was used.

To determine the relative impacts of difficulty of birth, high air temperature at birth, and early dam-calf separation on the plasma cortisol concentration, a multiple regression analysis was performed. The relationship between the plasma cortisol concentration 4 d after birth with the hair cortisol concentrations 1 and 2 wks after birth was investigated by means of Pearson's correlation coefficient.

RESULTS AND DISCUSSION

TB, MT, and TS ranged between10 and61 min, 16.7 and 33.8ºC, and 1 and 113 min, respectively. No calf required serious obstetric assistance at birth.

A regression analysis of results obtained from TB, MT, and TS yielded a significant multiple R (0.995, P=0.016). Partial correlation coefficients of TB, MT, and TS were 0.836 (P=0.164), 0.986 (P=0.014), and 0.296 (P=0.704), respectively. Pearson's correlation coefficients of the plasma cortisol concentration at four days after birth with the hair cortisol concentrations at one and two weeks after birth was r=0.509 (P = 0.302) and r=0.650 (P = 0.162), respectively.

In the multiple regression analysis, only the maximum temperature on the day of birth was independently correlated with the plasma cortisol concentration of newborn calves at four days after birth. As described above, subject calves in this study were all delivered without serious obstetric assistance. Thus, this result might be limited to cases of eutocia because dystocia has been shown to be a stressful event for calves (Murray and Leslie, 2013). Separation from the dam immediately after birth was not a crucial stress event for the calves either. This might be because it was performed before the dam-calf bond is formed. This assumption is supported by a previous study that showed increased blood cortisol levels of calves at weaning (Veissier *et al.*, 2013). As for high air temperature at birth, it is reported that even maternal heat stress during late gestation negatively affects prenatal as well as postnatal development of calves (Tao *et al.*, 2012; Monteiro *et al.*, 2014). In addition, our previous study indicated that stress is transferred from a dam to her newborn calf through the placenta during late gestation (Uetake *et al.*, 2014).

Plasma cortisol concentrations of calves at four days after birth tended to correlate with the hair cortisol concentrations at two weeks after birth. This result corresponds to the previous report demonstrating that cortisol concentration of bovine hair was greater on days 14 and 28 after adrenocorticotropic hormone challenges (González-de-la-Vara *et al.*, 2011).

CONCLUSION

High air temperature at birth may be the severest stress factor for newborn calves, and its impact is reflected in the hair cortisol concentration two weeks later.

ACKNOWLEDGMENT

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REFERENCES

Chan, W. W., F. H. Jonker, W. D. Rausch and M. A. M. Taverne. 1993. Plasma catecholamines and blood chemistry in newborn calves in relation to different obstetrical procedures and to neonatal outcome. Anim. Reprod. Sci. 34: 43-54.

Murray, C. F. and K. E. Leslie, 2013. Newborn calf vitality: Risk factors, characteristics, assessment, resulting outcomes and strategies for improvement. Vet. J. 198: 322-328.

Kirovski, D., 2015. Endocrine and metabolic adaptations of calves to extra-uterine life. Acta Vet. 65: 297-318.

Kälber, T. and K. Barth, 2014. Practical implications of suckling systems for dairy calves in organic production systems – A review. Landbau. Vol. 64: 45-58.

Ueno, T. 1997. Evaluation of heat stress based on a temperature-humidity index. In: Ito, M., T. Ueno, M. Kamo and K. Togashi (eds), Production Manual of High Quality Milk by Reducing Environmental Stress. Ministry of Agriculture, Forestry and Fisheries Hokkaido Agricultural Experiment Station, Sapporo, Japan. (In Japanese)

Peric, T., A. Comin, M. Corazzin, M. Montillo, A. Cappa, G. Campanile and A. Prandi, 2013. Short communication: Hair cortisol concentrations in Holstein-Friesian and crossbreed F_1 heifers. J. Dairy Sci. 96: 3023-3027.

Veissier, I., S. Caré and D. Pomiès, 2013. Suckling, weaning, and the development of oral behaviours in dairy calves. Appl. Anim. Behav. Sci. 147: 11-18.

Tao, S., A. P. A. Monteiro, I. M. Thompson, M. J. Hayen and G. E. Dahl. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J. Dairy Sci. 95: 7128-7136.

Monteiro, A. P. A, S. Tao, I. M. Thompson and G. E. Dahl. 2014. Effect of heat stress during late gestation on immune function and growth performance of

calves: Isolation of altered colostral and calf factors. J. Dairy Sci. 97: 6426-6439.

Uetake, K., K. Akiyama and T. Tanaka. 2014. Relationship between stress levels of the antepartum cow and her newborn calf. Anim. Sci. J. 85: 81-84.

González-de-la-Vara, M. R., R. A. Valdez, V. Lemus-Ramirez, J. C. Vázquez-Chagoyán, A. Villa-Godoy and M. C. Romano. 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. Can. J. Vet. Res. 75: 216-221.

PO-05-41

Effect of playing installation on productive performances of Hanwoo steers

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Objective

This study was conducted to provide basic information on productive performance, behavior, and blood composition of Korean native (Hanwoo) steers.

Methodology

Thirty-two steers (aged 18-months) with an average body weight of 410.6 ± 8.76 kg were used in this experiment. Steers were assigned to 4 treatment groups (Figure 1); control, treatment 1 (cow brush, CB), treatment 2 (wood equipment, WE), and treatment 3 (cow brush and wood equipment, CBWE).

Result

Steers in WE and CBWE groups showed higher ($p<0.05$) growth performance than the control group (Table 1). Standing time was higher ($p<0.05$) in control group than the other treatment groups whereas feeding, lying and walking time were higher ($p<0.05$) in WE and CBWE groups (Table 2). Blood composition including white blood cells and cortisol levels were higher ($p<0.05$) in the control group than the other treatment groups (Table 3). No differences ($p>0.05$) were found in hemoglobin, albumin and globulin among control and treatment groups. Carcass traits including back fat thickness, longissimus muscle area, and marbling score were as follows in control to treatment groups(Table 4); control < CB < WE < CBWE. Profitability showed the highest rate in CBWE up to 1.8 million (Korean won) per head where showed the difference additional revenue of 0.8 million (Korean won) than the control group.

Conclusion

The using of a cow brush could give a positive effect on the steers as reducing stress by liberation from the itching and brush something aside on the there's hair.

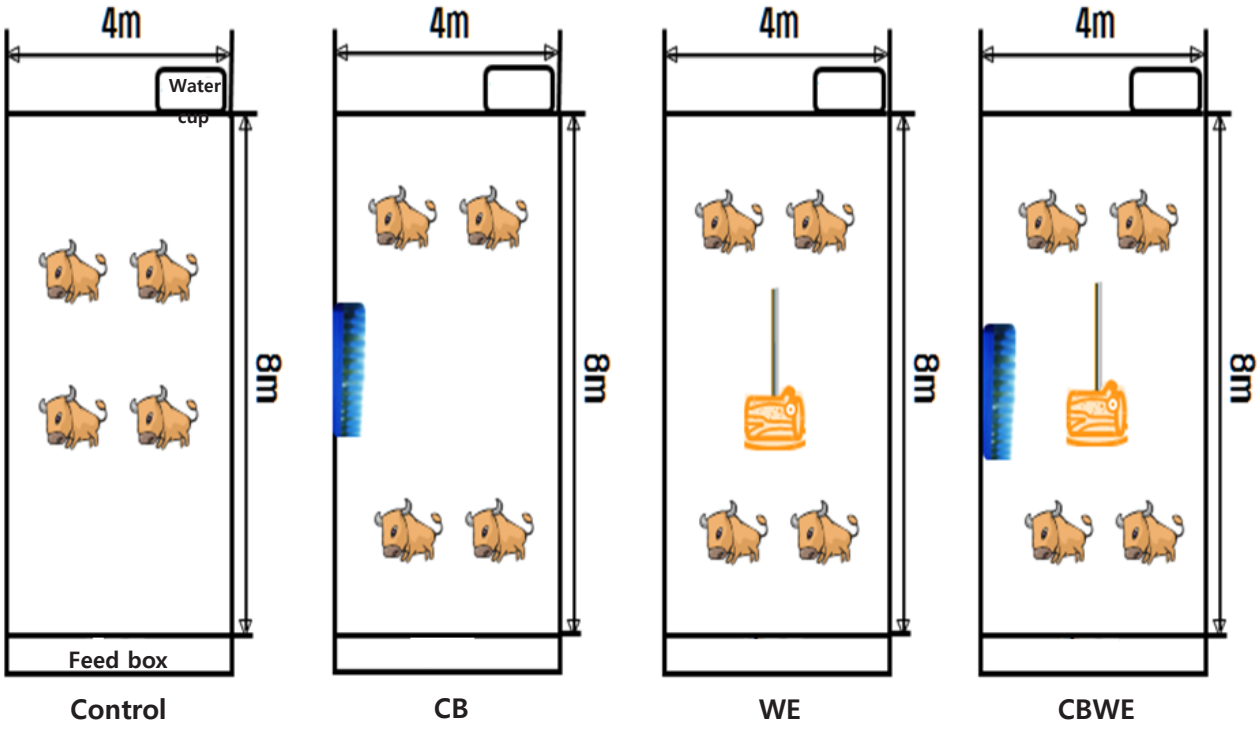

Figure 1. Experimental design.

Table 1. Effect of added assistant system on growth performance of Hanwoo steers

Item	Control	CB	WE	CBWE
IBW[1)](kg)	413.00±8.90	411.25±7.43	408.25±9.76	410.00±8.94
FBW[2)](kg)	656.75±11.56[b]	660.00±10.49[ab]	661.00±12.70[ab]	679.25±9.99[a]
ADG[3)], kg/day	0.81±0.09	0.83±0.07	0.84±0.12	0.90±0.05
FCR[6)]	13.23±0.76[a]	12.56±0.83[ab]	12.31±0.87[b]	11.13±0.92[c]

Values are mean ± standard deviation.

[a-b] Means within a row followed by a different letter are significantly different ($p<0.05$).

[1)] Initial body weight. [2)] Final body weight. [3)] Average daily gain. [4)] Feed intake. [5)] Dry matter intake. [6)] Feed conversion rate.

Table 2. Effect of added assistant system on behavioral characteristics of Hanwoo steers

Items	Control	CB	WE	CBWE
	Time (Minute)			
Feeding	53.19±5.27^{c}	55.63±6.00^{b}	61.54±4.67^{ab}	66.50±3.58^{a}
Standing	387.82±28.27^{a}	390.50±21.32^{a}	353.36±22.19^{b}	348.13±33.75^{b}
Lying	276.07±20.79^{b}	278.72±21.60^{b}	299.08±22.00^{a}	298.19±33.02^{a}
Walking	3.92±1.04^{c}	4.91±1.34^{b}	6.02±1.85^{b}	7.69±2.50^{a}

Values are mean ± standard deviation.

$^{a-c}$ Means within a row followed by a different letter are significantly different ($p<0.05$).

Table 3. Effect of added assistant system on blood parameters of Hanwoo steers

Items	Control	CB	WE	CBWE
WBC[1] (10^3/㎣)	8.84±1.57^{a}	7.53±1.28^{ab}	7.30±0.90^{b}	7.24±1.14^{b}
RBC[2] (10^6/㎣)	7.40±0.84^{c}	8.33±0.85^{bc}	8.71±0.90^{a}	8.83±0.81^{a}
HGB[3] (g/dL)	12.61±2.25	12.71±1.17	12.71±1.07	12.85±1.49
Albumin (g/dL)	4.31±0.18	4.38±0.23	4.25±0.23	4.34±0.21
Globulin (g/dL)	2.66±0.17	2.93±0.46	2.98±0.32	2.81±0.42
Cortisol (ug/dL)	2.47±0.88^{a}	1.58±0.91^{b}	0.92±0.22^{b}	0.71±0.19^{c}

Values are mean ± standard deviation.

$^{a-c}$ Means within a column followed by a different letter are significantly different ($p<0.05$).

[1] White blood cell, [2] Red blood cell, [3] Hemoglobin

Table 4. Effect of added assistant system on carcass trait of Hanwoo steers

Items	Control	CB	WE	CBWE
Yield traits				
CW[1)], Kg	411.25±15.67	410.50±15.00	412.75±18.41	421.25±11.15
BFT[2)], mm	13.75±2.22[a]	12.75±2.63[ab]	12.50±2.38[ab]	9.75±1.71[b]
LMA[3)],cm^2	86.25±3.10[c]	88.00±4.76[bc]	92.75±5.06[ab]	96.00±2.45[a]
YI[4)]	64.16±1.62[b]	65.03±1.31[b]	65.75±1.79[ab]	67.69±1.18[a]
Quality traits				
MS[5)], No.	4.25±0.82[b]	4.50±1.29[ab]	5.25±1.50[ab]	5.75±1.26[a]
MC[6)], No.	5.00±0.00	5.00±0.00	5.00±0.00	4.50±0.58
FC[7)], No.	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
FI[8)], No.	1.25±0.50	1.25±0.50	1.50±0.58	1.00±0.00
MA[9)], No.	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00

Values are mean ± standard deviation.

[a-c] Means within a row followed by a different letter are significantly different ($p<0.05$).

[1)] Carcass weight, [2)] Back fat thickness by ultrasound, [3)] Longissimus muscle area by ultrasound, [4)] Yield index, [5)] Marbling score by ultrasound, [6)] Meat color, [7)] Fat color, [8)] Firmness, [9)] Maturity.

References

Anderson NG. 2003. Observations on dairy cow comfort: Diagonal lunging, resting, standing and perching in free stalls. Proceedings of the fifth international dairy housing conference. pp. 26-35.

Browna SM, Klaffenböckb M, Nevisonc IM, Lawrence AB. 2015. Evidence for litter differences in play behaviour in pre-weaned pigs. App. Ani. Behaviour Sci. 172: 17-25.

Cho KH. 2006. A model of animal welfare farming in Korea. Ministry of Agriculture, Food and Rural Affairs the final report.

Devries TJ, Vankova M, Veira DM, Keyserlingk MA. 2007. Usage of Mechanical Brushes by Lactating Dairy Cows. J. Dairy Sci. 90(5): 2241-2245.

Yang KY, Lee KH, Kim GW, Yong SC, Ha JJ and Song YH. 2015. Studies on Behavioral Characteristics of Hanwoo Steers according to Pen and Group Size. Ann. Anim. Resour. Sci. 26(2): 128~134.

Valníčková B, Stěhulová I, Šárová R, Špinka M. 2015. The effect of age at separation from the dam and presence of social companions on play behavior and weight gain in dairy calves. J. Dairy Sci. 98(8): 5545-5556.

PO-05-44

The Evaluation of Homologous and Heterologous of Serum Neutralization against Newcastle Disease Virus using Vaccinated Chicken Sera

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Introduction

Newcastle Disease (ND) is one of the most important viral diseases of bird species which causes the high level of mortality and morbidity especially in chickens. It is the global issue leading to huge economic losses in poultry industry and trade restrictions (Alexander and Senne, 2008). Nowadays, ND is endemic in several developing countries with severe outbreaks have occurred in year by year. The causative agent of ND is avian paramyxovirus serotype-1 (APVM-1) that synonymous with Newcastle Disease Virus (NDV). It belongs to the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae,* and the genus *Avulavirus* (Mayo, 2002). NDV is an enveloped virus with a negative-sense, single-stranded RNA genome (Seal et al., 2000).

Based on pathogenicity, NDV is divided into three pathotypes including lentogenic (low virulence), mesogenic (moderate virulence) and velogenic (high virulence) (Alexander, 2000). Based on genome and sequences analysis, NDV isolates were classified into two distinct classes (class I and II). Class I strains were generally isolated from waterfowl and shorebirds while Class II strains were further divided into 16 genotypes that were typically from poultry and wild birds (Courtney et al., 2012; Miller et al., 2010). These viruses are circulating in avian population worldwide and the antigenic and genetic diversities of virus exhibit between different genotypes. The distributions of different NDV genotypes have become a main obstacle to protect ND by getting vaccine.

Objective

The aims of this study were to evaluate the immune response of vaccinated chickens with different NDV vaccine strains. Moreover, we evaluated various types of serum neutralization to assist the selection of appropriate NDV vaccine strains which match the field viruses concurrently circulating in flock chickens.

Methodology

Vaccines and viruses

Two attenuated live ND vaccines were used in this study namely Hitchner B1 and MET strains which were obtained from Nisseiken Co., Ltd., Japan and The Chemo-Sero-Therapeutic Research Institute, Kaketsuken Japan, respectively.

Both strains of viral vaccine and the virulent NDV (vNDV) obtained from Veterinary Medicine Faculty, Mahanakorn University of Technology, Thailand, were used for serum neutralization (SN) test.

Animal experiments

Chickens were divided into two groups. The first group was vaccinated with attenuated Hitchner B1 vaccine and another group vaccinated with attenuated MET vaccine following the manufacturer's instructions. Subsequently, all chickens were collected individual serum after 3 weeks post vaccination and kept at -30°C until tested.

Serum neutralization (SN) test

Cells: The 0.3% suspension of Chicken kidney (CK) and Chicken embryo fibroblasts (CEF) cells were prepared from 1-day old chick and 10-day-old chicken embryos, respectively. The cells (1.5×10^6 cells/ml) were seeded into the 96-well plate and the 60 mm petri dish, kept at 37°C with a 5% CO_2 incubator until monolayer and used for SN test.

Homologous serum neutralization test: Each vaccinated serum sample was diluted as two-fold serial dilution. The equal volume of 200 mean tissue culture infectious doses ($TCID_{50}$) of Hitchner B1 or MET NDV strain was added to all diluted serum samples. After that incubated at 37°C with a 5% CO_2 incubator for 60 min. Then, 25 µl of mixture was inoculated onto CK cells and incubated at 37°C with a 5% CO_2 incubator for 1 hour. Then, 100 µl of

maintenance medium (MM) was added into each well and incubated for 3 days. The cytopathic effect (CPE) was observed 2 times a day and confirmed by haemagglutination (HA) test at 3-day post inoculation (dpi).

Heterologous serum neutralization test: All samples were also tested with virulent NDV using SN test. Two SN methods were used including in the microplate and plaque reduction assays. Briefly, serum sample was diluted as two-fold serial dilution and equal volume of 100 $TCID_{50}$ of vNDV was added to all diluted sera. After incubated at 37°C with a 5% CO_2 incubator for 45 min, 200 µl of mixture was inoculated onto CEF cells and then incubated for 3 days. The CPE was observed 2 times a day and confirmed by HA test at 3 dpi. The plaque reduction assays was described by Takehara and colleague ((Takehara *et al*. 1991; Takehara *et al*, 1987)). Briefly, antiserum against MET and B1 NDV strains were diluted as four-fold serial dilution, and mixed with . Mixture was incubated at 37°C in water bath for 1 hour then inoculated onto CEF cells and incubated at 37°C in a 5% CO_2 incubator for 1 hour. After aspirated off the inoculum, the first overlay medium was added and incubated for 2 days. Subsequently, the second overlay medium was added and kept upside down in a CO_2 incubator for 2 days. The neutralizing antibody titer was calculated at 50% plaque reduction point by Behrens-Kaerber's method at 4-daypost inoculation (Matumoto, 1949).

Results

In order to determine the immune response of vaccinated chickens with the same (homologous) and different (heterologous) genotype vaccine strains of NDV, all sera were tested by serum neutralization (SN) test. The result of all SN test was showed in Table 1.

The mean neutralizing antibody titer of homologous and heterologous genotype was showed as 40.7 and 106.7 for B1 vaccination, and 102.5 and 48.0 for MET vaccination, respectively (Fig.1).

Conclusion

These results indicated that all vaccinated chickens could produce homologous and heterologous neutralizing antibodies against the same and different genotypes. MET vaccination showed higher homologous antibody level than Hitchner B1. On the contrary, Hitchner B1 vaccination could produce more heterologous antibodies against vNDV than MET. The present study suggested that both vaccine strains might protect ND including virulent NDV while Hitchner B1 trend to show more heterologous neutralizing titer than MET vaccine strain. These results suggest that the protection of high virulence NDV of Hitchner B1 vaccination might be more effective than MET. It could indicate that Hitchner B1 vaccine is more suitable for some countries that the velogenic strains are a predominant genotype or outbreak.

ND continues to be one of the important diseases in poultry because it causes huge economic losses in commercial and flock chickens worldwide despite the various vaccines licensed on the international market have been used. The protective efficacy of these vaccines may be limited as they do not provide clear immunity because NDV exhibits antigenic and genetic diversity between strains resulting in failing to prevent losses from morbidity and mortality. Therefore, vaccine selection that matched to field strains could contribute to robust protection against NDV infection. Our study indicated that all chickens were vaccinated with both virus strains could produce homologous and heterologous neutralizing antibodies. Hitchner B1 vaccine is suitable for heterologous NDV and potential for endemic countries of vNDV. However, MET vaccine showed the high homologous antibody level matching to field strains. Therefore, it may be crucial for diminishing of virus shedding.

Table 1: The antibodies titer of serum samples from vaccinated chickens using homologous and heterologous serum neutralization test.

Chicken number	Vaccine type	Homologous SN titer	Heterologous SN titer	
			microplate	plaque assay
N1	Hitchner B1	45	128	30.09
N2	Hitchner B1	45	64	≥32
N3	Hitchner B1	32	128	28.29
N4	Hitchner B1	45	32	30.09
N5	Hitchner B1	45	128	30.03
K1	MET	128	32	30.03
K2	MET	128	32	30.03
K3	MET	64	64	≥32
K4	MET	90	64	30.03

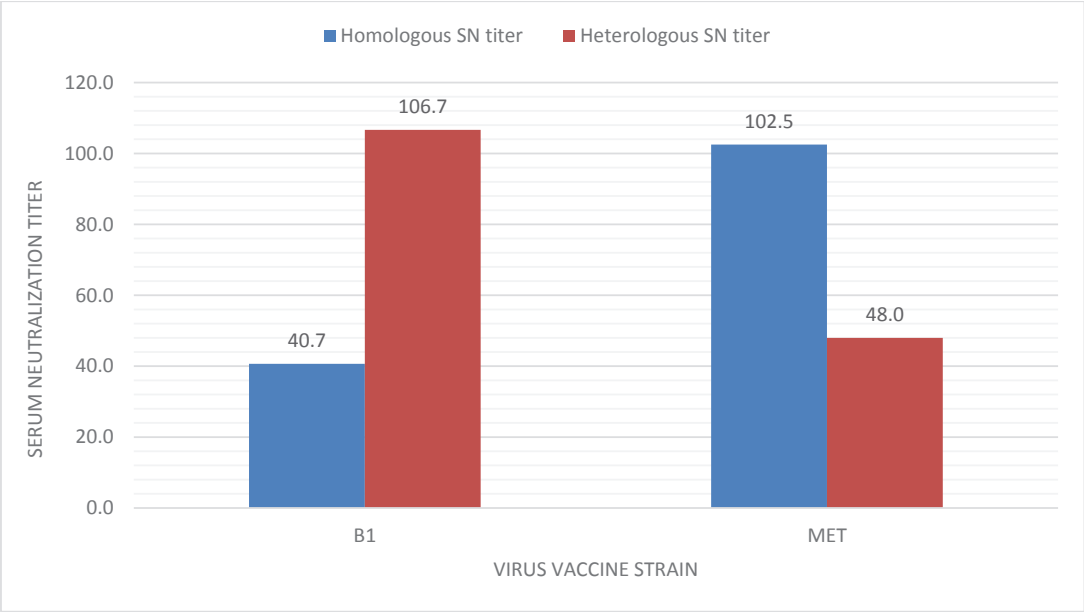

Figure 1: The mean neutralizing antibodies titer of vaccinated chickens with the same (homologous) and different (heterologous) genotype vaccine strains of NDV.

Reference

Alexander, D.J. 2000. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. 19: 443–462.

Alexander, D. and D. Senne. 2008. Newcastle disease, other avian paramyxoviruses, and pneumovirus infections, 12th ed Blackwell Publishing, Ames, IA

Courtney, S.C., L. Susta, D. Gomez, N. Hines, J.E. Pearson, C.C. Brown, P.J. Miller, and C.L. Afonso. 2012. Highly divergent virulent isolates of Newcastle disease virus from the Dominican Republic are members of a new genotype that may have evolved unnoticed for over two decades. J. Clin. Microbiol. 51: 508–517.

Matumoto, M. 1949. A note on some points of calculation method of LD50 by Reed and Muench. Jpn. J. Exp. Med. 20: 175-179.

Mayo, M.A. 2002. A summary of taxonomic changes recently approved by ICTV. Arch. Virol. 147: 1655-1663.

Miller, P.J., E.L. Decanini and C.L. Afonso. 2010. Newcastle disease: evolution of genotypes and the related diagnostic challenges. Infect. Genet. Evol. 10: 26–35.

Sakai, K., K. Yada, G. Sakabe, O. Tani, K. Miyaji, M. Nakamura and K. Takehara. 2006. Serological and virological studies of Newcastle disease and avian influenza in slaughter-age ostriches (Struthio camelus) in Japan. J. Vet. Med. Sci. 68: 491-494.

Seal, B.S., D.J. King and H.S. Sellers. 2000. The avian response to Newcastle disease virus. Dev. Comp. Immunol. 24: 257–268.

Takehara, K., H. Kiuchi, M. Kuwahara, F. Yanagisawa, M. Mizukami, H. Matsuda and M. Yoshimura. 1991. Identification and characterization of a plaque forming avian rotavirus isolated from a wild bird in Japan. J. Vet. Med. Sci. 53: 479–486.

Takehara, K., T. Shinomiya, H. Kobayashi, Y. Azuma, T. Yamagami and M. Yoshimura. 1987. Characterzation of Newcastle disease viruses isolated from field cases in Japan. Avian Dis. 31: 125–129.

PO-05-48

Biocontrol of Animal Pathogens by Lactic Acid Bacteria

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Objectives

The objectives of this study were to isolate and study lactic acid bacteria with the antimicrobial activity against animal pathogenic-bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

Methodology

Isolation of lactic acid bacteria Raw milk samples from dairy farms in the northeastern region of Thailand were used for lactic acid bacteria screening. The method used was modified from method describe by Luo. et al., 2011. All raw milk samples were diluted with MRS and 0.1 ml aliquot was spread-plated onto MRS agar, containing 1% $CaCO_3$. Colonies forming a clear zone in the MRS agar were selected and stocked in 30% glycerol at -20°C.

Antibacterial activity of lactic acid bacteria Antibacterial activity was tested against *S. aureus* TISTR 746, *E. coli* TISTR 746, *Ps. aeruginosa* TISTR 357 and *S. typhimurium* TISTR 1469 by agar-disc diffusion technique. Cell-free culture of bacteria suspension from each lactic acid bacteria was prepared by centrifugation and filtrated through 0.45 mm pore-size filter. Ten microliter aliquot of cell-free culture was dropped on 6-mm diameter discs, which were single placed on NA agar plates, covered with 100 µl of the indicator strain. The plates were incubated at 37ºC for 24 hours to check for an inhibition zone. The antimicrobial efficiency of each lactic acid bacteria against the indicator strains was defined by measuring diameter of inhibition zone in millimeter. Also, cell-free cultures with the inhibition zone were chosen for the study on the nature of inhibiting agents.

Study of the nature of inhibiting agents

3.1 Sensitivity of cell-free culture to pH change

Cell-free cultures were normalized to pH 7.0 by 1 M NaOH and tested for the remaining inhibitory activity.

3.2 Sensitivity of cell-free culture to proteolytic enzyme

Cell-free cultures were incubated with proteinase K at the final concentration of 1 mg/ml for 12 hour. The proteinase K-treated cultures were heated to kill proteolytic enzyme and tested for the remaining inhibitory activity.

3.3 Sensitivity of cell-free culture to high temperature

Cell-free cultures were incubated at 100°C for 10 min. After cooling down, the high temperature-treated cultures were tested for the remaining inhibitory activity.

Results and Discussion

It was found that the total bacteria count from raw milk samples ranges from 10^7-10^8 CFU/ml. By media selection, lactic acid bacteria were isolated and screened for their antimicrobial activity against indicator strains. The results of antimicrobial test showed that each cell-free culture of lactic acid bacteria possessed different degree of antimicrobial activity. Most lactic acid bacteria had a low level of antimicrobial property (their inhibition zones were lower than 7.00 mm, data not shown) while fourteen isolated could inhibit the growth of indicator strains in the moderated degree. Their inhibition zones were in the range of 7.32-10.80 mm, 7.93-8.94 mm, 7.56-9.08 mm and 9.41-10.64 mm for *S. aureus, E. coli, Ps. aeruginosa and S. typhimurium,* respectively (Table 1). These antimicrobial activities were compared to those of oxytetracycline (30 mg/disc), the commercial antibiotic drug and distrillation water (a positive and negative control, respectively). It was found that no any cell-free culture of lactic acid bacteria exhibited the antimicrobial activity greater than commercial drug. On the other hand, their antimicrobial activities were absolutely better than those of distillation water. Differences in antimicrobial activity were believed to as a result of the secondary metabolites produced by each lactic acid bacteria. Factors promoting the diversity of secondary metabolites, produced by lactic acid bacteria were environmental condition such as

climate, season and temperature Moreover, type and age of sample used in the study also had the impact on amount and diversity of lactic acid bacteria (Kwantrairat et al., 2009).

To study the nature of inhibiting agent produced by each strain, these isolated were tested for their anti-*S. aureus*, anti-*E. coli*, anti-*Ps. aeruginosa* and anti-*S. typhymurium* property against under different conditions. It was clear that the inhibition zone diameters of lactic acid bacteria were in the same range as normal condition (Table 1), proteolytic-treated condition (Table 2) and high temperature-treated condition (Table 3). The inhibition zone diameters of lactic acid bacteria under proteolytic-treated condition were between 7.32-9.91 mm, 7.29-8.56 mm, 7.13-8.97 mm and 8.29-9.84 mm for *S. aureus, E. coli, Ps. aeruginosa and S. typhimurium,* respectively (Table 2). In addition, the diameters of inhibition zone under high temperature-treated condition were 7.93-10.22 mm, 7.22-9.16 mm, 7.29-8.74 mm and 7.04-9.91 mm for *S. aureus, E. coli, Ps. aeruginosa and S. typhimurium,* respectively (Table 3). It date indicated that protease and high temperature treatment do not influence on the antimicrobial activity. It could be said that the inhibiting agents, produced by these lactic acid bacteria could not be protein-liked compound. Interestingly, the inhibition zone diameter dramatically dropped to 0 when adjusting pH of these cell-free cultures to 7.0 (data not shown), indicating that pH naturalization totally destroyed their ability of anti-microorganisms. It implied that the antimicrobial property of these lactic acid bacteria might come from producing secondary metabolite, which provided unflavored environment for the growth of indicator strains. The unflavored condition for the growth of indicator strains in this study seemed to acidic condition. It was because pH neutralization raised low pH value to neutral, resulting in no antimicrobial activity detection. This result agreed with the report by Kwantrairat et al., 2009, which examined that lactic acid bacteria isolated from gastrointestinal tract of Nile tilapia (*Oreochromis niloticus*) could inhibit the growth of *Aeromonas hydrophilia* by producing their organic acids. Though, some isolated lactic acid bacteria had been reported to produce another antimicrobial agents, aside from acidic condition, such as bacitracin (Todorov et al., 2006; Khunnajark et al., 2008; Petsuriyawong and Khunnajark, 2010; Saidi et al., 2011).

Conclusion

In conclusion, the isolation of lactic acid bacteria which were able to produce unflavored environment for the growth of indicator strains could be an alternative means to obtain natural anti-pathogenic bacteria agents. However, this study was only an introductory study on isolation of lactic acid bacteria with antimicrobial activity; the further study needs to be done on determination of the potential efficacy of these lactic acid bacteria as anti-pathogenic bacteria in animals.

Table 1 Inhibition zone diameter produced by lactic acid bacteria

Lactic acid bacteria strain	Mean of inhibition zone diameter (mm)			
	S. aureus TISTR 746	*E. coli* TISTR 746	*Ps. aeruginosa* TISTR 357	*S. typhimurium* TISTR 1469
KS7	9.52	8.50	8.62	10.64
KS8	10.30	8.53	8.95	9.64
KS27	10.02	8.94	9.08	10.43
KS29	10.11	8.30	8.56	10.37
KS36	10.05	8.81	8.00	10.55
KS37	7.32	8.35	7.56	9.86
KS39	10.80	8.47	8.38	10.49
KK23	9.34	7.93	8.11	9.83
KK27	9.84	8.18	9.08	10.43
KK36	10.17	8.05	8.49	9.25
KK38	10.04	8.66	7.98	9.41
R10	10.41	8.52	6.00	10.05
R41	9.97	8.77	7.68	10.40
MS5	9.73	8.23	7.84	9.87
Distillation water	6.00	6.00	6.00	6.00
Oxytetracycline	22.82	21.26	11.96	28.06

Table 2 Inhibition zone diameter produced by lactic acid bacteria under proteolytic-treated condition

Lactic acid bacteria strain	Mean of inhibition zone diameter (mm) under proteolytic-treated condition			
	S. aureus TISTR 746	*E. coli* TISTR 746	*Ps. aeruginosa* TISTR 357	*S. typhimurium* TISTR 1469
KS7	8.90	8.08	7.24	9.55
KS8	9.23	7.29	7.56	8.36
KS27	9.36	8.35	8.03	8.67
KS29	9.17	8.56	7.68	9.25
KS36	8.35	8.17	8.96	9.54
KS37	7.32	8.35	7.76	8.94
KS39	8.09	8.55	8.86	8.93
KK23	9.91	7.84	8.97	8.43
KK27	8.26	8.11	8.65	8.29
KK36	8.28	7.74	7.15	9.40
KK38	8.53	7.93	7.64	9.84
R10	8.89	7.68	7.13	8.59
R41	8.56	7.64	7.56	9.52
MS5	6.00	7.41	7.23	8.59

Table 3 Inhibition zone diameter produced by lactic acid bacteria under high temperature-treated condition

Lactic acid bacteria strain	Mean of inhibition zone diameter (mm) under high temperature-treated condition			
	S. aureus TISTR 746	*E. coli* TISTR 746	*Ps. aeruginosa* TISTR 357	*S. typhimurium* TISTR 1469
KS7	8.55	7.90	8.12	7.53
KS8	9.02	8.26	7.86	9.10
KS27	8.27	9.16	8.33	9.24
KS29	10.22	8.93	8.74	9.50
KS36	9.04	8.27	7.39	9.91
KS37	8.24	7.84	8.71	9.05
KS39	8.49	9.04	8.25	9.48
KK23	9.01	8.79	8.05	8.36
KK27	9.21	7.22	8.35	8.62
KK36	7.93	7.86	7.83	7.83
KK38	8.38	8.05	7.65	8.01
R10	9.21	7.22	7.35	8.62
R41	8.71	8.23	7.88	7.04
MS5	8.06	7.87	7.29	8.10

References

Khunajakr N., Wongwicharn A., Moonmanagmee D. and Tantipaiboonvut S. 2008. Screening and identification of lactic acid bacteria producing antimicrobial compounds from pig gastrointestinal tracts. KMITL Sci. Tech. 8: 8-16.

Kwantrairat S., Yossiri W. and S. siri. 2009. Isolation of lactic acid bacteria producing organic acids and possessing Anti- *Aeromonas hydrophila* activity from gastrointestinal tract of Nile tolapia (*Oreochromis niloticus*). KKU Res. J. 14: 811-821.

Luo F., Fend S., Sun Q., Xiang W., Zhao J., Zhang J. and Z. Yang. 2011. Screening for bacteriocin-producing lactic acid bacteria from kurut, a traditional naturally-fermented yak milk from Qinghai-Tibet plateau. Food Control. 22: 50-53.

Petsuriyawong B. and N. Khunajak. 2010. Screening of lactic acid bacteria isolated from piglet faces for antimicrobial activity. KKU. Res. J. 15: 446-48.

Saidi N., Hadadji M and Guessas B. 2011. Screening of bacteriocin-producing lactic acid bacteria isolated from west Algerian goat's milk. Global Journal of Biotechnology & Biochemistry, 6: 154-161.

Todorov S.D. and L.M.T. Dicks. 2006. Screening for bacteriocin-producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria comparison of the bacteriocins. Process Biochem. 41: 11-19.

PO-05-50 OPTIMIZE OF VEGETATIVE CONSERVATION MODEL FOR SUPPORT POOR FARMERS AGRICULTURAL SUSTAINABLE IN WEST TIMOR

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BACKGROUND

Amount of West Timor people is gradually increased, which their consequence are increased of food, feed and fuel. Now, West Timor population is 1.794.450 persons. If population growth is 1.76% per year, this population will be increased of twice on the middle of century XXI and be four times at late of century XXI. In the future, this challenge will be a big problem because agricultural land will be threatened for other used such as real estate and industry, while West Timor people still import of cereal 4.043 ton per year.

West Timor area is 1.512.948 hectares, which 1.466.604 hectares (97%) are dominated by dry land 291.554 and 70% of it area has hilly topography with slope higher than 25%. This condition caused high risk for erosion.

Land degradation is serious problem in Timor Island. It's caused not only shifting and burning cultivation practices, but also agricultural practices by farmers had not yet paid attention to land conservation aspects. Land farms were cultivated with local maize varieties and other perennial crops with low input productions.

Soil conservation must be informed continuously to the farmers. One of important information needed for present generation. Important aspect to soil conservation recommendation for Timorese must be considered for food and cattle feed. It needed combination of food crops and cattle feed crops compatibility.

Vegetative conservation practices opportunities to develop on the wide range compared than other conservation technique. Those technologies need a few labor and capital, supported cattle development, and effective to reduce soil erosion. Hedgerow crops on vegetative conservation had multiple functions to reduce soil erosion and cattle feed.

WEST TIMOR DRY LAND AGRICULTURAL PRACTICES

Dry land agricultural practices are appropriated with rainfall and day of rain. The farmers cultivated mixed cropping of food crops and annual crops. Generally, the farmers used to shiting cultivation practices. Shifting was done on seven to nine year, which they have 3-4 fields per household and each year they cultivated only 1-2 fields. In the first year, they cultivated first field. In the first field, they cultivated maize + squash + crop legumes + cassava alon 2-3 planted season (2-3 year), and then they left first field because this first field was not fertile. On the year three and four they moved to second field, year five and six on the third field, and year seven and eigh on the forth field, and year nine and ten back to the first field. On the next time, fallow long period will be gradually shorter than present because increasing of population. It caused fallow long period was not enough to bring back soil fertility and crops yield productivity were gradually decrease. If the same process recycled, West Timor agricultural lands qualities were gradually decrease and next generations were inherited poor land quality, while human population growth was increase. So shifting cultivation was not possible to continue in future time.

Furthermore, land degradation will be quickly increase because (a) Land preparation through burning cultivation caused organic matter easy to condensing and the dusty easy to erosion on sloping lands, (b) The farmers limited use organic and unorganic fertilizer, and (c) Conservation aspects have not paid attention.

Livestock, especially Bali cattle is an important component in contributing to the income of farmers because 59% farmers have Bali cattle with ownership 2.4 cattle per household and contributes 15-50% to farmer's income. Generally, cultivation method of cattle is extensive method, which it use grassing area. In a few decades, Timor Island becomes a cattle supplier to other regions, but cattle population decrease now. This is thought to be triggered by several things, especially the limited availability of feed in quantity and quality, and reduction grass protein content up to 4.5% in dry season.

THE IMPORTANCE OF VEGETATIVE CONSERVATION FOR TIMOR ISLAND

Sheet Erosion Predicted

Existing vegetative conservations were not enough to reduce soil erosion, nevertheless soil erosion lower than without conservation. USLE calculation showed that soil erosion of the fallow lands were 15 tons ha-1year-1. Soil

erosion increased after fallow, nevertheless with vegetative conservation was higher than without conservation, both increased 14 times and 136 times higher than fallow lands. This result was higher than tolerable soil lost value for Indonesian lands that were 0-30 t ha-1years-1.

Paired samples test showed soil erosion for five years cultivation with vegetative conservation lower than without conservation. Vegetative conservation practices reduced soil erosion and expressed with an exponential curve y = 42.89e0.474x and adjusted R2 = 0.334, while soil erosion on without conservation was fast gradually increased with an exponential equation y = 75.18e0.896x and adjusted R2 = 0.502.
This result showed that vegetative conservation practices increased soil erosion. Difference of sheet erosion with and without conservation was determined trough soil erodibility index, slope length, slope steepness, crop factor, and conservation practice factor.
a) Soil erodibility
Soil particles of conservation lands more difficult to erosion than without conservation. Soil erodibilty was smaller if very sand and silt percentage smaller and clay percentage higher. Silt and very sand percentage of with vegetative conservation lands was lower than without conservation that were 58% and 70%, while clay percentage 28% and 19%.
b) Slope length and slope steepness
Slope length and slope steepness value on vegetative conservation practices more and more smaller was caused slope length more and more shorter effect of more and more wider of hedge row crops. The observation showed that (a) slope length of without conservation lands range were 18.4 – 150 m and average 78.29 m, (b) average of vegetative conservation lands were 10.64 m, and (c) independent samples test showed that slope steepness of with conservation equal than without conservation that were 21% and 17%. Existing vegetative conservation must be shortened with different high 0.5 m.
c) Conservation practice factor
Hedgerows crops would slow the runoff water and then reduce the amount of soil it can carry. Observations from hedgerow crops showed that much of the soil eroded were filtered out of the runoff and soil eroded spread around hedgerow crops root zone.

Economic Value of Soil Erosion
Economic value of soil erosion was counted trough amount of soil eroded, soil nutrient content and fertilizer prize. Calculation result showed that total of economic value soil erosion along five years cultivation on without conservation lands was $USD 18,654.92 ha-1 and with vegetative conservation was $USD 3,516.57 ha-1. Thereby vegetative conservation prevented money lost $USD 15,138 ha-1.

EXISTING VEGETATIVE CONSERVATION PRACTICES

a. Production of Hedge Row biomass
The common species for hedge row is the elephant grass (Pennisetum purpureum) or Kaliandra (Calliandra calothyrsus). Usually planting of hedge row was done on the first year effort. Elephant grass can be harvested 2-3 months after planting, while Kaliandra after the second year. Biomass can be harvested 4-6 times per year.
West Timor farmers cut hedge row biomass for cattle feed 2-3 times per day, which weight of feed around 15-20 kg per intake. Intake of feed usually was done by family labor. Biomass taken for cattle feed, but the manure is not returned to the land.
Biomass production was gradually increased with plant age. Production will be stable after the fourth year, which their production reaches 25 tons ha-1year-1. The relationship between biomass production and effort time expressed with a linear equation:
y = 6.032,63x – 592,63 (y = biomass production of hedge row crops (kg ha-1year-1 and x = years cultivated).
While relationship between farmers biomass income and effort time expressed with a linier equation: y = 2.021.458,37x – 2.444.254,57 (y = farmers income from hedge row crops (IDR ha-1year-1 and x = years cultivated)
b. Food crop production
Although the vegetative conservation would reduce field for maize reaches 10-17% and reduce maize productivity, but this phenomena only until the fourth year. In the fifth year concession, maize productivity on the conservation land was higher than without conservation.

Decreasing of maize productivity expressed with exponential regression y = 4.559,18e-0.34x on the vegetative conservation land and y = -634,4x + 3.930 on the without conservation land.

Farmer's maize productivity on the first year of cultivation reached 3 ton ha-1. Productivity is further decreased in the second year and maize productivity is only 0.5 t ha-1 on the fifth year. This phenomenon caused farmer's cultivated maize on the same field 2-3 years only.

c. Land Equation Ratio

Farmer's revenue (revenue-cost) on the conservation land (intercropping maize + hedge-row feed) is greater than without conservation (maize monoculture). The land equivalent ratio/LER value is higher than 1 and its value increases with time effort. In the first, LER value is 1.63, it means that farmer's income on the conservation land is 1.63 times higher than without conservation, while in the fourth year 6.74 times higher than without conservation. This result showed that conservation land more efficient than without conservation land.

RECOMMENDATIONS OF VEGETATIVE CONSERVATION MODEL FOR TIMORESE FARMERS

The vegetative conservation that developed in the Timorese farmers is more profitable than without conservation. Even though, it's not save land degradation and save for food supply because:

a) Soil erosion is very high, which it's about 200-300 tones ha-1yr-1 on the fifth effort year, while tolerable soil lost is less than 12.5 tons-1ha-1 year-1.

b) The farmer has not organic matter added. These results indicate that farmers continually deplete natural resources for soil fertility.

c) Farmer's attitude was not support for sustainable agriculture because 26.17% farmers burn of crops biomass in the maize preparation land.

d) Maize productivity was gradually decrease, which it's less than 1 ton ha-1 on the fifth year effort. It's caused the farmers never fertilize their maize. So it is not possible to settled agriculture.

Some suggested for save food and feed for Timorese farmers are:

a) Soil erosion can be reduced by shortening of rows distance, which it's suggested height difference between the rows is 0.5 meter. West Timor slope land is 19% and existing of inter-row spacing on vegetative conservation farmers are 10.64 meter. If applied height difference between rows 0.5 meter, row distance will be 2.63 meter. So, based on the calculation of the USLE soil erosion equation can be reduced by 50%. Thus, soil erosion on the conservation 200-300 t ha-1 year-1 will be reduced to 100-150 t ha-1 year-1.

b) Utilizing hedge rows biomass as an organic material supplier. Increasing the number of rows in the point (a) will increase the biomass production about 4 times higher than existing biomass production, so the existing wet biomass production 26 tons on the fifth year effort or the equivalent of 6 tons of dry biomass will increase to 100 tons of wet biomass or equivalent with 24 tons of dry biomass. If the elephant grass biomass contain is 2% N, 0.25% P2O5 and K2O 4%, the biomass contain is 480 kg N, 60 P2O5 and 960 kg K2O.

c) Crops and livestock integration, which livestock as manure supplier for maize field. The potential supply of feed in points (b) is 100 tons of wet biomass per year. If it's assumed that biomass can be consumed by livestock by 50%, so the potential supply of feed from vegetative conservation by 50 tons per year. If the Bali cattle feed 10-15% of their body weight per day and average of Bali cattle weight 200 kg per cow will consumed 20-30 kg per day. For fattening for 6 months (a period of fattening) a cattle needs 3600-5400 kg. The potential supply of feed 50 tons per year, so potential supply of feed for 6 months is 25 tons. So, new pattern will supply cattle feed for 10 cattle per year.

d) Changing farmer's habits from to use manure. If a Bali cattle produced 3.69 kg/head/day, the potential supply of manure from five cattle are 6.5 tons. Furthermore, one ton of manure contains 21.6 to 25.7 kg N, 4.8 to 6.8 kg P2O5 and 14.5 to 16.2 K2O. So, the nutrients contained in 6.5 tons of manure are 140-167 kg N, 31-34 kg P2O5, and 94-105 K2O. This nutrient enough to support of maize growth because maize growth needs N, P2O5 and K2O respectively around 198 kg ha-1, 33 kg ha-1, and 205 kg ha-1.

e) Based on calculations such as points a, b, c and d, recommendation hedge-row spacing of each agro ecosystem zone are :

Recommendation of hedge row distance for West Timor Island

Slope (%) Prompts hedge-row spacing (m) Number of row per hectare Width of field for food crops

f) Integration of legume trees and grass, especially elephant grass and Kaliandra for hedge row crops. This technique was done because: (a) recommendation of cattle feed is 40% grass: 60% legume, (b) Kaliandra is a legume tree, which they have root nodules for Nitrogen fixation from the air, so it can pump leached nutrients

from depth soil back to the root crops zone.

g) Not to burning of crops biomass on land preparation, but left it in the field for compose. The way must be done because burning biomass effect are: (a) much nutrients will be evaporates, whereas maize stover contain 0.84 to 1.45% N, 0.01 - 0.04% P2O5 and 0.13 to 0.39% Ca and the natural grasses contain 0.74 to 1.03% N, 0.05 to 0.12 P2O5 and 0.22 to 0.45 Ca, (b) their ashes will be easily washed away carried out by rain water, and (c) heat of interfere can burn of hedge row crops.

h) Developing of strip cropping of maize and nuts for save Nitrogen

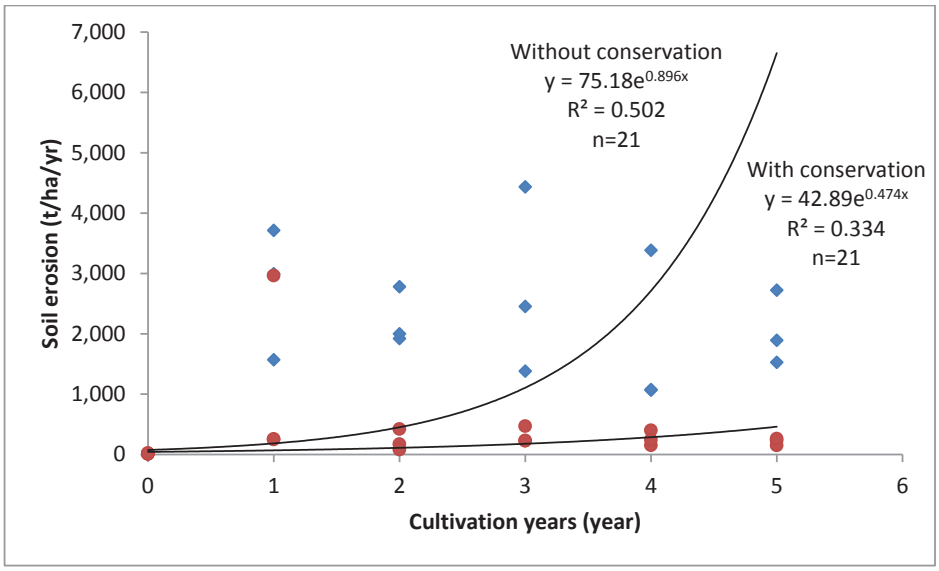

Figure Soil Erosion of With and Without Vegetative Conservation for Five Years Cultivation

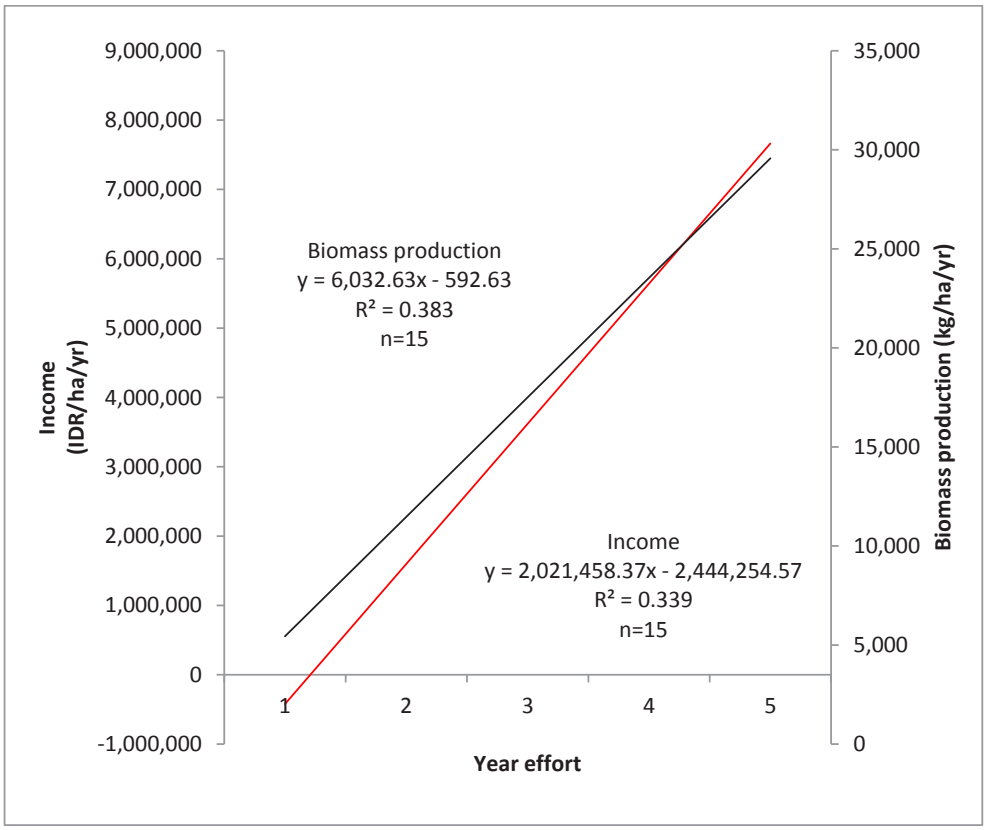

Figure regression equations of production and income of hedge row biomass on five year effort

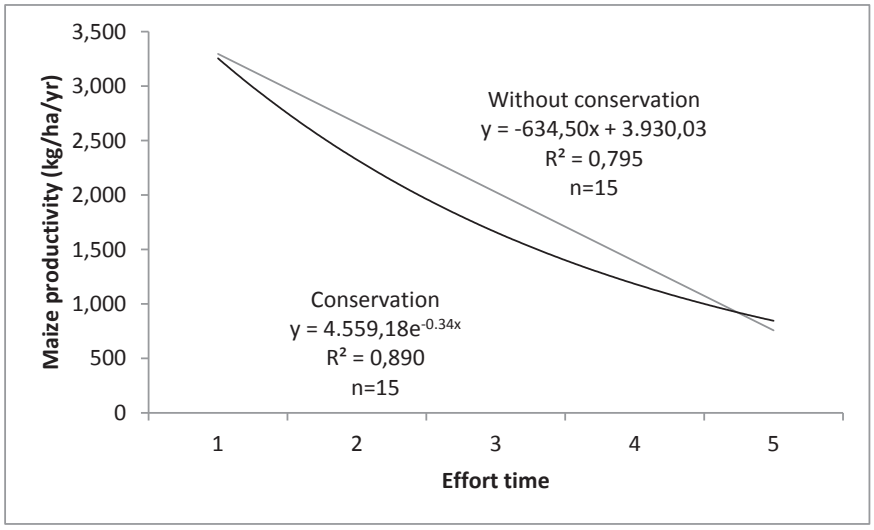

Figure maize productivity on the with and without conservation for five year

Table Soil Erosion Predicted

Konser vation	Cultivation years	R	K	LS	C	P	Mean annual soil lost (t ha^{-1} yr^{-1})
	0	1226.35	0.51	6.37	0.10	0.04	15
Without	1	1226.35	0.60	6.44	0.59	1.00	2,760
Without	2	1226.35	0.51	6.70	0.59	1.00	2,234
Without	3	1226.35	0.56	7.11	0.59	1.00	2,756
Without	4	1226.35	0.44	6.29	0.59	1.00	1,843
Without	5	1226.35	0.48	6.27	0.59	1.00	2,048
	0	1226.35	0.51	6.37	0.10	0.04	15
With	1	1226.35	0.43	4.16	0.59	0.60	1,157
With	2	1226.35	0.32	2.30	0.59	0.40	225
With	3	1226.35	0.39	2.68	0.59	0.40	309
With	4	1226.35	0.34	2.65	0.59	0.40	266
With	5	1226.35	0.35	2.13	0.59	0.40	214

Table Total of Economic Value Soil Erosion Lost Calculation on With and Without Vegetative Conservation for Five Years Cultivation

Soil conser vation	Culti vation years	Soil erosion (kg/ha/yr)	N (%)	P_2O_5 (ppm)	K_2O (mg/ 100g)	C org (%)	Urea lost value (urea prize $USD 0.27/kg)	SP36 lost value (SP36 prize $USD 0.33/ kg)	KCl lost value (KCl prize $USD 0.4/kg	CO_2 lost value (CO_2 prize $USD 10/ ton	Total of economic value soil erosion lost ($USD)
	0	15,318	0.16	15.83	0.60	1.65	14.59	0.21	0.79	9.41	25.00
Without	1	2,760,242	0.15	14.40	0.59	1.78	2,639.97	39.21	132.70	1,781.46	4,593.35
Without	2	2,234,468	0.17	11.44	0.56	1.60	2,263.90	24.46	105.87	1,370.45	3,764.67
Without	3	2,756,257	0.14	13.49	0.51	1.69	2,410.40	35.59	126.89	2,126.56	4,699.44
Without	4	1,842,643	0.14	14.16	0.54	1.57	1,504.58	23.84	94.26	1,038.37	2,661.06
Without	5	2,047,808	0.14	10.21	0.52	1.34	1,755.34	20.63	90.62	1,044.81	2,911.40
	0	15,318	0.16	15.83	0.60	1.65	14.59	0.21	0.79	9.41	25.00
With	1	1,157,290	0.14	21.50	0.57	1.57	1,125.87	34.91	59.71	809.76	2,030.25
With	2	225,352	0.14	22.43	0.53	1.22	193.70	4.12	12.44	99.74	310.01
With	3	309,410	0.16	19.38	0.49	1.30	260.31	5.98	12.28	136.21	414.78
With	4	266,091	0.15	22.00	0.56	1.63	246.88	6.69	13.55	175.45	442.56
With	5	214,105	0.12	17.37	0.54	1.37	166.66	3.69	9.62	114.00	293.97

Tabel *Land Equivalent Ratio*/LER on the with and without conservation on the five year effort

Years effort	Revenue on the without conservation	Revenue on the with conservation	LER value
1	4,604,444	7,480,843	1.63
2	4,382,222	7,474,165	1.71
3	2,931,111	8,176,754	2.79
4	1,613,333	10,865,899	6.74
5	-146,032	9,729,751	∞
Total	13,385,079	43,727,413	3.27

Recommendation of hedge row distance for West Timor Island

Slope (%)	Prompts hedge-row spacing (m)	Number of row per hectare	Width of field for food crops (m)	Forecast of wet biomass feed crop production (t year^{-1})	Forecast of maize production (t year^{-1})	Description
>40	-	-	-	-	-	Priority for forest
25-40	1,25 - 2	48 - 73	0,25 - 1	136 - 218	0 – 2,25	
15-25	2 - 3,3	29 - 48	1 – 2,3	82 - 136	1,14 – 2,72	
8 - 15	3,3 – 6,25	15 - 29	2,3 – 5,25	44 - 82	1,38 – 4,21	
<8	6,25 - 10	9 - 15	5,25 - 9	27 - 44	2,13 – 5,48	

Reference

Abdulrahman A and S. Sutono. 2002. Erosion Control Technology Dryland in Admihardja.A, Mappaona and Arsil Saleh. 2002. Management Technology Dryland Towards Productive and Sustainable Agriculture. The research and development of land and agro-climatic. Agricultural Research Agency.

Alexander, Kasterine. 2010. Organic Agriculture and Carbon Markets. From: FiBL & IFOAM 2010: The World of Organic Agriculture. Statistics and Emerging Trends 2010. Bonn and Frick.Arsyad, S. 2000. Soil and Water Conservation. Publisher IPB Press. Bogor Aspatria, utma.,

Fredrik L. Benu, I.W. Mudita and Remi. L.Natonis. 2007. Design Model Dryland Environmental Farming Systems To Improve Food Security Penggungsi Former East Timor in Belu, East Nusa Tenggara province. Proceedings of the National Seminar on Communication Research Results Agriculture And Livestock In Dryland Farming Systems. The Center for Agricultural Technology Assessment and Development (BBP2TP) Bogor

Bamualim, A and A.Saleh. 1992. Forage Production and Quality of Food Cattle in Nusa Tenggara. Annual Assessment Meeting 15 to 18 September 1992. Mataram, Lombok, Indonesia. Indonesia-Australia Development Programme.

Basuki, T., H. da Silva, J.Bobihoe, Wirdahayati RB, and A. Bamualim. 1998. Agroecology Zone Map Kupang District Scale 1: 100,000. Farming Systems Research Project East. BPTP Naibonat. Kupang.

Bobihoe, J., B.Murdolelono, A.Hipi and A.Bamualim. 1999. Farming Systems Assessment (SUP) Corn in Belu, East Nusa Tenggara. Proceedings of the Regional Workshop on Indigenous Technology and Advanced Technologies Supporting Agricultural Development in Nusa Tenggara. Kupang 1-2 March 1999.

BPS NTT province. East Nusa Tenggara 2007. In Figures. Kupang

BPTP NTT. 2010. Development of Climatic Data Climatological Station on Tobu village. 2003 - 2009. Budget Project Report BPTP NTT. Unpublished.

Clark, R. 1996. Methodologies for the Economic Analysis of Soil Erosion and Conservation. Working Paper GEC 96-13. Centre for Social and Economic Research on the Global Environment (CSERGE). Research Centre of the U.K. Economic and Social Research Council (ESRC). ISSN 0967-8875.

Da Silva, H., Wirdahayati RB and Bambang M. 1999. Map zoning Commodities Based on Land Suitability North Central Timor regency of East Nusa Tenggara Scale 1: 100,000. NTAADP project.

Dariah, A., U. Haryati, and T. Budhyastoro. 2004. Conservation of Mechanical Technology. P. 109-132 in Dryland Soil Conservation on slopes. Centre for Research and Development of Soil and Agro-climate. IAARD. Agriculture department.

Djauhari, A., and A. Sham. 1996. Management of Dryland in the Brantas River Basin upstream section. Forum agro-economic research. 14 (1): 24-40.

Foth, H.D. 1995. Fundamentals of Soil Science. Gajah Mada University Press. Yogyakarta.

Hafif, B., Maswar, M. Suhardjo, Supriya and A.A. Idjudin, 1995. Potential and effectiveness of some of Land Stabilization Techniques in Critical Nawungan Hills Region of Yogyakarta. Proceedings of the Workshop and Technology Exposure Conservation Farming Systems and Agricultural Machinery. Research Center for Soil and Agro-climate. Bogor.

Hariadi, S and Wawan Gunawan. 2007. Identification and Evaluation of Potential Land to Support Prima Tani in the village of Tobu-district. North Mollo Kab. South Central Timor, East Nusa Tenggara. Research Institute for Agro-climate and Hydrology. IAARD.

Haryati., D. Sudjarwadi and Kartiwa, 1998. Effect of Plant Strengthening terrace against erosion and Food Crop Production in Upstream Cimanuk Watershed. Proceedings of the National Workshop Discussion of Watershed Management Research: Alternative Technology Soil Conservation. Secretariat Control Team Reforestation and Afforestation Assistance Centre in collaboration with the Research Center for Soil and Agro-climate. Bogor.

Jones, Christine. 2006. Soil and Carbon Credits. http://soilcarboncredits.blogspot.com/. Downloaded October 20, 2010. Regional Office of the Provincial Agriculture Department NTT. 1998. Map zoning Commodities Based on Land Suitability South Central Timor regency of East Nusa Tenggara Scale 1: 100,000. Resource Development Project Agricultural Infrastructures.

Kartodihardjo, H. 2006. Problems and Forest and Land Rehabilitation Policy. Open Discussion Paper on Forest and Land Rehabilitation: Policy, operations and new ideas. Bogor Agricultural Institute.

Mahfouz. 2001. Critical Land Productivity Improvement: To Fulfillment of Food Through Conservation Farming. Papers Philosophy of Science. Graduate program. Bogor Agricultural Institute.

Margarath, W.B. and P. Arens. 1989. The Cost of Soil Erosion in Java: A Natural Resources Accounting Approach. Environment Dep. Working Paper 18, 1989. World Bank. National Watershed Management and Conservation Project (NWMCP). 1998. The Use of The Universal Soil Loss Equation in the RTL Process. Technical Report No. 30 DHV Consultants BV Amersfoort, the Netherlands - PT Mapalus Menggala Engineering. Bandung, Indonesia.

Nawastti, Dati. 2008. Shifting System With Slash And Burn, Cause Damage to Forests in NTT. Kabar Indonesia 13-Okt-2008 ttp://www.kabarindonesia.com/berita.php?pil=4&jd=PERLADANGAN-DENGAN-SISTEM-TEBAS-DAN-BAKAR,-PENYEBAB-KERUSAKAN-HUTAN-DI-PROVINSI-NTT&dn=20081013004840. Downloaded October 28, 2009

P3HTA, 1993. Final Report UACP-FSR. IAARD.

Pura WOHA, U. 1995. Developments and Opportunities for Development of Agriculture in East Nusa Tenggara, p. 1-4. In: I.K.Tastra, Sunardi and A.Winarto. Technology to Increase Productivity of Food Crops in East Nusa Tenggara province. Special Edition Balitkabi 3. 1995. Balitkabi. IAARD. Research Center for Soil and Agro-climate. 1996. Erosion Control With gulud terrace. IAARD. Agriculture department.

Rachman, A., R.L. Watung and U. Haryati, 1989. The role of cover crops in Erosion Control Tampingan patio bench in Latosol Soil Ungaran. Minutes of the Scientific Discussion Dryland Agriculture and Conservation in the watershed. Forest Rescue Research Project, Land and Water. IAARD.

Rahim, S.E. 2006. Soil Erosion Control in the Context of Environmental Conservation. PT. Earth Literacy. Jakarta

Roshetko, J.M and Mulawarman. 2001. Agroforestry in Nusa Tenggara: Summary of Workshops. Proceedings of the Workshop Agroforestry Se-Nusa Tenggara 11 to 14 November 2001 in Denpasar, Bali. International Centre For Research In Agroforestry and Winrock International

Sarief, S. 1986. Soil and Water Conservation. Reader Buana. Bandung.

Soelaeman, Y. 1999. Integration of Ruminant in Conservation Farming Systems in Bantul Regency of Yogyakarta Special Region. Bulletin Ranch. Extra Edition, December 1999. Faculty of Animal Husbandry. Gadjah Mada University in Yogyakarta. Spash, C. L. 1997. Environmental Ethics and Attitudes with Implication for Economic Valuation. Journal of Environmental Management. Vol 50. Academic Press.

Steer, A. 1996. Ten Principles of the New Environmentalism. Finance and Development.

Subandi, Jamaladin, E.O.Momuat and A.Bamualim. 1997. Dryland Farming Systems in Nusa Tenggara. Proceedings of the Regional Seminar Research Results Based Fisheries Agriculture, Animal Husbandry and Farming Systems Eastern Region of indonesia. BPTP Naibonat cooperation with the Department of Primary Industry and Fisheries Darwin, Northern Territory, Australia. IAARD.

Sudharto, T., Lintong R.B, Irawan and H.Suwardjo. 1994. Management of Dryland on the location of shifting cultivation in South Sulawesi. Proceedings of the Meeting of Consultation of Land Resource for Development of Eastern Indonesia. Soil and Agro-climate research center. IAARD.

Sukmana, S. and H. Suwardjo. 1991 Prospect of Vegetative Soil Conservation Measure for Sustainable Agriculture. Indonesia Agriculture Research and Development Journal Vol.13. Bogor

Taryono.1997. Land Resources. Lecture diktat. Faculty of Geography, University of Muhammadiyah Surakarta

Turner, R.K., D.Pierce and I.Bateman. 1994. Environmental Economics. Pearson Education Limited. London.

Utomo, W.H. 1987. Erosion and Soil Conservation. Communications Soil Science Unibraw no. 23. Brawijaya University of Malang.

Wikipedia. 2010. Wood Ash. http://en.wikipedia.org/wiki/Wood_ash. Downloaded December 15, 2010

Wirdahayati, R.B. 2007. Development Technology Support Against Cattle In East Nusa Tenggara. Proceedings of the National Seminar on Communication Research Results Agriculture And Livestock In Dryland Farming Systems. The Center for Agricultural Technology Assessment and Development (BBP2TP) Bogor Wischmeier, W.H and D.D. Smith. 1978. Predicting Rainfall Errosion Losses: A Gude to Conservation Planning. Sci. and Educ. Adm. USDA in Corporation with Purdue Agric. Exp. Sta.

Joseph and Bernard B. de Rosary. 2001. Study of Adoption and Impact of Application Package Recommendations Agriculture in the province of East Nusa Tenggara. BPTP NTT reports. IAARD.

PO-05-51

THE APPLICATION OF SCIENCE AND TECHNOLOGY THROUGH UTILIZATION WASTE OF CORN AS LOCAL CATTLE FEED IN SUBDISTRICT SANGKUB

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ABSTRACT

Local farms particularly cattle, is one of the subsector, contributing to the improvement of people's income. The problem, is a local cattle feed is not available continuously. This research has been conducted with the aim to analyze the extent of development of local beef, with regard to feed, as well as evaluating the empowerment of farmers through the application of science and technology. This research has been conducted using the method of the survey and field observation. Samples are farmers who are in the district Sangkub. Furthermore, the empowerment of 10 farmers through the application of science and technology for the preservation of grass (silage) and ammoniation. The results showed that the grass for cattle feed is not available continuously, and to acquire the farmer must provide about 1-2 hours. Most cattle consume corn waste. Farmers planting corn to meet the needs of cattle feed. Corn waste is only available within a certain time, so as to further local cattle consume corn straw is dry. In fact, the waste from corn, which is dry, low quality. Farmers then trained to make silage and ammoniation. In conclusion, farmers do not know how preservation/waste fermentation of corn, in the form of silage and ammoniation. Farmers who are trained, can respond well to the training. Suggestions submitted, need government intervention, to give presents silo, as a shelter silage and ammoniation.

1. Introduction

Sangkub is one area district in North Bolaang Mongondow, geographically, has a strategic position and significance for North Sulawesi province because it is located on border with the province of Gorontalo. Livestock, especially cattle, is one reliable subsector to increase incomes. However, in reality growth of cattle population in this area is slower than other regions. This is why government in area of research remains encouragement with regard to cattle development. However, this is possible if we are able to manage a strategy for provision of forage (Lesmana, 2011). The slow growth of the cattle population by Herianti and Subuharta (2013), one of which is determined by external factors such as the availability of feed is not sufficient. Government as a motivator in development of cattle need information about availability of feed in area of research. Saragi (2014) suggests that information about availability of feed is needed in development of cattle farming.

The problem is not yet available feed continuously. Feed is the main problem faced by farmers (Elly, 2008; Elly et al, 2008; Salendu 2012 and Susanti et al, 2013). Feed for cattle mostly in form of forage. This means that forage is main feed source for cattle. This phenomenon indicates, to increase cattle production should be followed by an increase in provision of sufficient forage. Provision of forage intended, both in quantity and quality. Forage consists of grass and legume, which is commonly given to cattle is usually derived from agricultural lands, dikes, and roadsides. Forage needed are not available continuously, due to several factors that inhibit. These factors include changes in land use into a residential area. Based on these problems has done research that aims to study extent of development of cattle, with regard to feed, as well as evaluating empowerment of farmers through application of science and technology.

2. Methods of Research and Application

This research was conducted using of survey and field observation. Samples of farmers determined by purposive sampling, that farmers in Sangkub and have a minimum of two head of cattle. Furthermore, empowerment conducted for 10 farmers through application of science and technology for preservation of grass (silage) and ammoniation. Community empowerment is an attempt to enable and independently, motivate, and raise awareness of potential of farmers to more efficiently and effectively (Mutiawardhana et al, 2013). The application of science and technology conducted using extension and training. Data was analyzed descriptively.

3. Results and Discussion

Results showed that type of grass that is known to farmer is a wild grass. But grass is not available continuously and to obtain farmers provide 1-2 hours. Forage availability was also affected by climate, so that there is a shortage in dry season, otherwise abundant in rainy season. Agricultural wastes derived from food crops have potential to feed according to Nurdiati et al (2012) one of which is corn waste. Farmers use corn waste as feed during forage shortage. That is, farmers in the area of research to develop cattle by utilizing corn waste. Corn waste are abundant, as farmers in this area develop a corn plant that is supported by government programs. Bahri (2012) reported a byproduct of corn is a source of feed ingredients available locally throughout year. Kusumaningrum and Suharyono (2013) suggested other than as a staple crop, corn waste (leaves and stems) can be used as feed.

The results showed cattle consume corn waste in form of fresh and dried. Feeding is not just limited to wet ingredients, but also material preserved (Lester, 2006). Farmers plant corn to meet needs of feed. Corn waste is only available within a certain time, then cattle consume corn straw is dry. Whereas dried corn waste, lower quality. However, Kusumaningrum and Suharyono (2013) declared nutritional content of corn waste can be improved through a process feed processing technology, including making of silage. Rauf (2013) suggested that one corn waste treatment technology that ammoniation.

The results showed that 100% of farmers do not have knowledge of feed processing. Farmers not knowing how to preserve or fermenting corn waste. The indication is necessary to empower farmers. According Syamsu (2011), farmers do not understand that feed technology, can improve quality of corn waste so as to increase productivity of cattle. Sugiyo et al (2013) stated government's role significantly affect empowerment process. Based on this research, farmers are empowered through technology introduction silage and ammoniation of corn waste.

Farmers were trained for preservation of fresh corn waste in form silage. This is done to overcome if there is excess production and can be used in dry season. Ratnada (2004) reported production of natural grass fluctuate depending on season. Silage is provided for needs of cattle feed. The main purpose making of silage by Schroeder (2004) and Jones et al (2004) is to preserve and reduce loss of nutrients fresh straw to be used later. Silage is produced by way, waste of fresh corn is cut 2-5 cm by farmers, then sprinkled rice bran (Figure 1), then put into an airtight plastic bag. Plastic bags are fully stocked and dense, then is sealed (tied). Making process for 21 days and after opening fragrant and a little acidic. Making silage is very responded by farmers.

Figure 1 shows process of making silage conducted with farmers. Silage, is feed from fermentation of forage, byproducts of agriculture and agroindustries with high water content are preserved using acids, either intentionally added or naturally produced (Bahri, 2012). Such materials during storage under anaerobic conditions, and these conditions were maintained until time opened.

Remains of agricultural produce, also can be used as a source of forage such as rice straw leaves and cobs of corn and others. Rice straw has a high fiber content and low energy levels so low digestibility value. Kardiyanto (2009) states need a treatment, that is easily digested, by fermentation process. Procedure of making straw ammoniation of corn (Figure 2), the straw has dried (water content of 60%) cut 2-5 cm, then stacked in a plastic bag until solid. Straw in a plastic bag sprinkled with probiotic and urea by comparison each 6 kg for every ton rice straw. Water was sprinkled into straw, until moisture 60%, to cultivate probiotics. Water looks pretty if straw kneaded and palms as if water would drip. Stages was repeated every straw 15 cm to plastic bags full, then closed plastic bag and tied up, were left for 21 days at the site, which is protected rain and direct sunlight. After 21 days of fermented ready to be given to cattle.

Figure 2 is a ammoniation process conducted with farmers. Objective ammoniation process, improve quality of feed materials low nutrient content and digestibility (Rauf, 2013). Advantages ammoniation process according to Directorate General of Livestock (2011) which increased digestibility, increased protein and inhibits growth of fungi. The application of science and technology conducted through empowerment of farmers with aim of increasing productivity of cattle. Utomo and Rasminati (2010) did introduction of forage processing technology with 100% success. Application of science and technology conducted by approach system integration cattle plants. Model community empowerment conducted by Mutiawardhana et al (2013) is integration of agriculture and animal husbandry. System integration of cattle and plants with zero waste approach is a refinement of intensification system that has developed in community (Wulandari, 2014).

4. Conclusion and Suggestion

Based on research and application can be concluded that farmers do not know how preserve/fermentation of corn waste in form of silage and ammoniation. Farmers who are trained to respond with good training silage and ammoniation process.

Suggestions submitted need government support to make the silo as silage and ammoniation shelters.

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Keywords: Science, Technology, Cattle, Silage, Ammoniation

PO-05-52 DEVELOPMENT OF AGRIBUSINESS FOR FARMING OF NATIVE CHICKEN IN THE SUBDISTRICT OF SOUTH KOTAMOBAGU

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Abstract

Native chicken is one alternative to businesses that do not require a lot of capital and a large area. Products of native chicken (eggs and meat) is favored by the people. In fact, most people, in the subdistrict of South Kotamobagu (about 65%) have a native chicken. The problem is, the development of the native chicken by society to do with the traditional system. The purpose of research that has been done, is to determine the extent of the role of chicken farming village, in providing benefits to farmers. This study was conducted using a survey method with direct observation in the study site. Results of research have shown the breeds of native chicken, are not yet available continuously. Breeds of native chicken obtained by incubation naturally. Native chicken reared in the home page and consuming feed from the remains of the kitchen, so the productivity is low. Feed, purchased from Poultry Shop at a price of about Rp 8,000 per kg. South Kotamobagu a district, which has, the largest irrigated land, among four other districts in the City of Kotamobagu, which is an area of 2143.50 ha (55.24%) of the total irrigated land in this area. The indication, that the chicken feed is not a problem if farmers take advantage of available resources. Prices of native chicken per head Rp 50,000. The value of R/C ratio greater one, thus it provides adequate reception for farmers. In conclusion, sub system of agribusiness native chicken has not been integrated. This is show business carried on not business oriented. Suggestions, need government intervention in developing the agribusiness of native chicken, with sustained.

Introduction Native chicken, to date, still has an important role in supporting needs of meat and eggs. The indication, development native chicken, according Hidayat (2012), it is located in a national livestock development in future. Siahaan et al (2013) suggested native chicken, is supporting development of poultry farm in countryside. Native chicken has an important role in development of animal husbandry (Melviyanti et al. 2013). It is mainly in supply of meat has a distinctive flavor and texture than broilers.

The demand for native chicken tend to increase due to an increase in population, income and awareness of importance of nutrition. This phenomenon indicates product native chicken important in supporting needs of consumption of animal protein. The government should encourage poultry industry in an effort to supply products native chicken. Wulyono and Daroini (2013) suggests that poultry industry in that it has a strategic value, especially in supply of animal protein.

In fact most societies South Kotamobagu Districts (about 65%) have a native chicken. The problem is development of native chicken by society, is still traditionally. Although, reality native chicken have better resistance to disease (Nataamijaya, 2010). Cultivation local poultry has opportunity to be developed mainly native chicken (Dewi et al. 2012). Based on these ideas, has done research on development of agribusiness farming native chicken. The purpose of this study to determine extent of role native chicken farming in providing benefits to farmers.

Methods of ResearchThis study was conducted using a survey method with direct observation. The data collected is a cross section taken over last month. South Kotamobagu villages in district have been determined by purposive sampling Village Poyowa Besar Dua with consideration of this village has highest number native chicken farmers. Respondents of 8 people who have been determined based on farmers who are members of a group native chickens. Analysis of data used is descriptive analysis.

Results and Discussion The village Poyowa Besar Dua, according to results of research, most earn income sourced from agriculture, including food crops (rice, maize), coconuts and livestock. Most societies (70%) are farmers. Farmers in question is owning farmers, sharecroppers and agricultural laborers.

Research results showed feed purchased from Poultry Shop at a price of Rp8,000/kg. South Kotamobagu is a district that has largest irrigated land among other districts in city Kotamobagu ie covering 2143.50 ha (55.24%) of total irrigated land in this area. The indication, native chicken feed is not a problem if farmers utilize available resources. Abun et al (2007) suggested cost of feed is largest production cost in native chicken farming

intensively. The indication is required effort to find alternative feed is cheap, easy to obtain, quality is good, and does not compete with food. Wulandari et al (2012) suggest chicken of any kind requires a good rations with adequate nutrient content. Feed is largest expenditure at around 70% of total production costs (Magdalena et al., 2013). According Aryanti et al (2013), if feed consumption is good then weight gain would also be good. Singarimbun et al (2013) state feed protein has an important role in improving quality of chicken carcasses.

Research results showed sales native chicken that has been done is still limited in area of research with price of Rp50,000/head. Value of RC ratio is 1.5 (greater than study of Yuwono and Prasetyo, 2013), thus it is still profitable for farmers. These results indicate development native chicken as food products complement in supply of poultry meat today has good prospects (Yuwono et al. 2012). The role native chicken can not be ignored because it is supporting efforts to increase contribution of local livestock in order to strengthen food security programs (Suthama et al. 2012). Farmers can determine strategy to support development of native chicken. David (2013) argues should farmers focus on strategies to reduce impact and probability of source of risk of disease.

Research results showed native chicken development in study area have not been oriented agribusiness. Agribusiness according Mappigau and Esso (2011) includes a upstream agribusiness subsystem, farming subsystem (technical production), downstream agribusiness subsystem and subsystems of supporting services. Activity in agribusiness sector include any one or a whole chain of production inputs, farming, processing and marketing including native chicken breeders (Kurniawan et al. 2013). Subsystems of production inputs focused on activities of procurement and distribution of production inputs, especially breed and feed. Research results showed native chicken breed is not available continuously. Breeds of native chicken have been produced by means of hatch naturally. This causes a slow increase in population. Breeding farm that produces eggs for incubation and hatching eggs is needed in this area, as stated Yuwono and Prasetyo (2013).

Subsystem of farming includes farming agro-climate physical condition of production, producers breeder structure and scale of business, performance and production constraints. In fact, native chickens were left in yard, not grounded. In fact, maintenance technology is a determining factor in overall native chicken farming (Suryana and Hasbianto, 2008). An important aspect in building a system of agribusiness, among others, to build a system of competitive livestock farming.

Processing subsystems, which play a role in initiatives forms and types of processed products, difficulties in processing, processing capacity, volume processed, and product prices. Farmers' knowledge about aspects of processing is still low. In this process added value native chicken products will be increased so that increase farmers' income. Livestock processed products needed to support food diversification program today and in future. Agribusiness development can improve availability, distribution and competitiveness native chicken products for industrial cake or bread (Saptana and Sartika, 2014).

Marketing subsystem includes a chain of domestic marketing and export (primary or processed products), composition of perpetrators of marketing and product marketing constraints. In fact, marketing of native chicken have been done only in area of research. In fact, domestic poultry have large market opportunities. Farmers can increase market share through improving quality and quantity of products, improve competitiveness, and cooperation with relevant parties (Kurniawan et al. 2013).

Supporting institutional subsystem includes a means trading system (infrastructure), banking services or credit and other supporting institutions. Cooperation with institutional support yet, so farmers do not have capital for business development toward agribusiness. Funding limitations is weakness of agribusiness development in Kurniawan et al (2013). The concept of native chicken agribusiness can be developed with reference to Elly (2012) as shown in Figure 1.

Figure 1 shows development of agribusiness, in this case not only development of farming subsystem (on farm agribusiness) but also includes up stream agribusiness subsystem and down stream agribusiness subsystem. Role between subsystems in agribusiness are interrelated and determine (Budiarsana and Hidayat, 2012). Native chicken agribusiness development provides opportunities in employment and income generation.

Conclusion and SuggestionBased on results of this study concluded, sub system of agribusiness native chicken has not been integrated. This is show business carried on not business oriented.

Based on research results suggested, need government intervention in developing the agribusiness of native chicken, with sustained.

PO-05-53

BENEFIT OF INTEGRATION CATTLE-CORN FARMING IN SANGKUB SUBDISTRICT

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Abstract

Corn is known as the dominant commodity in the Sangkub Subdistrict. The corn crop was developed in this district in supporting the development of cattle. Cattle have a comparative advantage to be developed in this area. The problem is, how the benefits of the integration of cattle corn in Sangkub Subdistrict. Based on these problems, this research has been done with the aim of analyzing the benefits of the integration of cattle corn. The research method that has been used is survey method, with the source of data is the primary data. Sample of respondents was determined by purposive sampling, namely corn farmers who develop cattle. The number of respondents as many as 30 farmers. Analysis of the data used is descriptive analysis. The results showed that the age of majority respondents considered productive (93 percent), with the highest levels of education, is a graduate (3.33 percent). Farmers plant corn, then the waste is utilized by cattle as feed. The results of the analysis of R/C ratio is greater than one, which indicates that the integration of cattle and corn farming provide higher revenue for farmers. Based on the results of this study concluded that the integration of cattle corn, providing benefits to the development of cattle. Waste of corn used as cattle feed, so in this concept no waste is disposed of. Based on the research suggested, it is necessary for the introduction of feed technology, from waste corn in order to feed continuously available and sustainable.

Introduction

Corn is known as dominant commodity in the District Sangkub. The corn crop, according Kusumaningrum and Suharyono (2013), is one of the plants that have an important role in achievement of food security. Corn plant can be grown in different regions, with different climates, from temperate zones to temperate sub-tropical/tropical wet (Bahri, 2012). The corn crop, according Wulandari (2014), is a plant which can be grown in sub-optimal land with various management. The corn crop was developed in this district in supporting development of cattle.

Cattle have a comparative advantage to be developed in this area. However, cattle production systems in Sangkub generally still done traditionally. Farmers and government in this regard should seek to maximize utilization of available resources to accelerate development of animal husbandry. Government by Bahri and Tiesnamurti (2012) need to prepare a livestock development strategy, in a sustainable manner, by utilizing availability of local resources. Cattle can be developed in an integrated manner with food crops and this approach is known as system integration of livestock and crops (Integrated Farming System). Cattle in study area are integrated with corn. Integration cattle-corn by Baba et al (2014) is an attempt to combine corn and cattle in the farming system by farmers. Corn wastes can be used as cattle feed in form of fresh or after being processed or preserved. Furthermore, cattle waste can be used as fertilizer for corn crop.

System of integration cattle-corn showed all potential resources possessed by each cattle farming are used optimally. Optimal use of available resources is known as principle of \"zero waste\". This activity shows no waste or byproduct which is wasted. Overall integration system is geared towards improving efficiency and value-added economy. Agricultural model zero waste by Sunanto and Nasrullah (2012) is a model of agriculture that does not let a byproduct becomes waste/not beneficial. The problem is extent of the benefits of the integration of cattle corn in Sangkub District. Based on these problems, this research has been done with aim of analyzing benefits of the integration of cattle corn.

Methods of Research

This research method used in Sangkub District is a survey method. Source of data collected is of primary data based on interviews with farmers. Determination of sample is purposive sampling namely corn farmers who have cattle. The number of respondents as many as 30 farmers. Analysis of data has been using descriptive analysis.

Results and Discussion

The success of farm cattle, in addition is determined by three elements, which are interrelated (use of breed, feed

and management), as well as by characteristics of farmers. Characteristics in question include age and education of farmers as respondents. The results showed that age of respondents largely categorized productive age (93 percent), with highest educational level undergraduate (3:33 per cent). Farmers, whose age is higher, to a certain extent, according Kiswanto et al (2004), led to ability to work is increasing, resulting in increased productivity. The level of formal education higher can cause a person more rational thinking (Kiswanto et al, 2004).

The results showed that corn crop farming combined with cattle. Farmers planted corn and waste given to cattle. Corn by Bahri (2012) is largest food crop after rice, as a byproduct is also potentially serve as a source of cattle feed. The indication, complementary cycles as waste is manageable in integration cycle becomes input for corn crops and livestock. Corn wastes managed and farmers are able to reduce dependence on external inputs for their farming activities. Reduction in use of external inputs impact on increasing added value of waste corn. Bahri (2012) reported that 50% of total weight of corn crop is a byproduct left after harvest. Fresh corn production by Rauf (2013) 9.40 tons/ha, dry production of 6.82 tons/ha and dry matter production of 5.86 tons/ha.

The quality of beef cattle production are closely linked to quality local feed (Harfiah, 2007). Cattle by Alfian et al (2012) can utilize feed material in form of forage, including from agricultural waste. This is due to grass and other forage in Sangkub District very limited. Integrated farming is right choice because of limited ability of agricultural resources (Wulandari, 2014). System integration corn-cattle (SIJS) according Wulandari (2014) is one of alternative model of integrated farming system on dry land.

The results showed that average income per year, which is received by farmers from sale of cattle Rp 5,663,333.33 at a cost of Rp 3,764,063. RC value ratio, obtained at 1.50, indicating that cattle farming provide higher revenue for farmers. Value of R/C, is still greater than results Wahyuni (2015) which shows value of R/C of 1.13. Rundengan research results (2013) show that agriculture-livestock farming systems integrated with farming of cattle-coconut-corn earn income as well as the optimal financing through farming system of cattle-coconut-corn.

Mansyur et al (2009) suggested the pattern of integration of crop-cattle has many advantages such as availability of feed resources, reduce cost of controlling weeds, improve soil fertility, increase yield of major crops and share risk of loss. Crop-Livestock Systems Integration Non Waste (SITT-BL) provides an opportunity to green and clean agricultural development (Haryanto, 2009). Integrated farm management which led to a cycle of mutual benefit, which in turn can increase productivity by utilizing by-products so as to provide maximum results (Utomo and Rasminati, 2010). Farming management in an integrated manner between cattle and crops by Rota and Sperandini (2010), is a key solution to improve livestock production and protecting environment through use of resources, efficient. Management of integration cattle-corn less risky, if managed efficiently can provide benefits and generate environmental health (Walia and Kaur, 2013).

Corn waste used by farmers as animal feed. Waste utilization plant food, as feed, including corn straw (Rouf, 2010 and de Lima, 2012). Cattle dung used as organic fertilizer and biogas to be used farm household. The results showed that majority of farmers has leveraged cattle dung as fertilizer. The integration of such a form interconnected with each other to form a continuous cycle and are able to shut down (Suroyo et al. 2013). This causes creation of sustainable agriculture. Sustainable agriculture is achieved with sustainable soil productivity, while sustainable land productivity can only be achieved if it is managed in an integrated manner. One of key according to Suroyo et al (2013) is to maintain soil organic matter content.

According Sutrisna et al (2014), there are some limiting factors if dry land used for food crops include low soil fertility, reacted sourly, containing Al, Fe or Mn in high amounts that can be toxic to plants. Dry land is an area with a lower quality (Syahriani, 2014). On that basis, the optimization of dry land resources require technology that is able to overcome fertility problems. Farming systems integration food crops such as corn and cattle was spot developed on dry land (Sutrisna et al, 2014), soil quality can be improved by establishment of nutrient cycling and soil structure more stable (Syahriani, 2014). The main characteristic of integration of crops and cattle is their synergism or association of mutual benefit between cattle and corn. This resulted in an increase in household incomes could further improve welfare. Wulandari (2014) argues that there are three main technology components in the system integration cattle-corn, namely: (i) animal breeding technology, (ii) corn cultivation technology; and (iii) the processing technology of fodder and compost as well as storage and improved nutritional quality of feed.

Conclusion and Suggestion

Based on results of this study concluded that integration of cattle corn crops provide benefits to cattle

development. Waste from corn crops used as feed so that this concept no waste is wasted.
Based on research results suggested introduction of feed technology needs, from corn waste, in order to feed continuously available.

Keywords: Cattle, Corn, Sustainable

REFERENCES

Alfian, Y., F.I. Hermansyah., E. Hardayanto., Utoyo and W.P.S. Suprayogi. 2012. Analysis of Carrying Capacity Ruminant on Drought in Region of Dryland Agriculture, Semin District, Gunung Kidul Regency. Tropical Animal Husbandry, Vol. 1 (1), Okt 2012, p:33-42.

Baba. S., S.N. Sirajuddin., A. Abdullah and M. Aminawar. 2014. Barriers to Adoption Integration of Corn and Cattle in Maros, Gowa and Takalar Regency. JITP, Vol 3 No 2, Jan 2014. p: 114-120.

Bahri, S. 2012. Response Silage Complete Rations Based Corn Straw as Bali Cattle Feed. Research Report. Department of Animal Husbandry Faculty of Agricultural Sciences, UNG, Gorontalo.

Bahri, S and B. Tiesnamurti. 2012. Sustainable Livestock Development Strategy by utilizing Local Resources. J. of Agricultural Research. Vol. 31 No. 4, Des 2012.p:142-152.

deLima, D. 2012. Production of Agricultural Waste and Livestock Waste and Utilization in District of Rear Huamual and Tanivel, West Seram Regency. J. Agroferestri, VII No. 1 Mart 2012,p:1-7.

Harfiah. 2007. Consumption of Feed Voluntary (Voluntary Feed Intake) Some Forage Sheep. Bulletin of Animal Nutrition and Feed, Vol 6 (1).p: 19-24.

Haryanto, B. 2009. Feed Technology Innovation in Crop-Livestock Systems integration based Waste Supports Efforts to Increase Production of Meat. Centre for Research and Development of Livestock. Development of Agricultural Innovation 2 (3). 2009: 163-176.

Kiswanto., A. Probowo and Widyantoro. 2004. Structural Transformation Fattening Beef Cattle in Central Java. Proceedings of Seminar, and Institutional System Crop-Livestock Farming. Agency for Agricultural Research and Development Department of Agriculture.

Kusumaningrum, C.E and W.T. Suharyono. 2013. Influence use of Complete Feed Based on Maize and Sorghum Waste in Productivity Sheep. Proceedings of National Seminar on Animal Husbandry and Veterinary Technology. p:356-362.

Mansyur., N.P. Indrani., I. Susilawati and T. Dhalika. 2009. Growth and Productivity of Forage Crops under Shade Banana Plantation. Research institutions, University of Padjadjaran, Bandung.

Rauf, J. 2013. Potential Plant Food Waste as a source of feed for the Development of Beef Cattle. Thesis. Graduate program. University of Hasanuddin, Makassar.

Rota, A and S. Sperandini. 2010. Integrated Crop-Livestock Farming Systems. Livestock Thematic Papers. Tools for Project Design. IFAD, International Fund for Agricultural Development, Rome, Italy. www.ifad.org/lrkm/index.htm

Rouf, A.A. 2010. Potential of Agricultural Waste as Cattle Feed in Gorontalo Province. National Seminar, Technology of Animal Husbandry and Veterinary, p:235-242.

Rundengan, M.L. 2013. Optimization of Integrated Farming between Beef Cattle with Coconut Plantation, in South Minahasa Regency of North Sulawesi Province. Doctoral Dissertation. Doctoral Program Livestock, Agribusiness Interests. Faculty of Animal Husbandry, Universitas Brawijaya, Malang.

Sunanto and Nasrullah. 2012. Study Agriculture Model Zero Waste, with Systems Integration Approach Corn-Cattle in South Sulawesi. Proceedings Insinas. p:223-228.

Suroyo., Suntoro and Suryono. 2013. Intercropping and Livestock Integration System to Change Physical and Chemical Properties Land Litosol. Soil Science, Journal of Soil Science and Agroclimatology 10 (1) 2013. P:71-80.

Sutrisna, N., Y. Surdianto and N. Sunandar. 2014. Model Design Integration Crop Sorghum Farming and Cattle on Suboptimal land in West Java. Proceedings of Seminar on Land Suboptimal 2014. Palembang 26-27 Sept 2014. ISBN: 979-587-529-9.p:111-123.

Syahriani. 2014. Repair Quality Dryland through Integrated Agricultural Rambutan, Corn and Gamal in Gowa Regency. Thesis. Journal of Soil Science, Faculty of Agriculture, University of Hasanuddin, Makassar.

Utomo, S and N. Rasminati. 2010. Introductions Forage Processing Technology and Cattle Waste as an Integrated Farming Systems in Tepian Watershed (DAS) Progo District of nLendah Kulon Progo. J. Inotek. Vol 14 No.1,Feb 2010.p:66-71.

Wahyuni, R. 2015. Mastery Structure Land Resources and Contribution of Beef Cattle on Farmer Household Income. Widyariset, Vol 18 No. 1, April 2015.p:79-90.

Walia, S.S and N. Kaur. 2013. Integrated Farming System-An Ecofriendly Approach for Sustainable Agricultural Environment-A Review. Greener Journal of Agronomy Forestry and Horticulture. Vol. 1 (1). Sept 2013, p: 001-011.

Wulandari, W.A. 2014. Integration of Cattle and Corn in Sub Optimal Land in province of Bengkulu. Institute for Agricultural Technology, Bengkulu.

PO-05-54

Effect of days from calving to first insemination on conception rate during the first three lactations of Japanese Holstein cows

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Objectives

Female fertility is important in improving the lifetime productivity of dairy cattle. Genetic improvements in cow fertility have been achieved in many countries by monitoring various reproductive traits (Interbull, 2016). Conception rate (**CR**) or non-return rate for first insemination, and number of inseminations, indicate the cow's ability to conceive. Days open and calving interval are defined as combined traits for the two abilities, i.e., the ability to recycle after calving and the ability to conceive. Genetic evaluation of **CR** and days open for dairy sires and cows has been performed in Japan. However, because the heritabilities of reproductive traits are low (e. g., Jamrozik et al., 2005; Liu et al., 2008; Abe et al., 2009; Hagiya et al., 2013; Yamazaki et al., 2014), optimal reproductive management of cows is also indispensable to improving female fertility.

The **CR** of lactating cows changes with the number of days from calving to first insemination (**DCF**) (e. g., Dhaliwal et al., 1996; Huang et al., 2008). Dhaliwal et al. (1996) reported that **CR**s were significantly ($P < 0.001$) lower for high-yielding cows inseminated within 60 **DCF** than for those inseminated thereafter. Precisely predicting the effect of **DCF** on **CR** in the first and later lactations could help to reveal the optimal voluntary waiting period (i.e., number of days from calving to breeding) during each lactation to maximize female fertility.

Therefore, our objective here was to investigate the effects of **DCF** on **CR** during the first three lactations in Japanese Holstein cows.

Methodology

Data

Insemination records during the first three lactations in Holstein cows whose first inseminations had been recorded between 2007 and 2011 were obtained from the Livestock Improvement Association of Japan (Tokyo, Japan), in which about 8400 herds are enrolled. Monthly test day (TD) milk records within 305 days in milk (DIM) were collected through the Dairy Herd Improvement program of Japan. The data set for the first lactation consisted of records for 475,446 cows; for the second lactation, 379,483 cows; and for the third, 266,652 cows. Each cow had at least eight TD records. Age at first insemination ranged from 20 to 46 months at the first lactation (for second calving), from 32 to 66 months at the second, and from 44 to 86 months at the third. **DCF** ranged from 20 to 200 days. **CR** = 1 indicated that the first insemination achieved pregnancy, and 0 indicated otherwise. The pedigree data for the first lactation included data on 1,212,017 animals; for the second, 1,038,356 animals, and for the third, 802,112 animals, each representing at least five generations.

Summary statistics for **DCF**, **CR**, and cumulative milk yield within 305 DIM (**MILK**) are shown in Table 1. **MILK** was estimated by using multiple-trait prediction (Schaeffer and Jamrozik, 1996) according to Wilmink's function (Wilmink, 1987).

Models

The **CR** data were analyzed within each lactation by using a single-trait linear animal model, even though threshold models theoretically are more appropriate for the analysis of binary data (Gianola, 1982). However, most routine national genetic evaluations of categorical fertility traits (including those in Japan) are based on linear models (e. g., Jamrozik et al., 2005; Liu et al., 2008), because analyses by using threshold models require excessive calculation time.

The **CR**s at various **DCF** were predicted by including the fixed effect of DCF group in the model. The model was:

$y_{ijklmn} = FHY_i + FM_j + FA_k + DCFG_n + s_m + u_l + e_{ijklmn}$,

where y_{ijklmn} is **CR** of cow *l*; FHY_i is the fixed effect of herd year *i* for first insemination (the levels of herd year were

38,906 for the first lactation, 37,761 for the second, and 34,860 for the third); FM_j is the fixed effect of month j at first insemination; FA_k is the fixed effect of age group k at first insemination with 7 levels (18, 19, 20, 21 to 25, 26 to 30, 31 to 40, and ≥41 months); s_m is the random effect of service sire m at first insemination (the numbers of service sires were 9,819 for the first lactation, 8,739 for the second, and 7,415 for the third); u_l is the random additive effect of animal l; and e_{ijklmn} is a random residual effect associated with y_{ijklmn}. The age effect at first insemination was not considered for the third lactation record. $DCFG_n$ is the fixed effect of **DCF** group n created by subdividing the overall range of **DCF** into consecutive 10-day intervals (18 levels: 21 to 30 [**DCF**21–30], 31 to 40 [**DCF**31–40], ... 181 to 190 [**DCF**181–190], and 191 to 200 days [**DCF**191–200]). The covariance structure for the model was defined as

(please insert chart 1),

where σ_s, σ_u, and σ_e are the variances of random service-sire effect for first insemination, random additive genetic effect, and random residual effect, respectively; **A** is the additive genetic relationship for animals; **I** is the identity matrix; and $\otimes$ indicates the Kronecker product.

Solutions for the fixed effects of **DCF** groups were obtained by using the BLUPF90 program (Misztal et al., 2002), which uses the preconditioned conjugate gradient algorithm with iteration on data. The variance components estimated previously by Yamazaki et al. (2014) were used to solve the effects (Table 2).

Results

The averages in the second and third lactations were slightly higher for **DCF** and lower for **CR** than the values in the first lactation (Table 1). The average of **MILK** increased with increasing lactation number. These trends across lactations are similar to previous findings in Japan (Abe et al., 2009; Hagiya et al., 2013). The number of cows in the **DCF**61–70 group as a ratio of total was the highest in each lactation, at 0.160 for the first lactation, and 0.155 for the second and third lactations (Figure 1).

Within each lactation, the **CR**s of the groups for which **DCF** was 60 days or fewer were lower than those of the remaining **DCF** groups (Figure 2). The difference in **CR** between the **DCF**21–30 and **DCF**61–70 groups was –0.14 in the first lactation, –0.12 in the second, and –0.12 in the third. These differences in CR among DCF groups in early lactation were consistent with the findings of Dhaliwal et al. (1996).

The within-lactation differences in **CR** among groups for which **DCF** was more than 60 days were smaller than those among groups for which **DCF** was within 60 days, especially in the first lactation (Figure 2). The difference in **CR** among groups for which **DCF** ranged from 61 to 200 days was, at most, 0.03 in the first lactation, 0.09 in the second, and 0.09 in the third. Cows with very large **DCF** might have experienced delayed recovery of reproductive function after calving and might therefore have been given hormone treatment. In using the insemination records, we could not clarify whether particular cows had been treated for reproductive disorders. Our results regarding **CR** in the large **DCF** groups should therefore be applied with care.

Conclusion

Our results suggest that, for the first three lactations, insemination during the first 60 days after calving decreases **CR** in Japanese Holstein cows. However, especially in the first lactation, delaying the first insemination beyond this point scarcely influences **CR**. These differences between the effects of **DCF** on **CR** should be considered when determining the optimal voluntary waiting period before insemination to improve female fertility in dairy herds.

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Chart 1 covariance structure for the model

$$\mathrm{Var}\begin{bmatrix}\mathbf{s}\\ \mathbf{u}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\sigma_s\otimes\mathbf{I} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \sigma_u\otimes\mathbf{A} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \sigma_e\otimes\mathbf{I}\end{bmatrix}$$

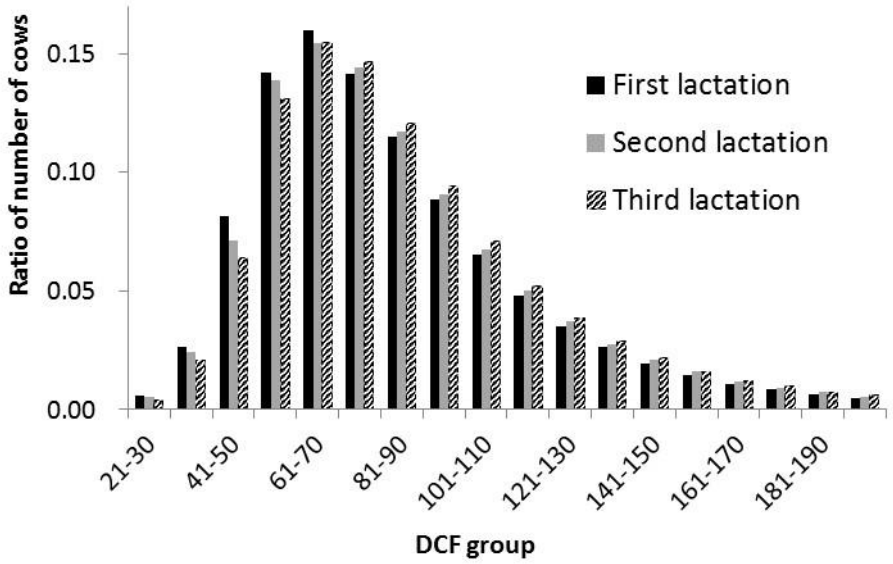

Figure 1 Number of cows in each groups of days from calving to first insemination (**DCF**) as ratio of the total within each lactation

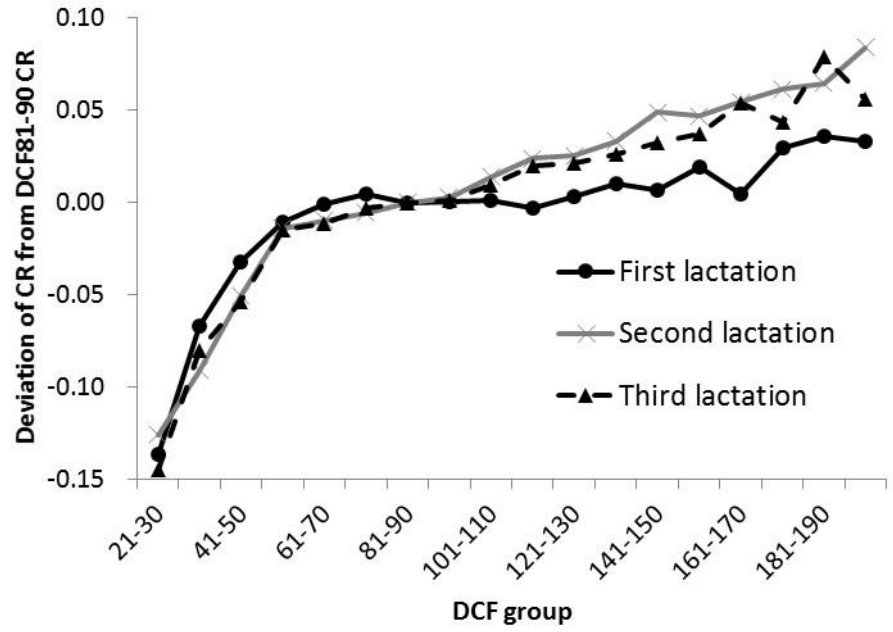

Figure 2 Deviations of conception rate at first insemination (**CR**) for different groups of days from calving to first insemination (**DCF**) from that for the **DCF**81–90 group in the first three lactations

Table 1 Summary statistics of number of days from calving to first insemination (**DCF**), conception rate from first insemination (**CR**), and cumulative milk yield within 305 days in milk (**MILK**) for the first three lactations

Lactation	First		Second		Third	
	Mean (SD)					
DCF (days)	83.1	(31.9)	84.6	(32.3)	85.8	(32.2)
CR	0.38		0.34		0.34	
MILK (kg)	8300	(1522)	9467	(1824)	9815	(1885)

Table 2 Values of variance components for random effects and heritability used to solve the mixed-model equation for conception rate in the first three lactations

Lactation	First	Second	Third
Variance components for random effects			
Service-sire effect for first insemination	0.00081	0.00061	0.00057
Additive genetic effect	0.00507	0.00448	0.00347
Residual effect	0.21883	0.20867	0.20817
Heritability	0.023	0.021	0.0016

These values were estimated previously by Yamazaki et al. (2014).

References

Abe, H., Masuda, Y., Suzuki, M., 2009. Relationships between reproductive traits of heifers and cows and yield traits for Holsteins in Japan. J. Dairy Sci. 92, 4055-4062.

Dhaliwal, G.S., Murray, R.D., Dobson, H., 1996. Effects of milk yield, and calving to first service interval, in determining herd fertility in dairy cows. Anim. Reprod. Sci. 41, 109-117.

Gianola, D., 1982. Theory and analysis of threshold characters. J. Anim. Sci. 54, 1079-1096.

Hagiya, K., Terawaki, Y., Yamazaki, T., Nagamine, Y., Itoh, F., Yamaguchi, S., Abe, H., Gotoh, Y., Kawahara, T., Masuda, Y., Suzuki, M., 2013. Relationships between conception rate in Holstein heifers and cows and milk yield at various stages of lactation. Animal 7, 1423-1428.

Huang, C., Tsuruta, S., Bertrand, J.K., Misztal, I., Lawlor, T.J., Clay, J.S., 2008. Environmental effects on conception rates of Holsteins in New York and Georgia. J. Dairy Sci. 91, 818-825.

Interbull 2016. National genetic evaluation forms provided by countries. Retrieved on 30 June 2016, from http://www.interbull.org/ib/geforms

Jamrozik, J., Fatehi, J., Kistemaker, G.J., Schaeffer, L.R., 2005. Estimates of genetic parameters for Canadian Holstein female reproduction traits. J. Dairy Sci. 88, 2199-2208.

Liu, Z., Jaitner, J., Reinhardt, F., Pasman, E., Rensing, S., Reents, R., 2008. Genetic evaluation of fertility traits of dairy cattle using a multiple-trait animal model. J. Dairy Sci. 91, 4333-4343.

Misztal I, Tsuruta S, Strabel T, Auvray B, Druet T and Lee DH 2002. BLUPF90 and related programs (BGF90). Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, 19-23 August 2002, Montpellier, France. CD-ROM Communication No. 28-07.

Schaeffer, L.R., Jamrozik, J., 1996. Multiple-trait prediction of lactation yields for dairy cows. J. Dairy Sci. 79, 2044-2055.

Wilmink, J.B.M., 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. Livest. Prod. Sci. 16, 335-348.

Yamazaki, T., Hagiya, K., Takeda, H., Yamaguchi, S., Osawa, T., Nagamine, Y., 2014. Genetic correlations among female fertility, 305-day milk yield and persistency during the first three lactations of Japanese Holstein cows. Livest. Sci. 168, 26-31.

PO-05-55

DEVELOPMENT FOR CATTLE UNDER COCONUT TREE BASED ON THE ENVIRONMENT IN THE DISTRICT OF SOUTH KOTAMOBAGU

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Abstract

South Kotamobagu is one of the areas that develop cattle to utilize the land under coconut trees. This condition is done because of the coconut, is a plant that predominate in the region. The problem, as far as the potential of land under coconut trees, in supporting the development of cattle, which are oriented environment. This research has been conducted in order to analyze the potential of land resources, in the District of South Kotamobagu. This study was conducted using a survey method. Village samples were determined by purposive sampling, the village that has the largest cattle population. Data analysis was performed using analysis of Livestock Development Potential Effective for dry land and analysis carrying capacity for land under coconut. The results showed that the value PMSL to dry land of 71,000.00 AU, KPPTR (SL) 70,403.91 AU, PMKK 24,831.00 AU, KPPTR (KK) 24,234.51 AU. The value of the carrying capacity for land under coconut is 7.65, which indicates that the area of land available coconut, then the real population may be increased to 7.65 times. In conclusion, the land under a coconut tree in the District of South Kotamobagu has the potential for the development of cattle, if the land is used as a source of forage. Suggestions that need to be addressed, this potential can be expanded through the approach integarsi cattle, environmentally friendly and sustainable.

Introduction

South Kotamobagu is one of the areas that develop cattle to utilize the land under coconut trees. This condition is done because coconut is a crop that predominate in the region. Cattle in the region has the potential to be developed as a farming reliable to meet demand tends to increase.

Cattle farming development in this area is still done traditionally. Cattle grazing in the coconut plantation and be moved from one land to another land. In fact, the cattle have an important role for farmers, namely as savings, which at times can be sold to meet the needs of farmers and their families. That is, cattle farming can provide added value in farming systems managed by farmers.

The problem is the productivity of cattle in this area is lower than the cattle that exist in other areas, one of the factors that cause is feed. Forage is the main feed for cattle, but its availability is still a problem for farmers. Feed in this case is one of the factors that determine the growth of cattle (Muslim and Nurasa 2008; Prawiradiputra, 2011). Several studies have been conducted Salendu (2012) and Susanti et al (2013), forage is a problem for farmers in different regions. Forage development needs to be done, but the farmers in this case has a problem of limited land for growing forage (Alfian et al, 2012). In addition, according Nurdiati et al (2012), the efficiency of cattle production on a traditional farm, in the area of dry land agriculture is low, so Sutrisna et al (2014) suggest optimizing the dry land resources require technology.

Salendu and Elly (2011) states the land under coconut potential for the development of cattle, because in the land can be developed forage (grass and legume). South Kotamobagu have coconut land that can be used as a source of forage for cattle. The development can be integrated, and this system can be considered as a step forward. The problem is the extent of the potential of land under coconut in supporting the development of cattle which are environmentally. Based on the problems above, has done research on the development of cattle under coconut are environmentally. The study aims to analyze the potential resource of dry land and coconut farm in South Kotamobagu.

Materials and Methods Research

This research material is cattle and coconut farm. This study was conducted using a survey method in South Kotamobagu. The data used as the primary data is published BPS Kotamobagu City (2014). Village samples was determined by purposive sampling, the village which has the largest cattle population. Analysis of data using analysis of the potential development of livestock effective for dryland, and analysis of indexes carrying capacity of land under coconut.

Results and Discussion

Farmers in the area of research, continue to develop cattle by utilizing agricultural lands. This development needs to be done with due regard to the area of agroecosystem. The utilization of agroecosystem is based on the potential of an effective livestock development, and the carrying capacity index of the area. Results of research on the potential of an effective livestock development, and carrying capacity index of land in the South Kotamobagu can be seen in Table 1.

Data Table 1 shows that the maximum potential of dry land resources (PMSL) for South Kotamobagu is 71,000.00 AU. That is based on dry land resources in the District is still able to accommodate the cattle population in the amount of PMSL. Hartono (2012) suggested cattle development can not be separated from the development of farming. Cattle are strategic commodities with multiple functions for dryland farmers (Hermawan and Utomo, 2012). The important factor that must be considered in order to increase the productivity of cattle is the availability of food, throughout the year, both quantity and quality.

The value of the capacity increase in the cattle population by land resources (KPPTR(SL)) in the district of South Kotamobagu as much as 70,403.91 AU. This means that to meet the maximum potential of land resources, the cattle population still can be increased to as much value KPPTR(SL). These efforts can be made to optimize the dry land there. Optimizing the utilization of land resources in support of agricultural development in the future needs to be improved (Mulyani et al, 2011).

Rasminati and Utomo (2010) suggested the availability of forage land will determine the amount of forage as feed. Alfian et al (2012) stated the cattle population exceeds the available capacity so that basic needs have not been met. In contrast to Nugraha et al (2013) which showed that the capacity of ruminants, larger than the population, resulting in the rainy season forage production available in large quantities.

The maximum potential based head of family farmers (PMKK) in South Kotamobagu is 24,831.00 AU. That is based on the availability of labor, with each having three AU, the cattle population can be increased up to the amount of the PMKK. The results of the analysis of the increase in the cattle population by the head of the family (KPPTR(KK)) amounted 24,234.51 AU. This means that the cattle population by heads of family farmers could be increased up to a value KPPTR(KK). Barus (2004) suggested an important aspect in increasing the carrying capacity of resources is the availability of labor. In the agricultural system, which is a community-based, labor is generally derived from farm household (Abdullah et al, 2012).

Carrying capacity index (IDD) according to the results of analysis of 7.65. These results indicate that the carrying capacity of coconut land in South Kotamobagu quite high. This means that the maximum potential coconut land resources are greater than the needs of feed. Based on the potential coconut land, the real population could be increased up to 7.65 of times. Tola et al (2007) suggested the diminished fertile lands caused the development of livestock face a tough challenge, especially to the availability of land resources. This condition indicates that the land under coconut trees in the study area has the potential to be developed. However, this condition needs to be supported by technology forage. Rahmansyah et al (2013) stated the strategy to be successful cattle farming, one of which is the need of the intake technology. Land under coconut trees in the study area has not been used so that cattle only consume agricultural waste and grasses that grow wild. It is supported by Rusdiana and Adawiyah (2013), the plantation land use is not maximized.

Cattle development can be done through the cattle-coconut integration. Management of livestock integrated, both technically and economically by Jayanthi et al (2009) feasible to be developed. This system approach can improve productivity and profitability compared to conventional farming (Mohanty et al, 2010). Studies have been done on the assessment of sustainable integrated farming according to quantitative standards of environmental and socioeconomic benchmarks (Rodrigues et al, 2010). Integrated systems according Syahriani (2014), can improve the quality of dry land and increasing farmers' income. Swarnam et al (2014) suggested an integrated farming systems approach causes the an increase in household nutrition, income and employment creation.

Conclusion and Suggestion

Based on the results of this study concluded the land under a coconut tree in the District of South Kotamobagu has the potential for the development of cattle, if the land is used as a source of forage.

Suggestions that need to be addressed, this potential can be expanded through the approach integration cattle, environmentally friendly and sustainable.

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Table 1. Potential for Effective Livestock Development, and Carrying Capacity Index of Land in South Kotamobagu

Coeffisient/Variable	Development Potential Values
PMSL	71,000.00
KPPTR(SL)	70,403.91
PMKK	24,831.00
KPPTR(KK)	24,234.51
IDD	7.65

Description:

PMSL = The maximum potential of land resources
KPPTR(SL) = The capacity increase in the cattle population by land resources
PMKK = The maximum potential based head of family farmers
KPPTR(KK) = The capacity increase in the cattle population by head of family farmers
POPRIL = The real population of cattle (AU) in the district studied
IDD = Carrying Capacity Index, equation: $IDD = \frac{PMSL}{TK}$
TK = Total feed requirements (TK=kxPOPRIL)
k = The constant need for digestible dry matter (BKC) by one animal unit is:1.14

References

Abdullah,A., M.A.Asja and Zulkarnaen. 2012. Potential analysis capabilities livestock breeders in (KPT) cattle based integration on regional center for beef cattle South Sulawesi. Proceedings of the Seminar's National animal husbandry, sustainable livestock systems revitalization based integration with plantations to achieve food security. Medan, Aula Soeratmat FP-USU. pp 34-39.

Alfian,Y., F.I.Hermansyah., E.Handayanto., Lutojo and W.P.S.Suprayogi, 2012. Analysis of the capacity of ruminants in the dry season in dryland farming areas in Semin Gunung Kidul Regency. Tropical Animal Husbandry, Vol. 1(1). Okt. p:33-42.

Barus,B. 2004. Determination of status of land carrying capacity in labor absorption in agricultural regions vegetables with Geographic Information Systems, Case Study District of Semarang, Garut, West Java. Land and Environment Journal. Vol. 6 No.2,Okt. p:57-69.15/12/2011.

BPS Kotamobagu City. 2014. City of Kotamobagu in figures. Central Board of of Statistics City Kotamobagu.

Hartono,B. 2012. Role of regional carrying capacity to Madura cattle farming development. Journal of Economic Development. Vol. 13. No2, Dec 2012. p316-326.

Hermawan,A and B.Utomo. 2012. Role of ruminant in conservation farming systems development in dryland das upstream section. Proceedings of the National Seminar on Sustainable Livestock 4, Farmers Agribusiness Innovation for Food Security. Faculty of Animal Husbandry, Padjadjaran University. Bandung.

Jayanthi, C., C.Vennila., K.Nalini and B.Chandrasekaran. 2009. Sustainable integrated management of crop with allied enterprises. Ensuring Livelihood Security of Small and Marginal Farmers. Tech. Monitor, Jan-Feb 2009.p:21-27.

Mohanty,D., S.C.Patnaik., P.J.Das., N.K.Parid and M.Nedumchezhiyan. 2010. Integrated Farming System for Sustainable Livelihood: A Success Story of a Tribal Farmer. Orissa Review. p:41-43.

Mulyani,A., S.Ritung and I.Las. 2011. Potential and availability of land resources to support food security. Journal of Agricultural Research. Vol 30(2). p:73-80. 30/12/2011.

Muslim,C and T.Nurasa. 2008. Policies of development of cattle, in region production center, crop food based, in Indonesia. Centre Socio Economic and Policy. Research and Development Agency, the Department of Agriculture.

Nugraha,B.D., E.Handayanta and E.T.Rahayu, 2013. Analysis of the capacity of ruminants in the rainy season in

dryland farming areas in the district of Semin, Gunungkidul Regency. Tropical Animal Husbandry, Vol. 2 (1). Jan. 2013: 34-40.

Nurdiati,K., E. Handayanta and Lutojo. 2012. Cattle production efficiency in dry season in ranch regional people dryland agriculture Gunung Kidul. Tropical Animal Husbandry, Vol 1(1),Oct, 2012.p:52-58.

Prawiradiputra,B. 2011. Ebb and flow of research and development of forage in Indonesia. Livestock Research Institute, Bogor.

Rahmansyah,M., A.Sugiharto., A.Kanti and I.M.Sudiana, 2013. Alertness feed on a small scale cattle as a adaptation strategy to climate change through the utilization of local flora biodiversity. Bulletin Ranch,Vol 37(2): 95-106.

Rasminati,N and S. Utomo. 2010. Cattle development potential in the DAS Progo Kulonprogo, Yogyakarta. Agrisains Journal, Vol 1, No 1 March. p:15-22.

Rodrigues,G.S., I.A. Rodrigues., C.C.A. Buschiselli and I. de Barros. 2010. Integrated farm sustainability assessment for the environmental management of rural activities. Environmental Impact Assessment Review. 30 (2010) 229-239.

Rusdiana,S and C.R.Adawiyah, 2013. Economic analysis and business prospects crops and cattle, in the coconut plantations. SEPA. Vol. 10. No. 1. Sept 2013: 118-131.

Salendu, A.H.S., Maryunani., Soemarsono and B.Polii, 2012. Integration of cattle-coconut in South Minahasa Regency. Proceeding : The 2nd International Seminar on Animal Industry 2012 (ISAI) Faculty of Animal Science Bogor Agricultural University.

Salendu,A.H.S and F.H.Elly. 2011. Coconut and cattle integration model as an approach ecofarming in North Sulawesi. Proceedings of National Seminar. Livestock Development Strategy Approach The Future of Ecofarming. Faculty of Animal Husbandry. Sam Ratulangi, Manado, North Sulawesi.

Susanti,A.E., A.Prabowo and J.Karman. 2013. Identifying and problem solving provision cattle feed, in support traditional livestock farming in South Sumatera. Proceedings. National Seminar on Sustainable Livestock. Agribusiness Innovation for Food Security. Faculty of Animal Husbandry, Padjadjaran University, Bandung. p:127-132.

Sutrisna,N., Y.Surdianto and N.Sunandar. 2014. Farming model design integration sorghum crops and cattle in Sub Optimal Land in West Java. Proceedings of the National Seminar on Land Sub-Optimal. Palembang 2014. p1-13 Sept 26-17.

Swarnam,T.P., A.Velmurugan., Z.George., N.Ravisankar., T.P. Sai., S.D. Ray and P. Srivastava. 2014. Integrated farming system for sustainable livelihood in Tribal Areas of Nicobar Island, India. Journal of the Andaman Science Association, Vol. 19(1):19-22.

Syahriani. 2014. Quality improvement of dryland through integrated agricultural (rambutan), corn and gamal in Gowa. Essay. Department of Soil Science, Agriculture Faculty, Hasanuddin University, Makassar.

Tola,T., P.T.Balla, and B.Ibrahim. 2007. Carrying capacity and productivity analysis of crop land in District Batang Jeneponto of South Sulawesi. Journal of Soil Science and Environment. Vol. 7 No. 1.p:13-22.

Wibowo,S.A and F.T.Haryadi. 2006. Characteristics factors affecting farmers' attitudes toward cattle loan program farmers group Andinihardjo Sleman, Yogyakarta. Media Ranch Vol 29. No. 3.p:176-186.

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DEVELOPMENT OF ORGANIC FERTILIZER OF WASTE CATTLE IN THE REGENCY OF NORTH BOLAANG MONGONDOW

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Abstract

The government of North Mongondow Bolaang continue to encourage farmers to develop cattle farming in order to increase their income. But in reality, the revolution of cattle can aggravate environmental problems. Cattle waste can cause pollution to the environment. This research has been conducted to analyze the benefits of the development of organic fertilizers derived from cattle waste. The method used is survey and direct observation. The location of the sample is the Subdistrict Sangkub, the respondent is a member of the group "Keong Mas". The results showed that Subdistrict Sangkub, has 447 head, can produce 1 957 860 kg feces and urine as much as 978 930 kg per year, which could substitute inorganic fertilizer for 391.57 hectares of rice fields. This cattle waste is the raw material that is excellent in making organic fertilizer. Organic fertilizer has been developed by members of the group in the form of liquid fertilizer. The group has a cattle housed as many as 20 birds. Members of the group have built a biogas digester and installation biourin, or a tank, as many as seven. The resulting liquid fertilizer, as much as 25 liters per day, which is sold at a price of Rp 30,000 per liter. In conclusion, the development of organic fertilizer, provide benefits for farmers, among others, as an alternative source of income, expenses for inorganic fertilizer can be minimized, and environmental pollution can be reduced. Suggestions, needs further research, to demonstration plots on food crops, by making use of liquid fertilizer from cattle waste.

Introduction

Cattle are a source of income for farmers. Cattle act as savings that can be sold at any time if farmer needs money. Cattle serve as a source of labor used by farmers to cultivate farmland. Cattle can also produce alternative energy from manure, and as a source of organic fertilizer which can be used to fertilize agricultural lands. North Bolaang Mongondow government, in this case, continue to supporting farmers to develop cattle farming in order to increase their income. The problem of livestock revolution can aggravate environmental problems. Cattle manure can cause pollution to environment. Cattle breeding is regarded as one of causes of CO2 emissions that lead to global warming increase. USEPA (2006) in Pratiwi et al (2012) stated that Indonesia was ranked sixth in world as a producer of greenhouse gas emissions (GHG) emissions from agricultural waste.

Breeding cattle in addition to producing resources in increasing food needs as a source of protein, also produces waste potentially negative environmental effects. Waste generated little is not a problem, but waste is produced in form of lots will cause environmental pollution (Harlia et al., 2012). This phenomenon is caused by nature are not able to decipher, do absorption and neutralizes the waste cattle.

According Kuruseng and Alianti (2011), waste in form of manure is base material, which is very good in making organic fertilizer. Cattle are biggest waste generators in comparison with other livestock. Cattle waste can be used as organic fertilizer, which is raw material is manure. Manure is animal waste that has been fermented properly and contains complete nutrients that plants need for growth. Introductions technology cattle waste utilization as organic fertilizer in North Sulawesi has been conducted through community service activities (Elly et al., 2010, Elly et al., 2011 and Elly et al. 2012).

Based on above problems, research has been done on use of cattle waste as organic fertilizer. The purpose of this study was to analyze benefits of development of organic fertilizers derived from cattle waste.

Methods of Research

The research method that has been done is a survey method and direct observation. Data have been collected are primary and secondary data. Sangkub Subdistrict sample locations are predetermined by purposive sampling, with consideration of farmers in this district has produced organic fertilizer (liquid). Respondents have been determined by purposive sampling are members of “Keong Mas” with consideration of this group have been

selling liquid fertilizer. Number of cattle as many as 20 head. Data analysis is descriptive analysis.

Results and Discussion

The results showed Sangkub has 447 head can produce 1,957,860 kg of solid waste and liquid water 978,930 kg per year, which could substitute inorganic fertilizer for 391.57 hectares of paddy fields. This phenomenon indicates approximately 4.75% of paddy fields in North Bolaang Mongondow can utilize liquid fertilizer produced from cattle in study area. Liquid fertilizer is used for plants that can improve soil texture, increases cation exchange capacity, ability to hold water, soil biological activity, soil pH, and others. Riley et al (2008) stated that application of organic materials, in addition to improving soil structure, can increase water holding capacity, according to Dinesh et al (2010) may also increase soil biological life.

This livestock waste is raw material that is excellent in making organic fertilizer. Dry land is an area with a lower quality (Syahriani, 2014). The indication is needed breakthrough in efforts to improve quality of dry land. Input use of manure by 10%, assuming other variables constant (ceteris paribus), according to research Suwandi (2005) can increase production by 1.25%. Mustard plant requires 10 tonnes per ha manure (according to results of research Nurshanti, 2009). Ohorella (2012) suggests a dose of liquid organic fertilizer waste cattle give a different response to growth and production of mustard greens. The organic fertilizer which is used to increase production of tomato (Pangaribuan et al. 2012). Dry land according Nurdiati et al (2012), generally nutrient-poor, less water and less fertile thus less productive to generate a source of food and feed ingredients. Haryono (2013) suggests use of compost is a choice in favor of an increase in productivity of upland rice on dry land.

The indication of manure can be considered in cultivation of plants in study area. According Widowati (2009), organic matter helpful in improving physical, chemical, biological, soil, and fertilizer use efficiency occurred. Organic fertilizer indicating able to maintain and even increase crop production (Rachmadhani et al. 2014).

The results showed organic fertilizer has been developed by Keong Mas members, in Sangkub in liquid fertilizer (Figure 1). This group has stabled cattle is 20 tails. Group members have built a biogas digester, and installation biourin, or sump seven. Liquid fertilizer produced is 25 liters/day are sold at a price of Rp 30,000/liter. Group members earn income Rp750,000/day, or Rp273,750.000/year. The indication, liquid fertilizer produced is a source of income, an alternative to group members. Dahono et al (2011) suggested economic benefits arising from use of a combination of NPK fertilizer and manure is higher than on use of NPK fertilizer without manure. The economic value of waste feces and urine increases and as new income sources led to farmers welfare is also increasing (Riyanto et al. 2012).

Figure 1 shows group members have resulted in liquid fertilizers as an alternative income. The indication cattle population and productivity can be improved in order to produce a liquid fertilizer more. Increased productivity and production of livestock, sustained, with pattern as developed Keong Mas group members can save resources, while reducing greenhouse gas emissions in order to realize concept of green economy (Bahri and Tiesnamurti, 2012). Group members can also use a liquid fertilizer produced for farming land. These conditions indicate group members to minimize costs for purchase of inorganic fertilizers are increasingly expensive and scarce. Cattle waste utilization is one alternative that is very appropriate to address rising prices of fertilizer and fuel shortages oil (Harlia et al, 2012). Alternative solutions to rising price of fertilizer is dependence of farmers on use of inorganic fertilizers can be minimized by utilizing organic fertilizer (Rachmadhani et al. 2014).

Government as a motivator give serious attention to development of cattle breeding oriented waste utilization. Wibowo and Sumanto (2012) states developing an integrated cattle farming need government support. This is because development of farms greater emphasis on economic growth could result in negative impacts on the conservation of natural resources and environment (Nainggolan and Aritonang, 2012). The results of study showing constraints faced by group members Keong Mas is marketing a liquid fertilizer that produced them. Sales of organic fertilizer is still limited to district of North Bolaang Mongondow. These conditions indicate need for socialization and promotions of liquid fertilizer in different areas. Market opportunities should be created both by members of group and government. Cattle Waste utilization as organic fertilizer which is of economic value, they are often be a constraint for farmers because marketing is still difficult and not continuous (Wibowo and Sumanto, 2012).

Conclusion and Suggestion

Based on results of this study concluded development of organic fertilizer, provide benefits for farmers, among

others, as an alternative source of income, expenses for inorganic fertilizer can be minimized, and environmental pollution can be reduced.
Based on research results suggested, needs further research, to demonstration plots on food crops, by making use of liquid fertilizer from cattle waste.

Keywords: waste, cattle, organic fertilizer

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A Comparison of Consumer Acceptability of Loin Steaks from Crossbred Fattening Pigs Derived from Pakchong 5 Boar and Commercial Boars

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INTRODUCTION

Currently, consumers prefer meat with excellent quality. Tenderness, juiciness and flavor of pork, which together compose of eating quality, are an important factor of the consumers overall judgments of quality. Previous studies indicated that several factors (e.g. breed, feed, and processing) can influence meat quality and eating quality of pork (Lo et al., 1992; Wood et al., 1994). Furthermore, the prominent boar can intensively influence pork quality. Crossbred pig has intermediate value of parents for meat quality and eating quality traits (Suzuki et al., 2003). The synthetic breeds become popular in pork production industry, especially for using as terminal boar (Chaweewan et al., 2012). Pakchong 5 pig (PC5), a genetic combination of Duroc and Pietrain, was developed by the Department of Livestock Development, Thailand. PC5 derived from the *inter se* mating and selected for 5 generations on important economy traits. It can be used as high growth and lean terminal boars. The results of our previous studies showed the growth performance and meat quality of PC5 (Chaweewan et al., 2012; Lertpatarakomol et al., 2015). But sensory characteristics evaluated by consumers have not been studied. Therefore, our objective was to evaluate consumer acceptability on the eating quality of pork loins from crossbred fattening pigs derived from PC5 boar compared to crossbred fattening pigs derived from two commercial boars.

MATERIALS AND METHODS

Animals and Samples collection

The experimental treatments consisted of crossbred pigs sired by Pakchong 5 boar (PC5) and crossbred pigs sired by two commercial boars (CB1 and CB2), which included 16 pigs (8 gilts and 8 barrows) in each treatment. All boars were sired with hybrid sow (Large White x Landrace). At about 103.54 ± 6.9 kg BW, all animals were slaughtered and their *Longissimus dorsi* (LD) muscles were collected to evaluate the consumer acceptability. Each LD sample was cut into 3.50 cm thickness and stored at -20°C until further evaluation.

Sensory evaluation

All LD samples were thawed overnight at 2 ± 2°C, andcooked by electronic broiler ovens (Sharp Corporation, Japan) at 180 ± 1°C until core temperatures reached 71 ± 1°C. Then cooked samples were cut into 1.30 cm^3 cubes, and held in water bath (Labec: Laboratory Equipment PTY. LTD, Australia) at 54 ± 1°C until served to the consumer. Consumers were not informed about the experimental protocol, and samples were individually labeled with three-digit random numbers and served once at a time in random order. Consumers were served water and crackers before evaluating the first sample and between samples for palate cleansing(AMSA, 1995). Pork eating quality attributes were evaluated byconsumers which include tenderness, juiciness, flavor, and overall liking, using a nine-point hedonic scale, where 9 is extremely like, 5 is neither like nor dislike, and 1 is extremely dislike. The experiment was a 3x2 factorial treatment structure in Completely Randomized Design. The influence of breed and sex were analyzed using Analysis of Variance.

RESULTS AND DISCUSSION

The results of consumer acceptability evaluation on eating quality of pork loins are illustrated in Table 1 and 2. The results (Table 1) showed no influence of breed and sex main effects ($P>0.05$) on tenderness, juiciness, flavor and overall liking of pork loins. This could be explained by all animals in this study were three-line crossbred pigs, which had a genetic variation from sire and dam. While interaction of breed and sex was found to affect ($P<0.01$) consumer acceptability in all sensory attributes (Table 1).

The results (Table 2) showed that eating quality (tenderness, juiciness, flavor and overall liking) of pork loins from PC5 and CB2 gilts could be better than those from their barrows. But eating quality of pork loins from CB1 barrows were better (P<0.05) than pork loins from CB1 gilts. However, pork loins from PC5 gilts had higher (P<0.05) overall liking score than PC5 barrows and CB1 gilts, but did not differ (P>0.05) from CB1 barrows, CB2 gilts and CB2 barrows.

CONCLUSION

By consumer acceptability evaluation, eating quality of pork loin from crossbred fattening pigs sired by Pakchong 5 boar was as good as eating quality of loins from those sired by the two commercial boars. Furthermore, consumer evaluation showed that pork loins of gilts from crossbred fattening pigs sired by Pakchong 5 boar were better in flavor and overall acceptability than those from barrows.

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Table 1 Effects of breed and sex of crossbred fattening pigs derived from Pakchong 5 boars (PC5) or commercial boars (CB1 or CB2) on consumer acceptability[1] on eating quality of pork loins (n = 100)

Attributes	Breed[Ψ]			Sex		SEM	P-value		
	CB1	CB2	PC5	Gilt	Barrow		Breed	Sex	Breed*Sex
Tenderness	6.01	6.27	6.22	6.15	6.18	0.09	0.35	0.81	<0.01
Juiciness	5.91	6.04	5.82	5.95	5.89	0.09	0.49	0.68	<0.01
Flavor	5.85	6.07	6.00	5.98	5.96	0.09	0.46	0.87	<0.01
Overall acceptability	5.92	6.13	5.99	6.02	6.00	0.09	0.36	0.83	<0.01

[Ψ]CB1 = crossbred pigs sired by commercial boars 1, CB2 = crossbred pigs sired by commercial boars 2, PC5 = crossbred pigs sired by Pakchong 5 boars
[1]9-point hedonic scale; 9 = extremely like, 5 = neither like nor dislike, and 1 = extremely dislike
SEM = standard error of mean

Table 2 Interaction effect of breed and sex of crossbred fattening pigs derived from Pakchong 5 boars (PC5) or commercial boars (CB1 or CB2) on consumer acceptability[1] of pork loin eating quality (n = 100)

Attributes	CB1[Ψ]		CB2		PC5		SEM	P-value
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow		
Tenderness	5.55^{b}	6.47^{a}	6.43^{a}	6.10^{a}	6.46^{a}	5.98^{ab}	0.16	<0.01
Juiciness	5.44^{c}	6.37^{a}	6.39^{a}	5.68^{bc}	6.02^{ab}	5.62^{bc}	0.16	<0.01
Flavor	5.50^{c}	6.19^{ab}	6.19^{ab}	5.94^{abc}	6.25^{a}	5.74^{bc}	0.16	<0.01
Overall acceptability	5.53^{b}	6.30^{a}	6.29^{a}	5.97^{ab}	6.25^{a}	5.72^{b}	0.15	<0.01

[Ψ]CB1 = crossbred pigs sired by commercial boars 1, CB2 = crossbred pigs sired by commercial boars 2, PC5 = crossbred pigs sired by Pakchong 5 boars
[1]9-point hedonic scale; 9 = extremely like, 5 = neither like nor dislike, and 1 = extremely dislike
[a,b,c]Means in the same row with different superscripts are significantly different (P≤0.05)
SEM = standard error of mean

REFERENCES

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. American Meat Science Association and National Live Stock and Meat Board, Illinois. 48 p.

Cannata, S., T.E. Engle, S.J. Morller, H.N. Zerby, A.E. Radunz, M.D. Green, P.D. Bass, and K.E. Belk. 2010. Effect of visual marbling on sensory properties and quality traits of pork loin. J. Meat Sci. 85: 428-434.

Chaweewan, K., S. Nakavisut, V. Jumparat, and V. Srisuriya. 2012. Genetic parameters of selection for economic traits over five generations of Pakchong 5 swine. In Proceedings of the 15th AAAP Animal Science Congress, Thammasat University, Rangsit Campus, Thailand, AAAP: 1347-1351.

Lee, S.H., J.H. Choe, Y.M. Choi, K.C. Jung, M.S. Rhee, K.C. Hong, S.K. Lee, Y.C. Ryu, and B.C. Kim. 2012. The influence of pork quality traits and muscle fiber characteristics on the eating quality of pork from various breeds. J. Meat Sci. 90: 284-291.

Lertpatarakomol, R., C. Chaosap, R. Sitthigripong, R. Limsupavanich, W. Champarat, W. Thaenthanee, and K. Chaweewan. 2015. Meat quality of crossbred fattening pigs sired by Pakchong 5 boars and commercial boars. In Proceedings of the 5th Sustainable Animal Agriculture for Developing Countries, Dusit Thani Pattaya Hotel, Thailand, SAADC: 733-736.

Lo, L. L., D. G. McLaren, F. K. McKeith, R. L. Fernando, and J. Novakofski. 1992. Genetic analyses of growth, real-time ultrasound, carcass, and pork quality traits in Duroc and Landrace pigs: I. Breed effects. J. Anim. Sci. 70: 2373-2386.

Suzuki, K., T. Shibata, H. Kadowaki, H. Abe, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64: 35-42.

Wood, J. D., G. R. Nute, G. A. J. Fursey and A. Cuthbertson. 1994. The Effect of Cooking Conditions on the Eating Quality of Pork. Meat Science. 127-135.

PO-05-61

Effect of farming activity included horticulture and interacting with domestic animals on person with mental disorders

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Introduction

In recent years, the actions that apply the horticulture and/or the interacting with animal to medical care and/or human welfare have been spread into many European countries (e.g. Relf, 2006). As the target person and the work forms are really various in these actions called "care farming", accumulating the scientific evidence about the efforts is needed. Aiming to establish the agricultural therapy in Japan, we investigated the effect of the farming activities at the Field Science Center, Ibaraki University that included the horticulture and interacting with domestic animals on the psychological and physiological state of person with mental disorders.

Materials and Methods

The subjects included nine men and one woman attending hospital or support facilities for persons with disabilities. They were almost the patients with schizophrenia (Table 1). The activities were conducted at 10: 00-11: 30 every Wednesday from April to December, 2015. The horticultural activities included seeding, planting, weeding, harvesting, and others according to the season and the timing. The interacting with domestic animals included brushing, feeding, and others for cattle and goats. Data measurement was conducted monthly from June to December on fine day, and for horticultural activities and interacting with domestic animals, the implementation order was switched round every month. Before and after the farming activities, self-assessment questionnaire of psychological state (Stress Response Scale: SRS) that was modified for State Anxiety Inventory (STAI: Spielberger *et al*., 1970) was carried out in the measurement day. Heart rate measurements by a heart rate monitor (POLAR RS800CX and V800) were continuously conducted through the whole activities, and the excitation index of sympathetic (LF/HF) and parasympathetic nervous system (HF nu) were calculated from heart rate variability (HRV) analysis (Matsuura *et al*, 2004, Motooka *et al*., 2007, Watanabe *et al*., 2014). The significant difference between before and after each activity was tested by Wilcoxon-test that the measurements of the same subject were paired. The present study was approved by the clinical and epidemiological ethics committee of Ibaraki University (No. 140300).

Results and Discussion

Table 2 shows the results of SRS. In SRS, there was no difference in scores between before and after the whole farming activity, and improvement of psychological condition was not found. Table 3 shows the results of HRV analysis during resting phase at before and after each activity. In all measurements (n=24), both LF/HF and HF nu did not changed between before and after each activity and whole activity. Whereas, the decreasing trend of LF/HF ($p<0.10$) and the significant increase of HF nu ($p<0.05$) were observed between before and after the whole farming activities in only the month when horticultural activities were conducted at first (n=13). As these changes in LF/HF and HF nu were not observed in the month when the interacting with animal were conducted at first (n=11), it is suggested that the effect of the farming activity are varied according to the implementation order of each activity. Furthermore, these changes in LF/HF and HF nu were only observed in the month when crop residues were fed to animal (n=6) in the month when horticultural activities were conducted at first. Because the feeding practice of self-harvested residues to animal could not be included in the month when interacting with animal were conducted at first, the feeding practice of self-harvested residues might be the reason why the tension of autonomic nervous system was relieved in only the month when horticultural activities were conducted at first.

Conclusion

From these results, the effect to relieve the tension of autonomic nervous system in the person with mental

disorder could be expected by conducting horticultural activities at first and then interacting with animals include feeding of self-harvested residues.

Table 1 Sex, age and diagnosis of the subjects

Subject no.	Sex(F/M)	Age(years)	Diagnosis
2	M	47	Schizophrenia
6	M	41	Schizophrenia
7	M	29	Schizophrenia
8	M	21	Schizophrenia
9	F	55	Schizophrenia
13	M	29	Schizophrenia
14	M	58	Schizophrenia & Higher brain dysfunction
15	M	41	Schizophrenia & Higher brain dysfunction
16	M	55	Higher brain dysfunction
17	M	23	Developmental disorder

Table 2 Comparison of stress response scale (Mean±SD) between before and after the whole farming activity (n = 33)

	Total score	Glumly / Anger	Depression / Anxiety	Anemic
Before activity	23.5±7.89	6.52±1.80	7.70±2.74	9.30±4.98
After activity	23.5±8.44	6.52±2.18	7.58±2.67	9.36±5.26

Table 3 Results of the HRV (heart rate variability) analysis before and after each activity

	LF/HF						HF nu(%)					
	Horticulture		Animal		Whole activity		Horticulture		Animal		Whole activity	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
All measurements (n = 24)	4.42	3.79	3.83	3.91	4.18	3.63	22.1	24.2	25.0	25.2	24.7	26.9
Horticulture – Animal (n = 13)	4.55	3.89	3.89	3.61	4.55^{a}	3.61^{b}	21.4	22.0	22.0	27.0	21.4^{A}	27.0^{B}
Animal – Horticulture (n = 11)	4.27	3.66	3.75	4.27	3.75	3.66	23.0	26.8	28.6	23.0	28.6	26.8
Horticulture – Animal												
with residual feeding (n = 6)	4.99	3.46	3.46	3.76	4.99^{a}	3.76^{b}	19.5	23.7	23.7	27.8	19.5^{A}	27.8^{B}
without residual feeding (n = 7)	4.27	3.46	3.46	4.15	4.27	4.15	21.6	24.0	24.0	22.4	21.6	22.4

A,B (P<0.05) and a,b (P<0.10) means the significant difference between before and after the each activity (Wilcoxon-test).

【References】

Matsuura, A., Takita, N., Hata, H. and Kondo, S. (2004). Spectral analysis of heart rate variability before and after equitation. Japan. J. Hum. Relat, 14:32-36.(in Japanese with English summary)

Motooka, M., Koike, H., Matsui, H., Yokoyama, T. and Kennedy, N.L. (2007). Benefits of a pet on the human autonomic nervous system. Japan. J. Hum. Relat, 19:87-92. (in Japanese with English summary)

Relf, P.D.(2006). Agriculture and health care ―The care of plants and animals for therapy and rehabilitation in the United States. In: Farming for Health. Hassink, J. and van Dijk, M.(eds.) p.309-343. Springer. Netherlands.

Spielberger, C.D., Gorsuch, R.I. and Lushene, R.E. (1970). *Manual for the state-trait anxiety inventory*. Palo Alto, CA: California Consulting Psychologists Press.

Watanabe, K., Ishida, M., Ito, S., Kasuya,E., Shutoh, M. and Yayou, K. (2014). The relationship between awake-sleep states and autonomic nervous balance in cattle. Animal behavior and Management, 50:119-126. (in Japanese with English summary)

PO-05-67

Measurement of Rib Eye Area and Backfat Thickness of Kamphaeng Saen Beef Carcass by Using a Computer Program

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Introduction

The yield grades (yield grade) were divided by the quantity or grade of carcass weight criteria such as the amount of meat or carcasses that have cut the amount of space available for retail sale. The criteria used in determining the quantity or grade of meat by carcass weight, including hot carcass weight, backfat thickness, The amount of fat around the abdomen, kidney, heart and rib eye area (Kanthapanit, 1986). The hot carcass weight and amount of fat around the abdomen, kidney, heart can be measured by weighing. However, backfat thickness and rib eye area can be measured by tool. Thailand, the measurements of rib eye area were measured by bring clear plastic sheet was grafted onto carcass and drawn a cross-sectional area picture. The picture cross-sectional area was calculated the area of a square sheet by estimates from the table.

Current has developed a computer program that can be used to calculate the area. A report from Kapetch et al. (2011) found that the area of paper photo were calculated by using Photoshop CS3 have the closest to real space (R2 = 0.9999). Rattanatham et al. (2008) found that applied Photoshop CS3 Extended to measured digital photos of the rib eye area of pork and measured with planimeter have a the average and the standard deviation was not significantly different.

Therefore, The objective of this experiment was to evaluate the possibility of using the computer program Photoshop CS3 Extended in a measure rib eye area and backfat thickness of beef carcasses for apply to cattle carcass yield grade precisely.

Materials and methods

1. Carcass

The rib eye area were measured by cut through the ribs 12^{th} and 13^{th}. Backfat thickness were measured on position of muscle sirloin by measured from the perpendicular to the length of the cross-sectional area of the spine and vertebrae (chine bone) in carcass of Kamphaeng Saen beef cattle remains 432 samples.

2. Treatments

The rib eye area and backfat thickness of beef carcass each sample were measured by three methods. Method 1: Measured by drawing a picture on the clear plastic and calculated the area and thickness of the table. Method 2: Measured by real carcass digital photos and calculated by Adobe Photoshop CS3 Extended program and Method 3: Measured by digital photos from drawing a picture in Method 1 and calculated by Adobe Photoshop CS3 Extended program.

3. Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using a completely randomized (CRD) models procedure and the differences three method groups were then compared by Ducan's Multiple Range Test. Results are expressed as means and the standard deviation (*SD*).

Results

The results showed that no significant differences ($p > 0.05$) were found three methods groups in that scale of rib eye area of Kamphaeng Saen beef carcass (79.24 ± 12.15 cm^2, 78.14 ± 14.79 cm^2, 79.75 ± 12.62 cm^2, respectively; mean ± SD.) and backfat thickness (0.81 ± 0.35 cm, 0.85 ± 1.31 cm, 0.85 ± 1.15 cm, respectively; mean ± SD.) (Table 1).

Discussion

In this study, the rib eye area and backfat thickness of Kamphaeng Saen beef carcass did not differ between three method groups. The similar study by Rattanatham et al. (2008) found that average of rib eye area of pork by measured with Grid method were no different when compared with analysis of digital image using a Adobe Photoshop CS3 Extended. the average backfat thickness of pork by measured with a ruler was similar as well as measured with analysis of digital image using a Adobe Photoshop CS3 Extended. Allen (1999) found that rib eye area of cattle fattening hybrid analysis with digital photos by using a computer program to compare the measurements of the area of the picture drawn on a plastic sheet (acetate sheets) and measured with Planimeter tool were not different. The thickness of backfat were measured using a ruler on carcass compare to use a ruler to measure on pictures drawn on a plastic sheet (acetate sheets) and measurement of digital photos using a computer is no difference as well. Santos et al. (2013) report that measured of rib eye area and backfat thickness of beef carcass (Charolais, Limousine and Retinta) with analysis of digital image using a computer program were no different when compared the measurements with planimeter and calliper.

Conclusion

Kamphaeng Sean beef carcass can measurement of rib eye area and backfat thickness by a computer program (Adobe Photoshop CS3 Extended). Thus, there is the possibility in used Adobe Photoshop CS3 Extended program applied in measurement of rib eye area and backfat thickness of beef carcass by use of digital image analysis. This method is precisely, conveniently and quickly.

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Table 1 Comparison methods for measuring of Rib eye area and Backfat thickness of beef carcass (Mean ± SD).

Variable	Methods of measuring		
	Method 1	**Method 2**	**Method 3**
Rib eye area (cm^2)	79.24 ± 12.15	78.14 ± 14.79	79.75 ± 12.62
Backfat thickness (cm)	0.81 ± 0.35	0.85 ± 1.31	0.85 ± 1.15

References

Allen, T. 1999. Use of a Digital Camera to Collect Carcass Data from Experimental Cattle. Beef Research Report, Iowa State University. http://www.extension.iastate.edu/Pages/ansci/beefreports/asl-1650.pdf [October 23, 2015]

Kanthapanit, C. 1986. Meat Science. Thai Watana Panich Publishing Co., Ltd. Bangkok, Thailand, pp. 112-115.

Kapetch, P., Pakdeethai, C. and Sarawat, V. 2011. Estimation of leaf area using digital image. Khon Kaen Agriculture Journal. 39, 392-397.

Rattanatham, C. Rattanatabtimtong, S. and Sookmanee N. 2008. The evaluation quality of pork by analyzing digital photos with a computer program. Proceedings of Graduate Research at the National University 10th, Sukhothai Thammathirat University. Nonthaburi, Thailand, August 11-12, 2008, 67- 69.

Santos, R., Pena, F., Juarez, M., Aviles, C., Horcada, A. and Molina, A. 2013. Use of image analysis of cross-sectional cuts to estimate the composition of the 10th–11th–12th rib-cut of European lean beef bulls. Meat Science. 94, 312–319.

PO-05-68 IMPACTS OF CHANGE FROM CATTLE FREE GRAZING TO GROWING AND INTENSIVE FEEDING OF LEUCAENA LEUCOCEPHALA IN EAST NUSA TENGGARA, INDONESIA

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Introduction

Long dry season and erratic rainfall caused fluctuation in the provision of high quality feed for ruminants in East Nusa Tenggara province, of the Eastern Indonesia. The poor availability of high quality forages in suficient quantity has brought about poor productivity of ruminants in the region, esecially in beef cattle farming (Bamualim and Wirdahayati, 2002), having high calf mortality (in Bali Cattle), long calving interval (in Sumba Ongole Cattle), a great body weight loss during dry season (both Bali and Sumba Ongole cattle), as well as bad condition of feeder cattle for fattening purpose (both cattle breeds). Thus there is a need to introduce, develop and use forage species capable of producing year round high quality feed, especially able to provide forage during the long period of the dry season (May to Novembe/December).

Forage species that would be able to meet this condition should have deep root system that can tap the soil moisture in the depth of the soils, withstand frequent cuttings, and produce high quality forage in freh form as at the moment forage preservation during the wet season is not a practical way to the smallhoder farmers in the region. Thus *Leucaena leucocephala* cv Tarramba, develop and released in Australia meets the demand, and has been widely encourage for the develoment and use in the region (since 2012), although the adaptation trials have been conducted since 2001 in West Timor and East Sumba, in East Nusa Tenggara province (Nulik *et al*., 2004).

Material and Method

A series of studies and assessments has been conducted between 2012 to 2016, by determining 2 distinct sites in feeding practices. The two sites, consisted of the existing site and non-existing sites. The existing site was the site at which the cultivation and use of the deep-rooted tree legume forage i.e. *Leucaena leucocephala* has been a normal practice since 1970s, while the non-existing site where the cultivation and use of the forage for fattening of beef cattle was a rare practice where farmers generally practice free grazing to the current assessments. The selection of the sites was explained by Kana Hau *et al*., (2014).

Recording was conducted on both location to identify the benefits that can be obtained by assessing the technical and socio-economic aspects as well as environmental sustainability practices improvement by the introduction of the intensive feeding system in pen through the cultivation and development of psyllid tolerant *Leucaena leococephala* cv Tarramba introduced from Australia in mid of 2011 and transplanted in 2012, while its adaptation in the regions have been conducted since 2001 (Nulik *et al*., 2004).

Results and Discussion

A list of improvements and achievements has been recorded during the assessment period (Mid 2012 to Early 2016), as follows:

Better knowledge and skills of collaborating farmers in *Leucaena leucocephala* cultivation and use (from the process of village seed production, germination, nursery planting in pollybags and seedbed, transplanting, care and management of plants to the harvest stage, harvest techniques, and feeding to animals). Better achievements in body weight gain of Bali Cattle in the non-existing area when comparing the free grazing condition (only gain at 1 – 5 kg monthly) to that of the intensive feeding in pen using forage from the established plots of *Leucaena leucocephala* cv Tarramba (improved to achieve gain at 15-30 kg per head/monthly) (Nulik and Kana Hau, 2015, and Kana Hau and Nulik, 2015) as also found in Bali cattle fattening in Sumbawa with best daily weight gain of up to 0.8 kg/hd/day as obtained by Panjaitan *et al*., (2014). Established a village based *leucocephala*"Tarramba" seed production systems, able to distribute > 2 tons of seed of high quality (mainly came from Oebola Dalam, and Kuan Heun villages of Kupang Distirct in West Timor), to supply within the province as well as some other places outside the province (Sumatera, Kalimantan, Java, and Bali), and to Timor Leste. Consistant change of free grazing practices can be seen from the increase number of cattle put into the intensive feeding system introduced,

beginning with 5 heads to an average of 35 heads of bulls in the first period assessment (2.5 years of the first project period) in farmer group at Oebola Dalam village. The practices were then adopted by the role out farmer groups, i.e. at the Setetes Madu (a drop of honey) in Camplong 2 village who has able at the current condition to establish 34 ha of for the group and encouraged the neibour groups to establish 50 ha of Tarramba and started intensive feeding in pen (Picture 1.), and Amtoas group at Nunsaen village who started planting 43 ha of *Sesbania grandiflora* and now replacing with Tarramba for longevitty of plant purpose as well as started fattened > 40 Bali Cattle Bulls, both in Fatuleu Sub-District of Kupang. Good *Leucaena* feeding demonstration (Kana Hau and Nulik, 2015) has shown that significant body weight loss of free grazing Sumba Ongole Bulls can be prevented and that there is a great potential to improve beef production in the region of Sumba Island if cultivation and intensive feeding of the leaf of *Leucaena leucoephala* could consitently be practiced by farmers in the island. The assessments has demonstrated the importance of providing sufficient number of plants of *leucocephala* cv Tarramba, as the growth and weight gain of the cattle was inline with the availability of forage supply, where the higher number of plants available the higher the growth and weight gain can be achieved (Nulik and Kana Hau, 2015). Beside better animal weight gain (thus shorter time to reach market weight and better income from selling cattle), additional cash was also obtained from selling of *leucocephala* cv Tarramba seed and forage. Forage was cut at a regular interval during the dry season (may to December) and sold at nearby cattle market, enabled farmers to improve their houses, purchase their own cattle, able perform cultural ceremony duty (paid with adult improved breed pig), buy motorcycle, and send children to schools. Livelihood improvements can be seen for example from the improvement of housing conditions, where at the start of the project (early 2012) the family houses were constructed using palm roof and dirt floor, while during the assessment period the houses were improved to the brick constructions and tin roofs and installing bettter timber for windows and doors. Make use of cattle manure to provide alternative energy supply such as cooking stove and night light (no government electricity access yet to the village) by using biogas produced, introduced by environmental department to reduce the effect to the glasshouse impacts and for village better sanitations. The system thus make farmer save cash from buying carosine for stove for cooking and light at night. The development *Leucaena leucocephala* cv Tarramba planting which may have reached upto > 700 ha in the region should have contributed to carbon sequestration and creates a better microclimate to the environment for food crop cultivation such as in alley pattern plantings, and thus as well as supply additional organic matter to the quality improvement of soils in the villages.

Conclusion

The smart introduced model of "improving small holders cattle fattening systems based on *Leucaena leucocephala* cv Tarramba" in the region, especially in west Timor and East Sumba, in East Nusa Tenggara of Eastern Indonesia has shown a great success in overcoming the classical problem of low beef cattle productivity caused by high quality feed shortages, and have demonstrated some positive impacts (improved cattle productivity, improved farmer's family income, created better microclimate condition for food crops cultivations in the dryland region, improved farmers livelihood and housing condition. Great achievements in the roll out of the technology which can be seen in the increasing number of adoption by farmers (currently may have reached > 3000 farmers in the province, from 250 farmers targeted by the project) as well as adoption by other stakeholders in the region (government policy makers, workers, and intelectual individuals). The evidence of efforts to uptake the technology through the demand for the seed of *Leuceana leucocephala*"Tarramba" into other provinces in Indonesia (Sumatera, Kalimantan, and East Java), and to the neiboring country Timor Leste, is increasing.

Picture 1. Introduction of *Leucaena leucocephala* "Tarramba" cultivation (left), develop a simple pen system (middle) and change into intensive feeding (right) to former free grazing animals.

Picture 2. Intensive feeding based on *L.leucocephala* on Sumba Ongole bulls (middle and right) demonstrated a great potential for improving beef production in Sumba Island by preventing the classical weight loss problem during the dry season.

Picture 3. Manure use as an alternative energy source (for stove and light) plays an important role, in the adoption of change from free grazing into intensive pen feeding systems using *L.leucocephala*"Tarramba" in west Timor.

References

Bamualim, A.M., Wirdahayati Ramli Barkri (2002). Peternakan Lahan Kerin di Nusa Tenggara (Dryland Animal Husbandry in Nusa Tenggara). BPTP Nusa enggara Timur (The East Nusa Tenggara Assessment Institute for Agriculture Technology). 95 pages.

Kana Hau, D., T. Panjaitan, J. Nulik, Dahlanuddin, and E. Van de Fliert. 2014. Barriers to and opportunities for the use of forage tree legumes in smallholder cattle fattening systems in Eastern Indonesia. *Tropical Grasslands – Forrajes Tropicales*, (2,1): 79-81.

Kana Hau, D and J. Nulik, 2015. Body weight gain response of Sumba Ongole cattle to the improvement of feed quality in East Sumba District, East Nusa Tenggara, Indonesia. Proceedings of the 16th International Seminar on Tropical Animal Production, October 20-22, Yogyakarta, Indonesia, pp. 143-146.

Nulik J., D. Kana Hau, P. Th. Fernandez dan S, Ratnawati. 2004. Adaptasi beberapa Leucaena species di Pulau Timor dan Sumba, Nusa Tenggara Timur (Adaptation of several *Leucaena* species in Timor and Sumba, East Nusa Tenggara). Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, 2004, pp. 825-831.

Nulik, J and D. Kana Hau, 2015. Daily body weight gain of Bali Cattle fed with *Leucaena leucocephala* as the main ration in West Timor, East Nusa Tenggara, Indonesia. Proceedings of the 16th International Seminar on Tropical Animal Production, October 20-22, Yogyakarta, Indonesia, pp.147-150.

Panjaitan T., M. Fauzan, Dahlanuddin, M.J. Halliday, and H. M. Shelton. 2014. Growth of Bali bulls fattened with *Leucaena leucocephala* in Sumbawa, Eastern Indonesia. *Tropical Grasslands – Forrajes Tropicales*, (2): 116-118.

PO-05-69

Carcass Traits and Tenderness of Grass-fed Beef from Subtropical Pastures in Hawaii

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Introduction

The "Grass-fed beef" label indicates meat that is produced by feeding forages from start to finish without any grain supplementation (USDA-AMS 2007). Many healthful aspects of grass-fed beef have been identified, including lower total fat content and higher content of omega-3 fatty acids, conjugated linoleic acids (CLA), and antioxidants as compared to feedlot-finished beef (Razminowicz et al. 2006, Faucitano et al. 2008). The healthy nutritional profile of grass-fed beef, along with the perception that grass-finishing promotes animal well-being and environmental sustainability, has probably contributed to the recent increase in the demand for grass-fed beef.

Even though beef cattle production is the third largest agricultural commodity in Hawaii, only 20-30% of weaned calves are locally raised for slaughter with the majority being shipped to the mainland USA due to the high cost of shipping concentrates feed to Hawaiian Islands. Since year-round maintenance of pasture is possible in some regions of Hawaii due to the subtropical climate, grass-fed beef production appears to be a viable alternative to shipping out weaned calves. For the development of a sustainable grass-fed beef industry, we contended that supplying consistent high quality of grass-fed beef is an important element in light of some studies reporting that palatability of grass-fed beef is inconsistent, often leading to consumer dissatisfaction with this product (Van Elswyk and McNeill 2014). Therefore, the objective of this study was to examine the carcass and meat tenderness characteristics of Hawaii grass-fed beef in an effort to improve meat quality characteristics of Hawaii grass-fed beef.

Materials and Methods

Sample collection

Three hundred fourteen ribeye steak samples from grass-fed cattle were obtained from two slaughterhouses on Hawaii Island (HI, USA) between November 2013 and June 2015. The one-inch bone-in steaks were collected from the 12th rib a few days after slaughter, individually vacuum-packaged, then shipped in a cooler with ice packs to the Human Nutrition, Food and Animal Sciences Department's meat lab of University of Hawaii at Manoa, USA. Upon arrival at the lab, the packages were removed and the boneless ribeye steaks were trimmed to less than 2 mm of subcutaneous fat and vacuum-packaged again. Vacuum-packaged samples were aged in a refrigerator for 2 weeks from the slaughter date and then were stored at -20°C for later measurement. Approximate animal age (based on teeth), sex, carcass weight, breed type (based on skin color), and level of marbling were evaluated during slaughter mostly by personnel at the slaughterhouses, and some evaluations were done by the research team.

Cooking and Shear Force Measurement

Shear force measurements were carried out periodically when about 70 samples had been collected. Steak samples were thawed overnight in a refrigerator. The thawed, vacuum-packaged steaks were cooked in a water bath at 70°C for one hour, cooled at room temperature for one hour, and chilled overnight in a refrigerator, as described in a protocol by the USDA-ARS Meat Animal Research Center (Wheeler et al. 2005). The pouches were unwrapped, and cooled steaks were gently dried with paper towels. For a shear force measurement, 6 core samples (1.3 cm diameter) were taken parallel to the longitudinal orientation of muscle fibers of each of the cooled steaks. The force required to cut the cores was measured by a Warner-Bratzler machine (G-R Manufacturing, Manhattan, KS, USA). The Warner-Bratzler shear force (WBSF) value was the mean of the maximum forces required to shear each set of core samples.

Data Analyses

To examine the WBSF value as affected by age, three age groups were established: Group 1, less than 24 months old; Group 2, 24 to 30 months; and Group 3, greater than 30 months old. The effects of age, sex class, carcass weight, and marbling on shear force value were determined by ANOVA procedure using Prism6 program

(Graphpad, San Diego, CA, USA).

Results and Discussion

Animal sex and age

Heifers and steers comprised 45.3% and 54.7% of cattle, respectively. Considering that some heifers are retained as cow replacements, it is to be expected that the proportion of heifers for grass-finishing would be lower than that of steers. The majority (64%) of grass-fed cattle were between 24 and 30 months of age with 17% and 19% being below 24 months and over 30 months of age, respectively.

Carcass traits

Table 1 summarizes hot carcass weight, marbling, and shear force values of the grass-fed beef. The mean carcass weight was 279.4 kg with 17.8% coefficient of variation, indicating a large variation in carcass size. The mean carcass size was much smaller than the US national mean carcass size of 372 kg (USDA, 2016). The mean marbling value was low Modest. Several studies (Davis et al. 1981, Realini et al. 2004, Van Elswyk and McNeill 2014) reported that intramuscular fat content of grass-fed beef is much lower than that of feedlot-finished beef, with a marbling from Slight to Small range. In this regard, the high marbling score is somewhat unexpected, and further studies are needed to examine underlying factors leading to the high marbling of current grass-fed beef samples.

Shear force value

The mean WBSF value was 4.43 kg, with values ranging from 1.95 to 11.37 kg (Table 1). The distribution of WBSF values is shown in Fig. 1. Miller et al. (2001) reported that 86% of consumers expressed that they had had a satisfying experience when the WBSF value of their steaks was less than 4.3 kg. In 2013, USDA launched a program certifying beef tenderness, under which eligible beef products can carry “USDA Certified Tender” or “USDA Certified Very Tender” labels. The minimum tenderness threshold values (MTTV) to claim “USDA Certified Tender” and “USDA Certified Very Tender” are 4.4 kg and 3.9 kg WBSF value, respectively (American Society for Testing and Materials International 2011). If we apply the MTTV of “USDA Certified Tender” as a standard for tender grass-fed beef in Hawaii, about 60% of Hawaii grass-fed beef appears to fall into this category.

Shear force value within age group, sex class, carcass size, and marbling score

We examined whether WBSF value was associated with animal age, sex, carcass size, or marbling score. Animal age appears to have a significant association with WBSF value (Fig. 2A), with younger animals having lower values than older animals. In our previous study (Kim et al. 2007), it was also observed that steaks from cattle more than 36 months had significantly higher WBSF values.

Steers had significantly greater mean WBSF value, with more variation, than heifers (4.60 vs 4.28, Fig. 2B). In contrast to the current result, our 2007 study showed that steers had a lower WBSF value (4.96 vs 5.52). With regard to the effect of sex on beef tenderness, results of various studies are not consistent (Gracia et al. 1970, Prost et al. 1975, Choat et al. 2006, Wulf et al. 1996), suggesting that some factors other than inherent sex-related factors, such as animal age and marbling, come into play together to influence meat tenderness. In the current study, more than 30% of steers were in the age group greater than 30 months, while only 3.5% of heifers were in this age group (data not shown). Also, steers had in general a lower marbling score (data not shown). It is thus speculated that the older age of steers compared to heifers contributed to higher WBSF values of steers.

Neither the carcass weight nor the marbling score had a significant association with WBSF value (Fig. 2C and 2D). Similarly, our previous study found no significant correlation between intramuscular fat and WBSF value (Kim et al. 2007). Fig. 3 also demonstrates that marbling is not a significant factor affecting grass-fed beef tenderness when marbling reaches more than high Slight level.

Shear force value by ranches

Variation in meat tenderness was observed among ranches (Fig. 4). Examination of animal age group, sex, carcass size and marbling score separated by ranches did not show that any of those parameteres are associted with the variation in shear force among ranches (Data not shown). Future studies, thus, need to examine how the combination of various production factors influence the tenderness of grass-fed beef.

Conclusion

In conclusion, results of this study showed that about 60% of Hawaii grass-fed beef are meeting "UDSA-Certified Tender" standards based on cooked shear force value, suggesting that cattle finished on subtropical pasture can yield quality tender beef. Younger slaughter age appears to be an important factor in improving the tenderness of grass-fed beef. Marbling, beyond a certain level (probably high Slight), does not appear to influence the tenderness of grass-fed beef. Beyond tenderness, a taste panel study is needed to evaluate consumer acceptance and the overall palatability of grass-fed beef.

Acknowledgements

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Table 1. Carcass weight (lbs.), marbling, and shear force value (kg) of grass-fed beef (2013–2015)

Trait	N	Mean	SD	CV	Minimum	Maximum
Carcass wt., kg	311	**279.3**	49.62	17.8%	178.7	572.9
Marbling*	308	**13.3**	3.36	25.3%	4	20
Shear force, kg	314	**4.43**	1.12	25.2%	1.95	11.37

*Practically devoid (-, o, and +): 1, 2, and 3; Trace (-, o, and +): 4, 5, and 6; Slight (-, o, and +): 7, 8, and 9; Small (-, o, and +): 10, 11, and 12; Modest (-, o, and +): 13, 14, and 15; Moderate (-, o, and +): 16, 17, and 18; Slightly abundant: 19; Moderately abundant: 20; Abundant: 21

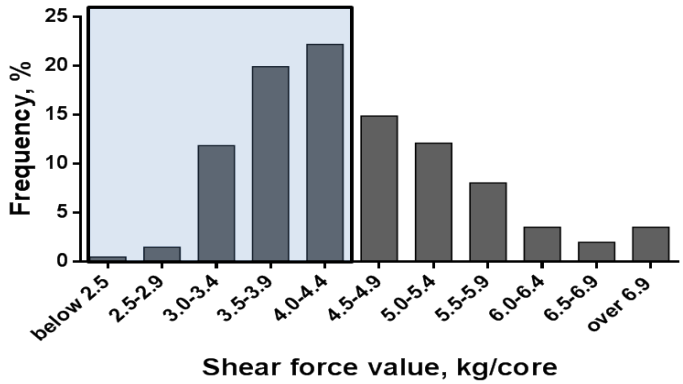

Fig. 1. Shear force value distribution of ribeye steaks from grass-fed cattle of Hawai'i. The rectangular region indicates the area below the minimum tenderness threshold value (MTTV) required to claim "USDA Certified Tender."

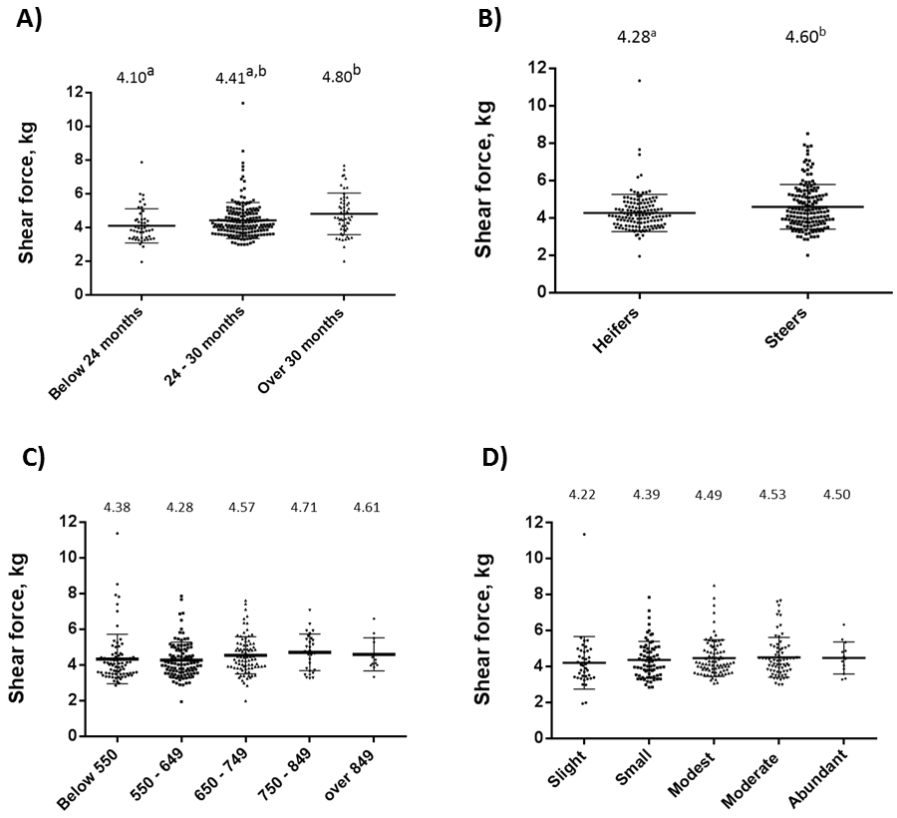

Fig. 2. Shear force value by age group (A), sex (B), carcass size (C) and marbling (D). Means not sharing the same superscript differ at P<0.05.

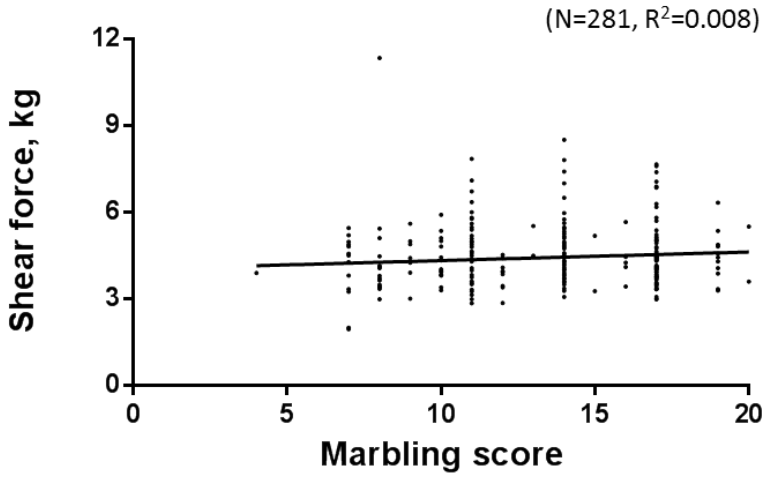

Fig. 3. Relationship between shear force and marbling score. The description of numerical marbling score is the same as in Table 1.

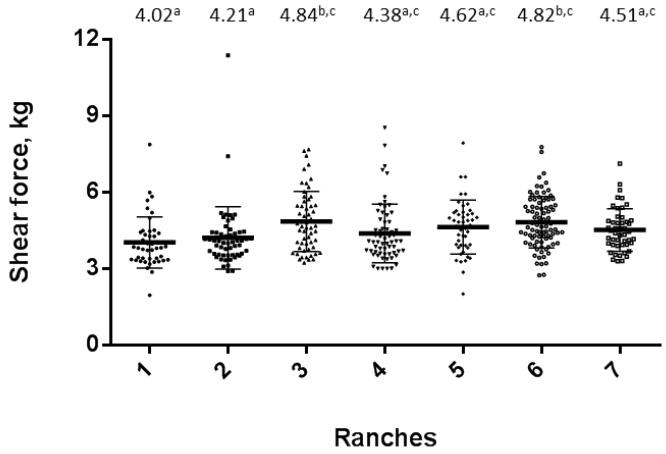

Fig. 4. Shear force value by ranches. Means not sharing the same superscript differ at P<0.05.

References

American Society for Testing and Materials International. 2011. Standard specification for tenderness marketing claims associated with meat cuts derived from beef. F2925-11. DOI: 10.1520/F2925-11.

Choat, W.T., Paterson, J.A., Rainey, B.M., King, M.C., Smith, G.C., Belk, K.E., and Lipsey, R.J. 2006. The effects of cattle sex on carcass characteristics and longissimus muscle palatability. *Journal of Animal Science* 84:1820–1826.

Davis, G.W., Cole, A.B., Backus, W.R., and Melton, S.L. 1981. Effect of electrical-stimulation on carcass quality and meat palatability of beef from forage-finished and grain-finished steers. *Journal of Animal Science* 53:651–657.

Faucitano L., Chouinard, P.Y., Fortin, J., Mandell, I.B., Lafreniere, C., Girard, C.L., and Berthiaume, R. 2008. Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 2. Meat quality, fatty acid composition, and overall palatability. *Journal of Animal Science* 86:1678–1689.

Gracia, E., Sink, J.D., Wilson, L.L., and Ziegler, J.H. 1970. Sex, sire and physiological factors affecting muscle protein solubility and other characteristics. *Journal of Animal Science* 31:42–46.

Kim, Y.S., Allison, O, Bobbili, N., DuPonte, M.W., and Fukumoto, G.K. 2007. Evaluation of meat tenderness of forage-finished cattle produced in Hawai‘i, and factors affecting the tenderness. FST-27, Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawai‘i, Manoa.

Miller, M.F., Carr, M.A., Ramsey, C.B., Crokett, K.L., and Hoover, L.C. 2001. Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Sciences* 79:3062–3068.

Prost, E., Pelczynska, E., and Kotula, A.W. 1975. Quality characteristics of bovine meat. II. Beef tenderness in relation to individual muscles, age and sex of animals and carcass quality grade. *Journal of Animal Science* 41:541–547.

Razminowicz, R.H., Kreuzer, M., and Scheeder, M.R.L. 2006. Quality of retail beef from two grass-based production systems in comparison with conventional beef. *Meat Science* 66:567–577.

Realini C.E., Duckett S.K., Brito G.W., Dalla Rizza M., and De Mattos D. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science* 66:567–577.

Van Elswyk, M.E. and McNeill, S.H. 2014. Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: The US Experience. *Meat Science* 96:535–540.

USDA Livestock, Poultry, & Grain Market News. 2016. https://www.ams.usda.gov/mnreports/lsddcbs.pdf

United States Department of Agriculture, Agricultural Marketing Service. 2007. United States standards for livestock and meat marketing claims, grass (forage) fed claim for ruminant livestock and the meat products derived from such livestock. *Federal Register* 72:58631–58637.

Wheeler, T.L., Shackelford, S.D., and Koohmaraie, M. 2005. Shear force procedures for meat tenderness measurement. Roman L. Hruska U.S. Meat Animal Research Center. http://www.ars.usda.gov/SP2UserFiles/Place/30400510/protocols/ShearForceProcedures.pdf.

Wulf, D.M., Tatum, J.D., Green, R.D., Morgan, J.B., Golden, B.L., and Smith, G.C. 1996. Genetic influences on beef longissimus palatability in Charolais- and Limousin-sired steers and heifers. *Journal of Animal Science* 74:2394–2405.

PO-05-73

Relationships between Rumen Acidosis, Energy Balance and Some Antioxidants, and Subclinical Mastitis in Postpartum Dairy Cows

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Introduction

Subclinical mastitis is more prevalent than clinical mastitis and could be approximately 30-90 % in dairy production. Losses in milk production were associated with high bulk milk somatic cell (BMSCC). The loss of milk production is 17-81 % when the SCC had 200,000 – 12,000,000 cells/ml (Philpot and Nickerson, 1999). Decreased milk production also correlated to positive CMT scores. Milk production decreased by 1.2 %, 6.3 %, and 33 % in quarters with California mastitis test (CMT) scores of 1, 2, and 3, respectively (Mungube et al., 2005). Benett et al. (1999)estimated that total economic impact of clinical mastitis was £119 per cow-case. Generally, pathogen-infected quarters of dairy cows may be clinical or subclinical depending on the degree of inflammation. Many risk factors on affecting subclinical mastitis have been reported in current articles. The epidemiological study has shown the relationship between mastitis, feedstuff and health indices. Minerals and vitamins are highly related to subclinical mastitis in the study. Supplementation of vitamin E resulted in lower incidence of mastitis. SCC had decreased when the farmer supplement cows with vitamins. The important of vitamin E for a well-functioning immune response around parturition has lately been given increasing interest (Weiss et al., 1997). Some vitamins and minerals have been reported to be associated with the prevalence and epidemiology of subclinical mastitis (Chassagne et al., 1998). A lower blood selenium concentration has high SCC compared with low SCC (Erskine et al., 1987). Factors regarding dairy cows were age, parity, breed, lactation stage, body condition score, and diets deficiency (Brand et al., 2001). However, the risk factors as regards changes in blood biochemistry, rumen ecology and serum antioxidants on subclinical mastitis have not been documented in the current articles.

Researchers have also shown that there is an association between a negative energy balance and the subsequent occurrence of mastitis (Janosi et al., 2003). Elevated blood ketone levels reduce the udder's ability to fight off invading bacteria through phagocytosis. Also during a subclinical or clinical ketosis situation, there is a reduced capacity for udder leucocytes to induce cell recruitment during an udder infection, and a reduced ability for the leucocytes to migrate into an infected mammary gland (Suriyasathaporn et al., 2000). Elevated SCC closely related to the subclinical ketotic cows as well. Over 29 % of those cows had elevated SCC versus 21 % of the non-ketotic cows. Over 21% had elevated SCC as compared to 13.6 % of the cows without chronic elevated blood ketone levels (Leslie et al., 2000).

Ruminal microflora has been well known for their functions in feed fermentation (Van Soest, 1994). Ruminal bacteria mainly co-work in the rumen ecosystem with protozoa and fungi in a complex fermentation process (Weimer, 1998). Ruminal ecology was regulated by pH level, volatile fatty acid concentration (VFAs) and temperature (Van Soest, 1994). The optimum fermentation depends on the quality and quantity of feedstuffs and high related with the milk production. The regulation of control system may impair ruminal function in situations where a high grain overload, low effective fibers and or lowered level of buffer agents from saliva occur (Beauchemin et al., 2003). Consequences are that ecology of rumen has changed from normal condition (Kleen et al., 2003). The physiological of rumination, absorption, buffering and microbiology are changes to acidity condition (Moller, 1993). Disadvantages of acidosis have affected to health and production of dairy cows including decreased dry-matter-intake (DMI) (Owens et al., 1998), perakeratosis-rumenitis-liver-abscess-complex (Nagaraja and Chengappa, 1998), low milk fat and laminitis (Enemark and Jorgensen, 2001). Recently, the association of rumen ecology and feeding system with subclinical mastitis has not fully described.

The aim of this study was designed (1) to study of subclinical mastitis (2) to determine the risk factors for subclinical mastitis in postpartum in smallholder dairy farms (3) to estimate their relationship between to occurrence of subclinical mastitis in smallholder farms at postpartum in the dairy cow.

Materials and methods

Study design

The study was carried out on small holder dairy farms kept in loose stall system. Twenty small holder farms in Khon Kaen cooperative, Thailand were selected to the study. Longitudinal study was performed to investigate subclinical mastitis in each dairy cow, which was sampled three times at 14 days before calving, 30 days and 50 days postpartum. Pregnant cows from each farm were selected to the study and categorized by parities. The dairy cows were fed by same concentrate source provided by the Dairy Cooperation. One hundred and thirty one dairy cows from the twenty small herds were selected. Concentrate was provided for cows in every selected herd during milking time. Fresh grasses were used as a roughage source by cut-carry or grazing. The rice straw was provided as major alternative roughage in all farms during a shortage of fresh grasses.

Samples collection

The questionnaire was used for feeding and milking data. Body condition score was evaluated at every farm visit during sampling times using a scale of 1 to 5 based on standard methodology (Brand et al., 2001). Blood samples were collected from coccygeal vein of each cow at 2-4 hours postfeeding in the morning by single use plastic tubes of blood clot, EDTA and NaF with EDTA (Vacuette, Greiner Bio-one GmbH, Kremsmünster, Austria). Serum and plasma were configured at 1000´ g for 15 minutes and kept in -20 °C until analysis. Rumen samples were taken using a stomach tube sampling device (Modified from Jorgensen Laboratories Inc., Loveland, CO), based on a previously described technique (Duffield et al., 2004). The distal end of the tube has a weighted metal device that penetrates the rumen mat, allowing collection of a sample from the ventral sac of the rumen. The other end is connected to a manual vacuum pump to aspirate rumen fluid. A volume of approximately 200 mL of rumen fluid was collected each time. Rumen pH was measured immediately after taking the sample with an electronic pH meter (MicropHep3 waterproof pH tester, Hannah Instruments, Woonsocket, RI). One milliliter of the ruminal fluid was placed in grass tubes with 9 ml of 10 % buffer formaldehyde and keep in room temperature until analysis. Composite milk samples were collected routinely in an aseptic technique in test tube and submitted for SCC, bacteriological identification and milk composition.

Laboratory analyses

Direct microscopic count was used to measure the number of bacteria, protozoa and zoospore by conventional laboratory (Holdeman et al., 1977). Commercially available kits were used to determine serum biochemical parameters including BHBA (Ranbut® RB1007, Ranbox Laboratories LTD, UK), BUN and glucose (Human GmbH, Wiesbaden, Germany). Milk samples were analyzed with standard procedures according to the guidelines of the International Dairy Federation (NMC, 1987) and Quinn et al. (1994) for bacteriological identification. SCC was determined by a fluoro-opto-electronic method (Fossomatic 250®, Foss Electric, Hillerùd, Denmark). HPLC was measured for serum levels of alpha-tocopherol and retinol. The serum samples of the purified extract were performed using a Shimadzu LC-10 system (Shimadzu, Shimadzu Corporation, Kyoto, Japan) according to Makimura et al (1991). The Se content in serum was determined by a modified digestion method (Norheim and Haugen, 1986) while the concentrations in the digestion solution were measured by an atomic absorption spectrometer (Shimadzu AA-680, Shimadzu Corporation, Kyoto, Japan). The feeding information of concentrate and roughage was also estimated by interviewing, inspecting and weighing.

Statistical analyses

The final data sets available for statistical analyses at farm and cow levels included 131 cows in 20 small holder farms. The null hypothesis was no relationships between feeding, rumen, and mastitis and milk quality. Data of rumen and blood chemistries were analyzed by ANOVA. Statistical analysis was conducted using Stata for windows (Stata, 2001). Linear regression models were used for subclinical mastitis and others variables. Multiple logistic-regression models were used separately for each indicator of variations. Ordinal outcome variables were dichotomized. The cut-off for dichotomization of ordinal variables was set on the level that was expected to impact subclinical mastitis. For each farm, the prevalence of positive results was calculated for each risk indicator. This was done by deriving odds ratios, p-value and 95% confidence intervals (CIs) of those selected risk factors when entered as explanatory variables the subclinical mastitis. The odds ratios that were greater than unity supported by P-values less than 0.05 were considered as significance at 95% confidence intervals.

Results

One hundred and thirty one dairy cows from twenty small holder farms were selected into the study. The eight and four dairy cows were excluded from the study in thirty days postpartum and 50 days postpartum, respectively due to the occurrence of clinical mastitis. Most farmers used homemade concentrate using rice bran, cassava chips, corn meal and soya bean meal. Eight out of twenty farms were used the same formula of concentrate for dry cows and milking cows. The Ruzi grass *(Brachiaria ruziziensis)* is the most roughage source in this area. The crude protein was 8.49 % and 16.57 % for abundant and intensive care pasture, respectively. The rice straw used for cows in all farms by ad libitum contains 3.97 % of crude protein composition. The average ruminal pH was not different significantly between prepartum and postpartum. However, the number of dairy cows had SARA problem was increased after parturition significantly. The number of zoospore was lower in 30 days postpartum significantly. The rumen ecology and SARA problem were shown in the Table 1.

Table 1 Rumen pH and microlfora in rumen fluid samples

Samples		14 days Ante-partum	30 days Postpartum	50 days Postpartum
pH	: Mean	6.08	5.90	5.89
	SARA	25.4^{A}	42.6^{B}	42.0^{B}
	(pH 5.5-5.8)	(15/59)	(23/54)	(21/50)
Rumen microflora :				
	Bacteria (x 10^{10} / ml)	4.11	4.11	3.14
	Protozoa (x 10^{4} / ml)	2.08	2.54	3.16
	Zoospore (x 10^{4} /ml)	3.97^{A}	2.75^{B}	4.33^{A}

$^{A, B}$ different letters in the same row mean significant difference ($p < 0.05$)

The prevalence of subclinical mastitis was 59.3 % and 58.8 % at 30-day and 50-day postpartum. The *CNS* and *Streptococci* were mostly isolated from in the infected quarters. The contagious pathogens consisted *Staphylococcus aureus* and *Streptococcus agalactiae* were found in the same dairy cows at the postpartum. The data of milk composition and prevalence of subclinical mastitis was shown in Table 2.

Table 2 Bacterial identification and milk composition in milk samples

Milk quality		30 days postpartum	50 days postpartum
Prevalence of subclinical mastitis		59.3 % (73/123)	58.8 % (70/119)
Bacterial identification :			
	Contagious pathogens	15.0 % (11/73)	17.1 % (12/70)
	CNS	79.4 % (58/73)	61.4 % (43/70)
	Environmental pathogens	26.0 % (19/73)	30.0 % (21/70)

The negative energy balance status in this study had lower than previous reports. The average body condition score were higher in prepartum than 30, 50 days postpartum significantly. The serum BHBA was increased in postpartum period significantly. Serum Se level had higher in 50-day postpartum than in 14-day ante-partum and 30-day postpartum significantly. The results of blood chemistry were described in Table 3. The risk factors on subclinical mastitis were also described in Table 4 of which all factors were not significantly related to subclinical mastitis.

Table 3 Blood chemistry level in samples

Blood chemistry	14 days Ante-partum	30 days Postpartum	50 days Postpartum
BCS	3.62^{A}	2.62^{B}	2.63^{B}
Blood chemistry			
BUN (mg/dl)	10.12	11.3	11.15
BHBA (mmol/L)	0.56^{A}	0.82^{B}	0.78^{B}
Glucose (mg/dl)	51.22	49.07	49.05
Serum antioxidant (μmol/L)			
alpha-tocopherol (normal 2-10)	3.56	2.69	3.31
Retinol (normal 0.15-0.60)	0.64	0.76	0.79
Se (normal 0.6-6.3)	0.58^{A}	0.66^{A}	0.76^{B}

$^{A, B}$ different letters in the same row mean significant difference ($p < 0.05$)

Table 4 Summary result of Mutivariable analysis of association between subclinical mastitis and potential risk factors

Risk parameters	Odd ratios	*P* values	95 % Confidence interval
Feed composition			
Crude protein	0.76	0.47	0.36 - 1.59
Energy	0.74	0.37	0.38 - 1.43
R:C	1.36	0.33	0.72-2.55
Rumen pH	0.64	0.27	0.28 -1.43
Serum Antioxidant			
Vit E	1.09	0.89	0.44-2.67
Se	1.27	0.57	0.53-3.04
Blood chemistry			
BHBA	1.29	0.62	0.46 – 3.61
BUN	0.81	0.48	0.47- 1.42
Glucose	1.15	0.72	0.51-2.55

Discussion

An incidence of SARA was 17% higher in postpartum cows and even in the cows fed by normal ration of concentrate. Nocek et al., (2002) has reported that rumen pH of dairy cows fed by 50, 60 and 70% of grain diets could be found SARA after feeding. Cows fed by 60 and 70% grain diets had more hours during the day less than pH 6.0 as compared with cows consuming diets with 50% grain. This study showed that zoospore was decreased, while the number of bacteria and protozoa did not change. Although, the rumen ecology involves many kinds of zoospore but decreasing number of zoospore is affected to the rumen ecology and could be alerted on high fiber diet fermentation (Russell and Rychlik, 2001). The prevalence of subclinical mastitis was found to be common in world-wide and the *CNS*s and *Streptococci* were found to be more common than contagious pathogens (Piepers et al., 2007). The incidence of subclinical mastitis in the current study, defined by SCC more than 200,000 cells/ml and bacterial identification positive (IDF, 1987). The prevalence of contagious pathogen was lower than others study (Roesch et al., 2007). However, this study was shown the infection still infected in the udder at 30-days and 50-days postpartum.

The serum analyzed of blood chemistry and antioxidants were described in Table 3. The serum glucose was in a normal range but quite lower than other experiments in dairy cows (Roesch et al., 2005). The lack of glucose has affected to cell metabolism in defense mechanisms (Ohtsuka et al., 2006). The low of BUN in serum is affected

from crude protein in feedstuff (Colmenero and Broderick, 2006). Negative energy balance was lower in this study, the low milk production of the small holder farm, high milk production had high incidence of ketosis. Serum vitamin E and Se were too low in the study. Many studies are showed relationship between Se and vitamin E in mastitis (Weiss et al., 1997).

Conclusions

The incidence of subclinical mastitis was high in all farms. Many factors were affected to rumen ecology and metabolism. The negative energy balance was found at postpartum periods and low levels of serum antioxidants in the studied farms. The relationships between subclinical mastitis and rumen pH and blood chemistry were not significantly related, as well as feed composition in the ration.

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References

Beauchemin, K. A., Yang, W. Z., and Rode, L. M. 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J Dairy Sci 86(2):630-43.

Bennett, R. M., Christansen, K., and Clifton-Hadlay, R. 1999. Preliminary estimates of the direct costs associated with endemic diseases of livestock in Great Britain. Prev. Vet. Med. 39: 155–171.

Brand, A., Noordhuizen, J.P.TM., and Schukken, Y.H., 2001. Herd health and production management. In: *Dairy Practice*, 3rd edition (Wageningen Pers Publ., Wageningen, The Netherlands), 351-415

Chassagne, M., Barnouin, J., and Chacornac, J. P. 1998. Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. Prev. Vet. Med. 35: 29–38.

Colmenero, J. J., and Broderick, G. A. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J Dairy Sci 89(5): 1704-12.

Duffield, T., Plaizier, J. C., Fairfield, A., Bagg, R., Vessie, G., Dick, P., Wilson, J., Aramini, J., and McBride, B. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J Dairy Sci 87(1): 59-66.

Enemark, J. M., and Jorgensen, R. J. 2001. Subclinical rumen acidosis as a cause of reduced appetite in newly calved dairy cows in Denmark: results of a poll among Danish dairy practitioners. Vet Q 23(4): 206-10.

Erskine, R. J., Eberhart, R. J., Hutchinson, L. J., and Scholz, R. W. 1987. Blood selenium concentrations and glutathione peroxidase activities in dairy herds with high and low somatic cell counts. J Am Vet Med Assoc 190(11): 1417-21.

Holdeman, L.V., Cato, E.P., and More, W.E.C. 1977. Anaerobe Laboratory Manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, VA.

IDF 215: 1987. Machine milking factors affecting mastitis - a literature review. In International Dairy Federation Bulletin 215, Machine Milking and Mastitis, pp. 2-32.

Janosi, S., Kulcsar, M., Korodi, P., Katai, L., Reiczigel, J., Dieleman, S. J., Nikolic, J. A., Salyi, G., Ribiczey-Szabo, P., and Huszenicza, G. 2003. Energy imbalance related predisposition to mastitis in group-fed high-producing postpartum dairy cows. Acta Vet Hung 51(3): 409-24.

Kleen, J. L., Hooijer, G. A., Rehage, J., and Noordhuizen, J. P. 2003. Subacute ruminal acidosis (SARA): a review. J Vet Med A Physiol Pathol Clin Med 50(8): 406-14.

Leslie, K.E., Duffield, T.F., Schukken, Y.H., and Leblanc, S.J., 2000. The influence of negative energy balance on udder health. National Mastitis Council Inc., 2000 Regional Meeting, Cleveland, Ohio, pp. 25–33

Makimura., S., Matuo, S., Usui, M., and Katayama, H. 1991. Simmultaneous determination of retinol, beta-carotene and alpha-tocopherol in serum and liver of Japanese Black Beef Cattle by HPLC. J.Jpn.Vet.Med.Assoc. 44: 328-332.

Moller, P. D. 1993. Acidosis in dairy cows. Acta Vet Scand Suppl 89: 111-2.

Mungube, E. O., Tenhagen, B. A., Regassa, F., Kyule, M. N., Shiferaw, Y., Kassa, T., and Baumann, M. P. 2005. Reduced milk production in udder quarters with subclinical

mastitis and associated economic losses in crossbred dairy cows in Ethiopia. Trop. Anim. Health Prod. 37(6): 503-12.
Nagaraja, T. G., and Chengappa, M. M. 1998. Liver abscesses in feedlot cattle: a review. J Anim Sci 76(1): 287-98.
National Mastitis Council. 1987. Laboratory and field hand-book on bovine mastitis. p.151.
Nocek, J. E., Allman, J. G., and Kautz, W. P. 2002. Evaluation of an indwelling ruminal probe methodology and effect of grain level on diurnal pH variation in dairy cattle. J Dairy Sci 85(2): 422-8.
Norheim G., and Haugen, A. 1989. Precise determination of selenium in tissues using automated wet digestion and an automated hydride generator-atomic absorption spectroscopy system. Acta pharmacol toxicol. 59 (Suppl VII):610–612.
Ohtsuka, H., Watanabe, C., Kohiruimaki, M., Ando, T., Watanabe, D., Masui, M., Hayashi, T., Abe, R., Koiwa, M., Sato, S., and Kawamura, S. 2006. Comparison of two different nutritive conditions against the changes in peripheral blood mononuclear cells of periparturient dairy cows. J Vet Med Sci 68(11): 1161-6.
Philpot, W.N., and Nickerson, S.C. 1999. Mastitis: Counter Attach. Babson Bross. CO. Illinois.USA. 150 p.
Piepers, S., De Meulemeester, L., de Kruif, A., Opsomer, G., Barkema, H. W., and De Vliegher, S. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J Dairy Res 74(4): 478-83.
Quinn, P.J., M.E. Carter, B.K. Markey, and G.R. Carter. 1994. Clinical Veterinary Microbiology. p. 648. Wolfe, U.S.A.
Owens, F. N., Secrist, D. S., Hill, W. J., and Gill, D. R. 1998. Acidosis in cattle: a review. J Anim Sci 76(1):275-86.
Roesch, M., M GD., Scharen, W., Schallibaum, M., and Blum, J. W. 2007. Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. J Dairy Res 74(1): 86-92.
Roesch, M., Doherr, M. G., and Blum, J. W. 2005. Performance of dairy cows on Swiss farms with organic and integrated production. J Dairy Sci 88(7): 2462-75.
Russell, J. B., and Rychlik, J. L. 2001. Factors that alter rumen microbial ecology. Science 292(5519): 1119-22.
Stata Corporation 2001. Stata reference manual: release 7. College Station, TX: Stata Press.
Suriyasathaporn, W., Heuer, C., Noordhuizen-Stassen, E.N., and Y. H. Schukken, Y.H. 2000. Hyperketonemia and the impairment of udder defense: a review. Vet. Res. 31:397–412.
Van Soest, P.J.1994. The Nutritional Ecology of the Ruminant, 2nd edition. Cornell University Press. Ithaca, NY. 476 pp
Weiss, W. P., Hogan, J.S., Todhunter, D.A., and Smith, K.L.1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J. Dairy Sci. 80(8):1728-37.
Weimer, P. J. 1998. Manipulating ruminal fermentation: a microbial ecological perspective. J Anim Sci 76(12): 3114-22.

PO-05-76

Association of fat protein ratio and non-genetic factors on Milk β - Hydroxybutyric Acid and Acetone Levels in Holstein Cattle

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Introduction

The ketone bodies acetone, acetoacetate, and BHBA can all be measured in milk and are useful as direct indicators of physiological imbalance and subclinical ketosis (Geishauser et al., 2000; Enjalbert et al., 2001; Nielsen et al., 2003).Subclinical ketosis in dairy cattle can be defined by the presence of increased levels of circulating ketone bodies, without the expression of typical clinical signs. (Zang et al., 2012). Early identification of diseases in dairy health management systems have been based on the measurements of milk constituents (Mottram et al., 2002), because milk is easier to sample than blood or urine. Furthermore, techniques have been developed for automated sampling and measurement of components in milk (Geishauser et al., 2000; Godden et al., 2002; Van Knegsel ., 2010), which makes milk a suitable medium for routine analysis.

Maintenance of energy balance is essential for ideal health and production of high yielding dairy cows. The fat protein ratio has been reported to be a better indicator of estimated energy balance (Duffield et al., 1997). Minimizing the occurrence, severity, and consequences of negative energy balance in the early lactation period has become an important issue for the dairy industry.

The proper maintenance of milk collection, well- organized herd management programs and assessment of BHBA and acetone measurements in milk should be considered for the efficient management on ketosis resistance and negative energy balance (Arnould et al., 2013). Apart from that, the non-genetic factors such as season of calving, season of test, parity, lactation stage and milk collecting time could be highly correlated with the maintenance of the ketone body concentrations, because metabolic disorders in early lactation indicate an overstressed ability of the animals to adapt to living conditions that do not appropriately provide the specific nutrient and energy requirements (Sundrum., 2015). The objective of this study was therefore to identify and quantify individual and herd factors affecting ketone body concentrations and association of early lactation fat protein ratio and on Milk β - Hydroxybutyric acid and acetone levels in holstein cattle.

Materials and methods

Fourier Transform InfraRed (FTIR) measurements for milk acetone (MAc) and milk BHBA levels along with routine monthly test-day records were collected from April 2012 to August 2014 by the Korea Animal Improvement Association (KAIA). Test-day milk sampling and analysis were performed according to the Korean milk-recording procedures (Cho et al., 2013).

The test day milk records included milk yield, fat, protein and lactose percentages of cows that were 1 to 305 days in milk at sampling. Milk samples were taken in the evening and morning. Test-day milk samples were analyzed by FTIR spectroscopy using a CombiFoss™ FT + system (Foss Analytical A/S, Denmark) with previously developed calibration equations for milk BHBA and milk acetone from the manufacturer. The original data set consisted of 264,221 test-day records.

For descriptive statistics of all parameters and statistical analysis of environmental factors affecting milk BHBA and acetone concentrations, SAS 9.2 package (SAS Institute Inc. Cary, NC, USA) was used. The following model, was used for the analysis:

$$Yjklmn = \mu + STj + SCk + Ll + Pm + AMPMn + ejklmn$$

Where Y is the observations for milk BHBA and acetone concentrations; μ is the mean of population. Fixed effects that were j^{th} season of test (ST_j) (j= 1~2), k thseason of calving(SC_j) (k=1~2), 1 thlactation stage(L_l)(l=1~5) , m^{th} parity(P_m)(m=1~12), and n^{th}milk collecting time ($AMPM_n$)(n=1~2); e_{jklmn} is random residual errors.

26,672 early lactation test day records from cows that were 1 to 60 days in milk at sampling were considered for the analysis of FPR. The cows were divided into two groups based on their FPR values, as less than 1.2 and greater than 1.2 and evaluate the mean ketone body concentrations of two groups.

Results and discussion

A total of 264,221 milk samples from Korea Animal Improvement Association (KAIA) were evaluated for their BHBA levels, acetone, protein, fat, contents fat protein ratio and peak milk yield. The control of energy metabolism in dairy cow along with milk composition including ketone bodies has concerned. The mean BHBA level for all the cows studied was 81.18 μmol/L with a range of 0 to 4,380 μmol/L while the mean acetone level was 268.62μmol/L with a range of 0 to 3880 μmol/L (Table 1).

Fluctuations of ketone body concentrations in this study was associated with individual factors and herd factors. According to the analysis of variance milk BHBA records, accounting for 52.26 % of the total variation along with season of test, season of calving, parity, lactation stage and milk collecting time while acetone records accounting for 49.61 % from the total variation. Mean squares from the analyses of variance are presented in Table 2. All sources of variation on milk BHBA were highly significant ($P<0.01$). Milk acetone mean squares also were highly significant except calving season. A similar study have been reported about non genetic effects on acetone concentration. According to that main factors were DIM class, lactation stage and test month. The R^2 of that analysis was 0.102. All of the main factors had highly significant effects (Wood et al., 2004). The means of milk BHBA and acetone concentrations in this study were high in first lactation stage (between 1 and 50 DIM) and low around second lactation stage (51- 100 DIM), after which they slightly increased with lactation stage (Figure 1).

Production of milk fat and protein can vary from one herd to another. In this study average milk protein ranged from 2.50% to 3.44%, with an average of 2.95%. Milk fat ranged from 1.76% to 5.25%, with an average of 3.46%. To evaluate FPR cut point for elevated ketone body levels, cows were divided into two groups: less than (<) or greater than (>) 1.2 FPR during the first 60 DIM.Within 1-20 DIM cows with FPR>1.2 showed the highest mean BHBA and acetone concentrations (110.95 μmol/L and 299.71 μmol/L respectively) while the mean BHBA and acetone concentrations were significantly reduced under FPR (Table 3).

During early lactation in dairy cows, a negative energy balance compels the cow to use body fat and proteins to meet its milk producing requirements increasing the availability of free fatty acid. At the same time, these changes in energy balance may cause an insufficient protein synthesis by ruminal bacteria. This negative energy balance may leads to elevated ketone body levels and prevalence of ketosis.

CONCLUSION

The risk of ketosis can be potentially predicted using measures of BHBA and acetone in milk and other environmental risk factors. Elevated levels of ketone bodies can be due to, lactation stage, parity, milk collecting time, seasons of calving and testing. Individual detection of ketosis can be performed via quantitative determination of milk ketone bodies. The means of milk BHBA and acetone concentrations in this study were high in between 1 and 50 DIM and low around 51- 100 DIM, after which they slightly increased with lactation stage. High milk FPR leads to increase the risk of sub clinical ketosis. Further testing and thorough validation using a dataset from cows from differing management systems is needed.

Table 1 . Descriptive statistics of milk ketone bodies acetone (AC) and beta-hydroxybutyrate (MBHBA) and milk composition data of the cows in the study

Variable	Mean	Std Dev	Minimum	Maximum	N
Milk(Kg/d)	38.43	8.71	19.10	58.40	18424
Milk Fat %	3.46	0.68	1.76	5.25	18306
Milk Protein %	2.95	0.19	2.50	3.44	18401
FPR	1.16	0.22	0.52	2.07	17585
MAc (μmol/L)	268.62	194.13	0	3880.00	19214
MBHBA (μmol/L)	81.18	120.85	0	4380.00	19214

Table 2. The Analysis of variance for milk β-hydroxybutyrate and acetone

		Milk β-hydroxybutyrate		Milk acetone	
Source	df	MS	F	MS	F
Season of calving	1	5000.02	13.38	83.02	0.09
Season of test	1	64440.28	172.40	7372.86	7.88
Lactation stage	5	8949.38	23.94	65095.88	69.54
Parity	4	2887.40	7.72	4559.40	4.87
Milking time	1	5461.06	14.61	9228.29	9.86

All sources of variation were highly significant ($P<0.01$) except for calving season in milk acetone.

Table 3. Effects of fat protein ratio on early lactation ketone body concentrations

	Milk β-hydroxybutyrate		Milk acetone	
Days in milk	FPR<1.2	FPR>1.2	FPR<1.2	FPR>1.2
5-20 DIM	55.83	110.95	171.24	299.71
20-40 DIM	39.28	75.87	133.58	191.64
40-60 DIM	27.21	50.34	119.39	141.29

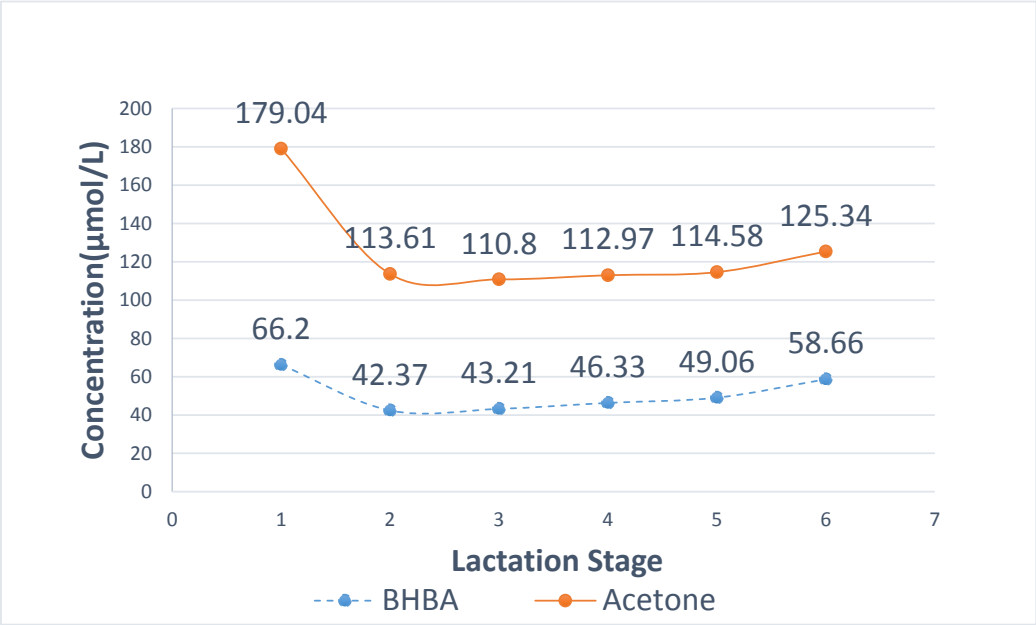

Figure 1. LS means of milk β-hydroxybutyrate (BHBA) and acetone level across lactation stages.

REFERENCES

Arnould V, Reding R, Bormann J, Gengler N, Soyeurt H. 2013. Review : milk composition as management tool of sustainability. Biotechnol Agron Soc Environ.;17(4): 613-621.

Cho, Kwang Hyun, Byoungho Park, Jaekwan Choi, Taejeong Choi, Yunho Choy, Seungsu Lee and Chungil Cho. 2013. Development of International Genetic Evaluation Models for Dairy Cattle. J. Animal Sci. & Tech. 55: 1-6.

Duffield TF, Lissemore KD, McBride BW, Leslie KE. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci.;92(2): 571-580.

Enjalbert F, Nicot MC, Bayourthe C, Moncoulon R. 2001. Ketone bodies in milk and blood of dairy cows: relationship between concentrations and utilization for detection of subclinical ketosis. J Dairy Sci.;84(3): 583-589.

Geishauser T, Leslie K, Tenhag J and Bashiri A. 2000. Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. J. Dairy Sci. 83: 296-299.

Godden S, Bey R, Farnsworth R, Reneau J, LaValle M. 2002. Field validation of a milk line sampling device for monitoring milk quality and udder health. J Dairy Sci.;85(6): 1468-1475.

Mottram T, Velasco-Garcia M, Berry P, Richards P, Ghesquiere J, Masson L. 2002. Automatic on-line analysis of milk constituents (urea, ketones, enzymes and hormones) using biosensors. Comp Clin Path.;11(1): 50-58.

Nielsen NI, Ingvartsen KL, Larsen T. 2003. Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. J Vet Med Ser A Physiol Pathol Clin Med.;50(2): 88-97.

Sundrum A, 2015 Review: Metabolic Disorders in the Transition Period Indicate that the Dairy Cows' Ability to Adapt is Overstressed. Animals, 5: 978-1020

Van Knegsel ATM, van der Drift SGA, Horneman M, De Roos APW, Kemp B, and Graat EAM. 2010. Ketone body concentration in milk determined by Fourier transform infrared spectroscopy: Value for the detection of hyperketonemia in dairy cows. J. Dairy Sci. 93 :3065–3069.

Wood GM, Boettcher PJ, Kelton DF, Jansen GB. Phenotypic and genetic influences on test-day measures of acetone concentration in milk. J Dairy Sci. 2004;87(4): 1108-1114.

Zhang Z, Liu G, Wang H, Li X, Wang Z. 2012. Detection of subclinical ketosis in dairy cows. Pak Vet J.;32(2): 156-160.

PO-05-79

Influence of environmental temperature on the measurement of udder surface temperature in Holstein cows using Infrared Thermometer

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ABSTRACT

The aim of this study was to evaluate the influence of environmental temperature on the udder surface temperature to detect mastitis in Holstein cows using infrared thermometer (IRT). IRT could be a useful method to measure body temperature of the animals, as it is a non-invasive and safe method. Our previous study, which was carried out in May 2015, revealed that surface temperature of the left hind quarter of the udder measured by using IRT was higher than that of the right hind quarter of the udder in Holstein cows, although we could not detect significant difference of udder surface temperature between animals infected and uninfected with mastitis. In this study, a total of 12 lactating Holstein cows (Mean Parity ± SE: 2.8 ± 0.4) ware examined from January to March, 2016. The cows raised on Sumiyoshi Livestock Science Station, Faculty of Agriculture, University of Miyazaki, were enrolled in the experiment. Temperatures of the posterior surface of the left and right hind quarters of the udder were measured directly behind the standing cows using IRT, totalizing 558 measurements (mastitis: n = 42, normal: n = 516). Definition of mastitis was based on Somatic Cell Count which was over 500,000/ml. Differences of temperature between hind half of the udder of the animals infected and uninfected with mastitis, and the environmental temperature were examined. There was a difference of udder surface temperature between animals infected and uninfected with mastitis in January ($p < 0.01$), although no difference was detected in February and March. There was no significant difference between left and right hind quarter of the udder in this study. Our results indicate the udder surface temperature could be influenced by environmental temperature. Further studies are needed to evaluate measurement of body surface temperature of the animals using IRT.

INTRODUCTION

Body temperature is an important indicator in the diagnosis of mammals' illnesses and for estimation of their physiological status. There are many methods to measure body temperature; however, non-contact and non-invasive infrared measurement is often the better method for the animals in the field. It would be valuable to develop new screening methods for the identification of animals infected with diseases without catching and restraining of affected animals.

Mastitis is one of the most serious diseases causing economic loss in dairy farms. It is a major endemic disease of dairy cattle, and is the inflammation of the mammary gland and udder tissue. It usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm. The most obvious symptoms of clinical mastitis are abnormalities in the udder such as swelling, heat, hardness, redness, or pain; and the milk has a watery appearance, flakes, clots, or pus. Other symptoms can also include a reduction in milk yield, the lack of appetite, and dehydration, especially an increase in body temperature.

Infrared thermography has potential application in the clinical monitoring inflammation (Dennis et al., 2015) and for assessment of animal welfare (Stewart et al., 2005). Infrared thermography measures the radiant energy from the surface of a source, which can be used to calculate its surface temperature. Contactless surface temperature measurement using an infrared thermometer (IRT) could be a modern, safe technique of thermal profile visualization to detect livestock diseases such as mastitis. Also, this technology may be useful for assessing the physiological responses of dairy cows to milking and feeding. Future development of ICT system such as alerting the producer of potential problem animals, by using automated IRT scanning mechanism, would be a helpful and effective method to improve livestock productivity.

Consequently, the aim of this study was to investigate the relationship between mastitis and the skin surface temperature of cow's udder using low-cost hand held infrared thermometers, in order to establish framework for detecting abnormalities in the cows.

MATERIALS AND METHODS

A total of 12 lactating Holstein cows (Mean Parity ± SE: 2.8 ± 0.4) were examined from January to March in 2016. The cows raised at Sumiyoshi livestock science station (total area = 50 ha), Faculty of Agriculture, university of Miyazaki, were enrolled in the experiment. Animals were pastured from 09:30 to 15:30 daytime on 0.5 – 3.2 ha of pasture (Italian grass and Bahia grass), and kept in a freestall barn from 15:30 to 09:30 during the experiment. Additional hay, wrapped silage, corn silage and concentrates that meet the demands of cows were also provided every afternoon at 15.00 hours. Lactating cows were milked twice daily at milking parlor. Morning milking commenced at 08.30 and afternoon milking at 17.30. Milk samples from each quarter for each cow were obtained, and samples were analyzed for somatic cell count (SCC) using cell counter (DeLaval). Definition of mastitis was based on SCC which was over 500,000/ml.

Temperatures of the posterior surface of the left and right hind quarters of the udder were measured from directly behind of the standing animals at a distance of 2m. Measurement of surface udder temperature was carried out using IRT (SANSYO, SIK-300), totalizing 558 measurements. Data of environmental temperature was obtained from Japanese Meteorological Agency to estimate the effect on body surface temperature of the animals. Differences of temperature between hind half of the udder of the animals infected and uninfected with mastitis, and the environmental temperature were examined. Mann–Whitney *U*-test and Tukey–Kramer test were used to determine differences in the groups. All of the protocols were approved by the Institutional Review Board for animal experiments of the University of Miyazaki.

RESULTS AND DISCUSSION

Forty-two out of 558 measurements were carried out from the cows infected with mastitis (Table 1). There was no difference of temperature between posterior surface of the left and right hind quarters of the udder in this study. There was a difference of udder surface temperature between animals infected and uninfected with mastitis in January ($p < 0.01$), although no difference was detected in February and March (Fig. 1).

In this study, there was no significant difference of temperature between posterior surface of the left and right hind quarters of the udder. This result is an opposite to that of our previous study, which was carried out in May 2015, revealed that surface temperature of the left hind quarter of the udder measured by using IRT was higher than that of the right hind quarter of the udder in Holstein cows (Nakagawa et al., 2015). The difference in human thermal asymmetry between opposite sides of the body is generally believed to be subtle, because blood flow governed by the autonomic nerve impulse seems to be symmetrical, both anatomically and histologically. Asymmetric temperature changes on the body surface may be diagnosed as diseases relating to autonomic functions (Niu et al., 2001). There may be some different heat radiation system in the body of dairy cows because distribution of stomach and intestines is asymmetrical in the ruminants. Ruminants have a big rumen which produces much heat by rumination in the left side of the body, and it may effect on temperature of the surface skin of udder in lactating dairy cows. Relatively cold environmental air temperature may affect to the result in which no significant difference of temperature between posterior surface of the left and right hind quarters of the udder was detected in this study.In the contrast, there was a difference of udder surface temperature between animals infected and uninfected with mastitis in January, and no difference was detected in February and March. It may be also caused by relatively cold environmental air temperature in January.

Thermographic equipment has found increasing applicability, as infrared thermography is a non-invasive safe method and only minimal restraint may be necessary. As we could detect difference of udder temperature between normal cows and cows infected with mastitis under a certain environment in the study, IRT has been used to predict changes in udder temperature for early diagnosis of mastitis in dairy cows (Scott et al., 2000, Berry et al., 2003, Colak et al., 2008). Increase in body temperature of cows with experimentally induced clinical mastitis was detected by thermal camera (Hovinen et al., 2008). Some researchers found that IRT may be useful for early diagnosis of laminitis (Nikkhah et al., 2005). It has also been reported that IRT have been tested for early detection of foot-and-mouth disease virus infected cattle (Rainwater-Lovett et al., 2009). The result of our study may be useful to understand these controversial results of the studies.

Some researchers found that infrared thermography has potential application for assessment of animal welfare (Stewart et al., 2005). One of the concerns regarding animal welfare is heat stress which is a serious problem for dairy cows in hot season, and it causes mastitis by depressing immune system in the cows. Many of the techniques used to measure stress involve invasive procedures, such as blood sampling, which may cause a stress response. Application for IRT in the management of cattle could be beneficial to improve animal welfare.

In Japan, the average number of animals per livestock farm has increased as the number of holding has declined. The equipment used in this study is easy to handle and quick to measure the body temperature of animals, however, it would be laborious for a producer to measure body temperature for the entire herd on a daily basis. Future development of technology such as automated scanning mechanism, simply alerting the producer potential problem animals, is required. Also, it is needed to understand the activities of animals, seasonal change of circumstances which may affect body surface temperature, animal physiology of the animals and so on. Further studies on the use of IRT are needed before it can be used as a reliable diagnostic tool, in order to establish framework for detecting abnormality in the cows.

Keywords: Holstein cows, Mastitis, Infrared thermometer, Environmental temperature

	January	February	March
Air temperature (Mean ± SEM)	4.6 ± 1.4	7.9 ± 1.2	11.3 ± 1.1
Number of Sampling day	9	9	9
Number of Sampling data	180	162	216
Mastitis	27	6	9
Normal	153	156	207

Table 1. Change of air temperature and number of sampling in the study.

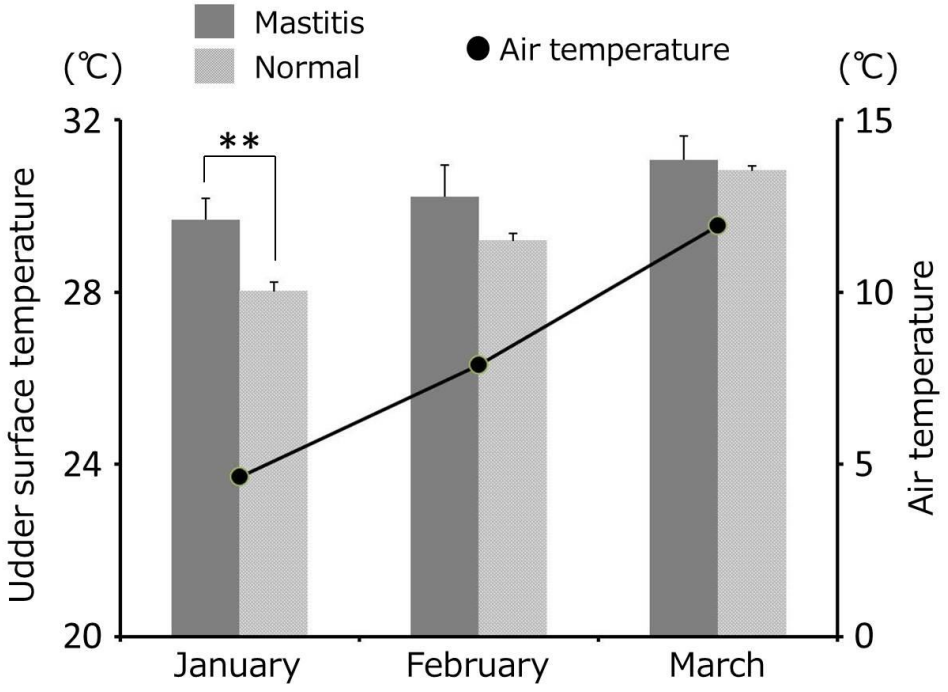

Figure 1. Difference of udder surface temperature between animals infected and uninfected with mastitis (**; $p < 0.01$).

REFERENCES

Berry, R.J., Kennedy, A.D., Scott, S.L., Kyle, B.L., Schaefer, A.L.: Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis detection. Can. J. Anim. Sci. 83, 687–693 (2003)

Colak, A., Polat, B., Okumus, Z., Kaya, M., Yanmaz, L.E., Hayili, A.: Early detection of mastitis using infrared thermography in dairy cows. J. Dairy Sci. 91, 4244–4248 (2008)

Dennis, D.S., Lene J.P.: Infrared skin temperature measurements for monitoring health in pigs: a review. Acta Veterinaria Scandinavica 57, 5 (2015)

Hovinen, M., Silvonen, J., Taponen, S., Hänninen, L., Pastell, M., Aisla, A.M., Pyörälä, S.: Detection of clinical mastitis with the help of a thermal camera. J. Daily Sci., 91, 4592–4598 (2008)

Nakagawa, Y., Noor, A., N., Fukuyama, K., Kobayashi, I.: Measurement of udder surface temperature in cows using infrared thermometer. Proceedings of the Ninth International Conference on Genetic and Evolutionary Computing, Advances in Intelligent Systems and Computing 388, Springer 1, 429-434 (2015)

Nikkhah, A., Plaizier, J.C., Einarson, M.S., Berry, R.J., Scott, S.L., Kennedy, A.D.: Infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. J. Dairy Sci. 88, 2749–2753 (2005)

Niu, H.H., Lui, P.W., Hu, J.S., Ting, C.K., Yin, Y.C., Lo, Y.L., Liu, l., Lee, T.Y.: Thermal Symmetry of skin temperature: Normative data of normal subjects in Taiwan. Chinese Medical Journal (Taipei) 64, 459–468 (2001)

Scott, S.L., Schaefer, A.L., Tong, A.K.W., Lacasse, P.: Use of infrared thermography for early detection of mastitis in dairy cows. Can. J. Anim. Sci. 80, 764–765 (2000)

Stewart, M., Webster, J.R., Schaefer, A.L., Cook, N.J., Scott, S.L.: Infrared thermography as a non-invasive tool to study animal welfare. Animal Welfare 14, 319–325 (2005)

Rainwater-Lovett, K., Pacheco, J.M., Packer, C., Rodriguez, L.L.: Detection of foot-and-mouth disease virus infected cattle using infrared thermography. The Veterinary Journal 180, 317–324 (2009)

PO-05-80

Factors affecting fleece characteristics of wool sheep in Thailand

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ABSTRACT

The objective of this study was to determine the effect of different genetic groups, gender and age of sheep on fleece characteristics. A total of 172 rams and 428 ewes, of the following genetic groups, were used Bond (n=162), Corriedale (n=216) and their crossbred sheep (n=222) maintained at Pang Tong Royal Development Project from 2012 to 2015. The animals were kept in a semi-intensive system with concentrated feed at 1% of body weight. Statistical analysis was performed by using the mixed procedure of the SAS statistical package. Analysis revealed that all fleece yield traits were significantly (P<0.01) affected by gender, and age of sheep. Triplicate fleece samples were obtained from the shoulder, right mid-side and back by hand shearing. The comparison of means among genetic groups revealed that the staple length of Bond (14.58 ± 0.23 cm) and Corriedale (14.18 ± 0.23 cm) were longer than crossbred sheep (13.78 ± 0.23 cm). Bond and Corriedale had greater fleece weight than crossbred sheep (2.92 ± 0.06, 2.91 ± 0.06, and 2.48 ± 0.06 kg, respectively). Fleece characteristics have economic significance in sheep. When assessing fleece characteristics, the importance of genetic groups, gender, age of sheep and the body location of the sample taken are important considered by producers.

INTRODUCTION

The sheep population in Thailand is relatively small (49,488). Breeding objective of sheep in Thailand is mainly to produce meat sheep suitable for optimum growth and well adapted to such a tropical climate as in Thailand. Wool sheep are found in small numbers in rural areas However, the import value of wool was more than 2,000 million baht by textile industry a year. Wool sheep are predominantly raised by hill tribe in the northern part of the country. In February 1995 when visiting the people of Mae Hong Son province Her Majesty the Queen suggested the Department of Livestock Development (DLD) to improve the breed of sheep for a better quality of wool and mutton and to promote improved breeds of sheep to the farmers and wool weaving to have a product from a soft fleece quality. DLD imported Bond and Corriedale sheep from Australia for breeding purpose to cross with the existing sheep. Sheep farmers can make products of high quality wool to generate income. (Anothaisinthawee, 2005). The Corriedale produces bulky, high-yielding wool ranging from 31.5 to 24.5 micron fiber diameter. The fleece from mature ewes will weigh from 4.5-7.7 kg with a staple length of 9-15 cm.(Timmins, 1989)
Genetic selection should consider which traits are more heritable or more likely to pass to the lambs. Traits that are highly heritable include variation in fiber diameter, face covering, staple length, crimps and skin folds. Traits that are moderately heritable include fleece weight, clean wool yield. The heritability estimate of fleece weight and length were 0.40-0.45 and 0.60-0.65 respectively (Carrick, 1992). The mean heritability for the annual fleece weight of adult ewes was 0.37 (Notter and Hough, 1997). The genetic correlation of lamb fleece weight with live weight at shearing was 0.29. Staple length is an important determinant of quality; it influences the type of manufacturing process used. Staple length is a function of individual fibre lengths and the extent of crimping; the fibre length component is related to growth rate and the duration of the growth period. However, research on factors of economically important traits in tropical climate of wool sheep is not well de?ned or even studied. Therefore, the objective of this study was to evaluate the effects of genetic group, gender, and age of sheep on wool production and quality. Moreover, in order to better classify and remunerate this kind of wool, it is necessary to understand its characteristics, including defects.

MATERIALS AND METHODS

Animal and management

All the animals were raised at the DLD research stations is located in the north (19°, 18'N and 97°, 58'E). Sheep were pastured on pastures and supplemented concentrates (16% crude protein and 2600 kcal/kg ME) at 1% of

body weight. They were treated for internal parasites and vaccinated against FMD twice annually against the general epidemic diseases. Sheep shearing starts in March and April (hot season) of each year.

Data collection and statistical analysis

Data of records on fleece weights and lengths were obtained from 126 Bond, 206 Corriedale and 102 their crossbred with native sheep in 2012 to 2015. Triplicate fleece samples were obtained from the shoulder, right mid-side and back by hand shearing. The comparison of means among genetic groups revealed that the longest staple. Preliminary analysis of fixed effects on fleece weight and length traits were performed using the Generalized Linear Model (GLM) procedure of a Statistical Analysis System (SAS, 1996). Fixed effects were genotype group, gender and age of sheep. The response variables that showed homogeneity of variances and normal distribution for the errors were subjected to analysis of variance according to the following statistical model: $Y_{ijkl} = \mu + G_i + S_j + A_k + E_{ijkl}$; in which Y_{ijkl} is the value observed for the response variable; μ is the overall mean; G_i is the effect of genetic group i; S_j is the effect of sex j; A_k is the effect of age k; and E_{ijkl} is the random error associated with each observation.

RESULTS AND DISCUSSIONS

Description of the fleece weight and staple length traits in all fixed effects

The least-squares means and standard errors of fleece weight and staple length traits in various fixed effects are given in Table 1. All the fleece weight and staple length traits were significantly affected by genetic group, gender, and age at shearing (P<0.01). The fleece weights of male and female were 3.04 ± 0.06 and 2.50 ± 0.04 kg respectively. Staple length of male were higher than female. The effects of gender in this study agree well with those found in literatures Akhtar *et al*. (2014) reported wool yield traits were significantly affected by sex of lamb. The difference between ewes and rams was statistically significant (P<0.01). Because of large body size, males produced heavier fleece weight than females. While as Wuliji *et al*. (2011) reported that the males Romney sheep were significantly heavier than females, but females had higher wool than males, and there was no significant effect of sex on each wool characteristics. Another factor that relates to wool production is body weight because larger sheep have more surface area to produce wool.

The results of this research showed that age of sheep significantly affect the fleece weight traits (P<0.01). Least-squares means of fleece weight show a peak value in the two to four years of age, and then tend to decline. Maximum fleece weights in sheep have been observed as from three to five years of age, with variable rates of decline in wool production thereafter (Corbett, 2001). The average wool staple length was 14.24 ± 0.41 cm with an individual sheep range of 7.33-25.67 cm. Males had longer staple length than females and one to two years old sheep had longer length than older sheep. There was no significant difference in the staple length of different breeds. The staple length of crossbred sheep is lower than that of Bond and Corriedale sheep. Table 2 show the result the body location of shearing at shoulder and side were longer than side in all genetic groups.

CONCLUSIONS

Wool quality can be affected by genetic and environmental influences. Genetic influences would be to select sheep with higher quality wool, however the environmental influences might include sheep management, and shearing management. It would be possible to produce finer wool effectively in Thailand

Key words: Fleece weight, Staple length, Wool Sheep

Table 1 Least square mean and standard errors of fixed effects of fleece weight and staple length traits

Effect	N (heads)	Fleece weight (kg)	Staple length (cm)			
			Shoulder	Side	Back	Average
Overall means	600	2.74±0.15	14.47±0.58	14.37±0.61	13.90±0.54	14.24±0.41
Genotypes		**	**	**	**	**
Bond	162	2.92±0.06^{a}	14.57±0.23^{a}	14.24±0.24^{a}	14.21±0.21^{a}	14.34±0.21^{a}
Corriedale	216	2.91±0.06^{a}	14.18±0.23^{ab}	13.94±0.24^{ab}	13.88±0.22^{a}	14.00±0.21^{a}
Crossbred	222	2.48±0.06^{b}	13.78±0.23^{b}	13.47±0.25^{b}	12.97±0.22^{b}	13.41±0.21^{b}
Gender		**	**	**	**	**
Male	172	3.04±0.06^{a}	14.58±0.24^{a}	14.16±0.24^{a}	14.11±0.22^{a}	14.28±0.21^{a}
Female	428	2.50±0.04^{b}	13.79±0.15^{b}	13.62±0.25^{b}	13.27±0.14^{b}	13.56±0.14^{b}
Age of Sheep		**	**	**	**	**
1 year old	94	2.54±0.14^{c}	15.58±0.52^{a}	15.44±0.55^{a}	14.75±0.49^{a}	15.26±0.47^{a}
2 years old	209	2.86±0.13^{ab}	15.41±0.48^{a}	15.42±0.51^{a}	14.64±0.45^{a}	15.16±0.43^{a}
3 years old	130	2.95±0.13^{a}	13.70±0.50^{b}	13.77±0.53^{b}	13.52±0.47^{b}	13.66±0.45^{b}
4 years old	60	2.99±0.15^{a}	13.68±0.56^{b}	13.41±0.59^{bc}	14.59±0.52^{bc}	13.39±0.50^{b}
5 years old	37	2.65±0.17^{bc}	13.33±0.62^{bc}	12.79±0.66^{bc}	14.59±0.59^{bc}	13.06±0.57^{bc}
6 years old	36	2.25±0.17^{d}	12.59±0.63^{c}	12.15±0.67^{c}	14.59±0.59^{c}	12.41±0.57^{c}
7 years old	34	2.05±0.17^{d}	13.12±0.63^{bc}	12.82±0.67^{bc}	14.59±0.59^{bc}	12.80±0.57^{bc}

^{a,b,c,d} the different superscripts in the same column for each trait mean significant;
** = (P<0.01)

Table 2 Least square mean and standard errors of staple length traits at 3 body locations

Genetic group	N (heads)	Staple length location (cm)		
		Shoulder	Side	Back
Overall means	600	14.52±0.12^{a}	14.41±0.12^{a}	13.96±0.12^{b}
Bond	162	15.06±0.22^{a}	14.82±0.22^{a}	14.61±0.22^{b}
Corriedale	216	14.47±0.19^{a}	14.51±0.19^{a}	14.04±0.19^{b}
Crossbred	222	14.02±0.19^{a}	13.90±0.19^{a}	13.23±0.19^{b}

^{a,b} the different superscripts in the same row for each trait mean highly significant (P<0.01)

REFERENCES

Akhtar, M., K. Javed, M. Abdullah, R. H. Mirza and M. A. Elzo. 2014. Environmental factors affecting greasy wool yield traits of Buchi sheep in Pakistan. The Journal of Animal & Plant Sciences, 24(3): 2014, Page: 685-692 ISSN: 1018-7081

Anothaisinthawee, S. 2005. Wool characteristics and economic return of wool raising. Progress report 2005. Department of Livestock and Development, Thailand. 10 pp (in Thai).

Carrick, M. 1992. Breeding objective in wool sheep. In Animal Breeding. The modern approach. Plublished by Post Graduate Foundation in Verterinary Science, University of Sydney.257p.

Corbett, J.L. 2001. Variation in wool growth with physiological state. Univ. New England Publishing Unit, Armidale. 79-98.

Notter, R.D. and J.D. Hough. 1997. Genetic parameter estimates for growth and fleece characteristics in Targhee sheep. J. Anim. Sci. 75, 1729.

SAS, 1996. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Taherpour, N., M. Salehi and F. Mirzaei. 2012. Evaluation certain environment factors on wool characteristics of Arabi sheep breeds. Open Journal of Animal Sciences. 2(1):11-14. http://dx.doi.org/10.4236/ojas.2012.21002

Timmins, L. 1989. Handbook of Australian Livestock. Australian Meat and Livestock Corporation. 145 p.

Wuliji, T., K.G. Dodds, R.N. Andrews and P.R. Turner. 2011. Selection response to fleece weight, wool characteristics, and heritability estimated in yearling Romney sheep. Livestock Science. 135, 2631.

PO-05-81

The Japanese white, an Akita-improved rabbit variety: Current status and problems of its farming in Japan, and possible strategies for sustenance

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INTRODUCTION

Modern lagomorphs are classified into families Ochotonidae and Leporidae. In particular, subfamily Leporinae consists of 11 kinds of animals (Matthee et al., 2004), including the hare (genus *Lepus*) and the domestic rabbit (genus *Oryctolagus*).

Phoenicians first came across the European rabbit (Oryctolagus cuniculus) in the Iberian Peninsula ca. 1000 BC (Lebas et al., 1997). Since then, the European rabbit has been domesticated for meat in as many as 70 countries, where in 2013 alone 1,781,618 tons of rabbit meat were produced (FAOSTAT, 2015, Raharjo and Bahar, 2016).

It is believed that rabbits have existed in Japan at least since the Edo period, because in 1786 the artist Oukyo Maruyama depicted rabbits in the painting "*Tokusa* (scouring rush) *& Rabbits*". It is also believed that domestication of rabbits in Japan was started in the Meiji period, because the book "*Method for breeding meat rabbits*" (1889), the oldest publication on rabbit breeding for meat production, was written in that era by Senji Nara, an agricultural instructor.

Rabbit meat production in Akita prefecture, in northeast Japan, dates back to 1890 when Kagawa Prefecture-born Senji Nara brought rabbits to Akita Prefecture. Thereafter, rabbit meat production has remained a lively activity in Akita. For example, through a century-long improvement of rabbits, local people in Akita succeeded in increasing the size of the rabbits, which then was renamed Japanese White Akita-improved variety (JW-AKT) (Fujimoto. 2003, unpublished data). In addition, a competition show of the JW-AKT that started in 1939 has continued to date, although the name was changed in 1988 to the current "*National Jumbo Rabbit Festival*" (Fujimoto. 2003, unpublished data).

As described above, rabbit meat production has been carried out worldwide; in Japan, however, while there is a strong recognition of rabbits as laboratory models and companion animals, their value as meat-producing animals is yet to be fully acknowledged. Nonetheless, if fully exploited, the rabbit industry could be an important source of food for Japanese people, because high-protein meat can be produced from rabbits mostly fed on grass in only 70 days (Lebas *et al*., 1997). Moreover, rabbit meat could become an important alternative source of protein for people with food intolerance to beef, pork and/or chicken.

In the present study we examined the data from interviews with rabbit farmers and a rabbit competition show held in Akita to elucidate the current status and problems of rabbit farming in Japan, as well as to propose possible strategies for the sustenance of the JW-AKT and the promotion of the rabbit meat industry in Japan.

MATERIALS AND METHODS

To survey the current conditions of rabbit farming in Japan, interviews with rabbit farmers were carried out in September, 2014 and October, 2015. The resulting data from the interviews were used for this study. In addition, data from a rabbit competition show at Nakasen Festival held in Daisen City, Akita Prefecture, and kindly provided by the National Jumbo Rabbit Festival Executive Committee, were also used for this study. All data were collected by a single worker.

Figures were prepared using data from the rabbit competition show and created using Microsoft Excel®.

RESULTS

Several differences were found between the breeding methods used by Japanese rabbit farmers and those used in other countries (unpublished data). For example, the length of the fattening period in overseas rabbit meat production is usually 70 days and the body weight at slaughter is 2 kg (Lebas *et al*., 1997; McNitt *et al*., 2013). However, in Japan JW-AKT are reared for at least six months and the body weight at slaughter is six kg. In addition, in Europe rabbits are mostly housed in wire-mesh cages (Lebas *et al*., 1997), whereas Japanese rabbit farmers rear JW-AKT in homemade wood cages with straw or towel litters (Figure 1). Moreover, while the rabbit

meat industry in other countries gives balanced pelleted feeds to rabbits (Lebas *et al*., 1997), in Japan wild grass, rolled barley and concentrated antibiotic-free feed for beef cattle are given as feeds to rabbits. Finally, while in France an average of 66 rabbits/doe are produced in elaborate breeding systems (Lebas *et al*., 1997), rabbit production in Japan is still modest, as an average Japanese rabbit farm rears about 20 rabbits that reproduce by natural mating.

Regarding the management of rabbit breeding, Japanese rabbit farmers face a number of problems ranging from decreased fertility due to excessive inbreeding and pododermatitis (sore hock) caused by increased body weight, to a lack of viable successor to pass on the business after aging farmers retire. Nonetheless, insofar diseases do not seem to pose a problem for the rabbit farming in Japan. For example, although a viral rabbit hemorrhagic disease (RHD) first emerged in 1984 in China, and since then it has spread worldwide (Abrantes *et al*., 2012), occurrence of RHD in Japan was not confirmed during the interviews with the rabbit farmers.

With respect to the rabbit competition show, the data showed that a number of parameters are measured during this event including body weight, body length and rabbit meat production per pen (weight of 3 rabbits). Furthermore, the data also indicated that rabbits are judged by the quality of appearance of legs, ears, hair, etc. (Table 1). Interestingly, it was found that in the last 28 years the average maximum body weight and body length have been 9.57 kg and 59.15 cm, respectively (Figure 2 and 3). Unfortunately, it was also found that the number of rabbits entered in the competition has been steadily decreasing on a yearly basis (Figure 4).

DISCUSSION

Rabbit meat production in Japan is still extremely small. The reason is that rabbit meat production in Japan has been inherited from generation to generation more as a way of preserving the JW-AKT as species than as a form of animal production. Therefore, unlike in other countries, there are no hybrid meat rabbits in Japan, the use of which could dramatically increase the production of rabbit meat. In addition, the rabbit breeding methods in Japan are very different in comparison with those used in other countries. However, not all breeding methods used in Japan can be considered optimal. For example, the use of flat floors for rearing can cause health problems in rabbits such an increase in the risk of pododermatitis, due to the significant weight of modern JW-AKT and sole infection by pathogens found in the floor dirt.

According to the Executive Committee, the National Jumbo Rabbit Festival is held mainly for JW-AKT promotion purposes. However, the number of rabbits entered in the competition show of the festival has been steadily decreasing on a yearly basis and it is predicted that the competition show will fold in 2064 (Figure 4). Likewise, JW-AKT may die out as species earlier than expected due to the inability of aging rabbit farmers to carry on rabbit farming. One good strategy to reverse the declining production trend in rabbit breeding and the rabbit meat industry in Japan would be to adopt husbandry techniques from countries where a robust rabbit meat production has already been developed and is currently thriving. In addition, an additional approach would be to develop a social network where Japanese rabbit farmers could exchange information about nutrition and rabbit breeding to tackle problems such as the decline in productivity due to low fertility and diseases. These strategies should help develop a more sustainable rabbit industry in Japan that would eventually entice a greater number of people to work in rabbit breeding and the rabbit meat industry.

In conclusion, rabbit meat production in Japan is still very small to due current techniques and the lack of sufficient farmers working in the industry. To support meat rabbit production in Japan and the sustenance of JW-AKT, it is advised to adopt husbandry techniques from overseas as well as to develop a social network where rabbit farmers could exchange nutrition and rabbit breeding information to tackle productivity and disease problems. These strategies could eventually entice more Japanese people to work in rabbit breeding and the rabbit meat industry.

Figure 1. Homemade wood cages used for rabbit meat production in Japan.

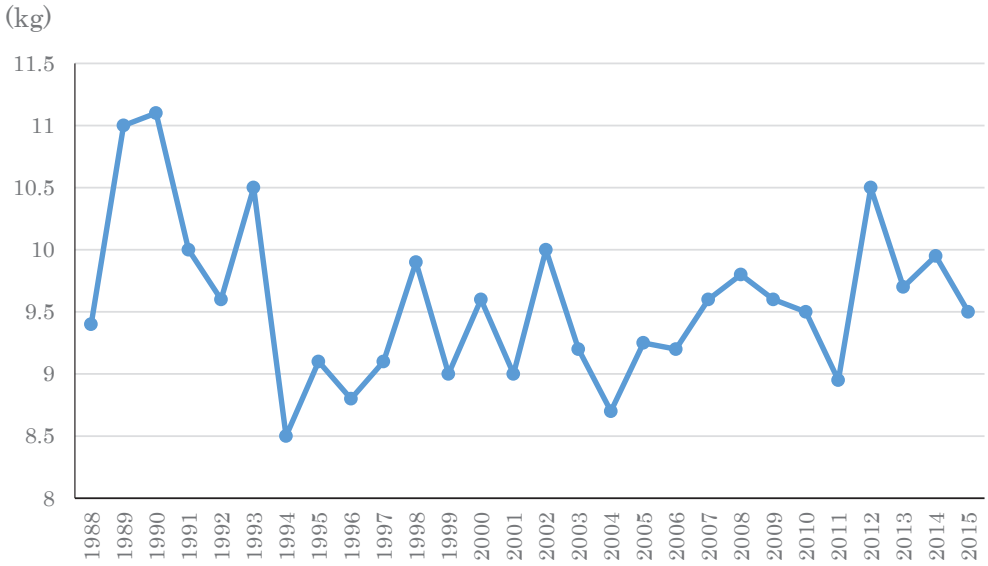

Figure 2. Body weight of champion rabbits at the National Jumbo Rabbit Festival.

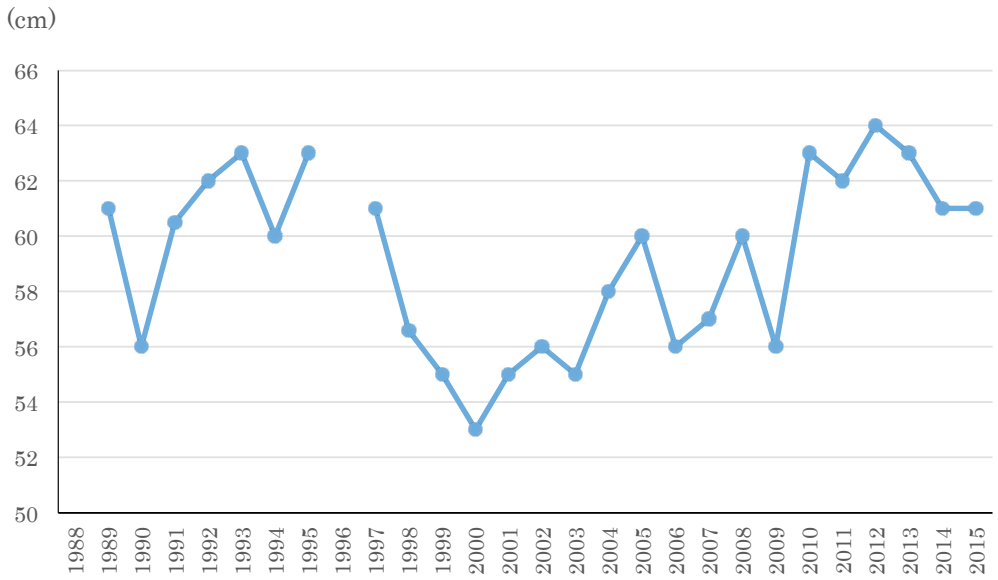

Figure 3. Body length of champion rabbits at the National Jumbo Rabbit Festival.
*There were no data of the body length of champion rabbits in 1988 and 1996.

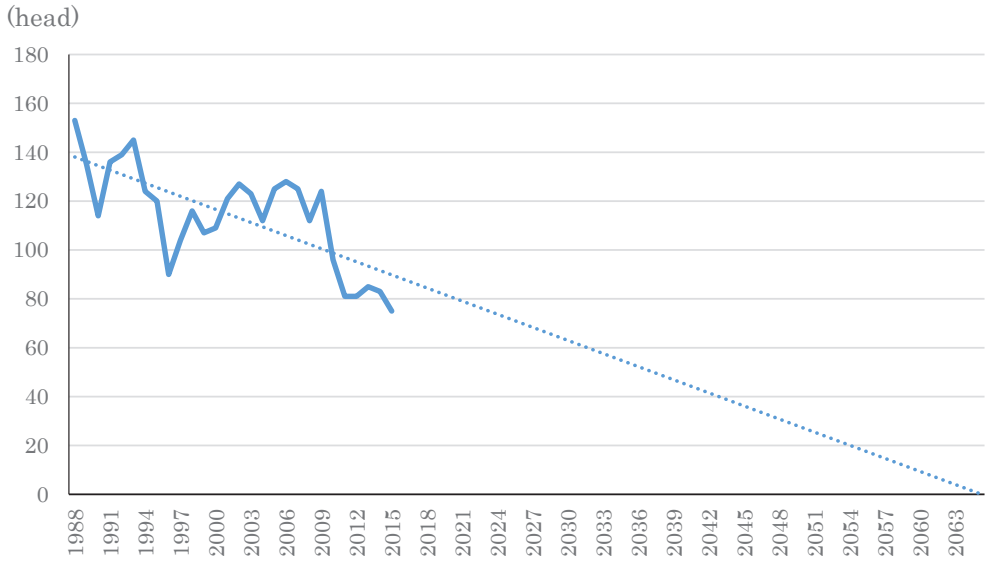

Figure 4. Number of participating rabbits at the National Jumbo Rabbit Festival.

Parameter	Description	Score
1. General apparent condition		60
Head and neck	The head is in good balance with the body; moderately short neck is good.	5
Face	Elegant facial form; nose, mouth and eyes color are good.	3
Ears	Big and straight standing ears are good.	2
Body	Long and wide body is good.	20
Shoulders	Elegant form of shoulders is good.	5
Chest	Thin and deep chest is good.	5
Loin	Long and wide loin is good.	3
Belly	Wide belly is good.	3
Hip	Long and wide hip is good.	1
Tail	Strong, straight and thick tail is good.	1
Legs	Elastic and normal-shaped leg is good.	1
Genitals	Symmetrically shaped genitals are good.	1
Constitution and dignity	Robust-looking and elegant rabbit is good.	10
2. Fur and skin condition		20
Fur	Sleek and woolly fur is good.	10
Skin	Elastic and pliable skin is good.	10
3. Body weight		20
Body weight	Rabbit weight of at least 4.5 kg at 7 months post-birth is good.	20
Total		100
Disqualification	1. Malformation, sickness, lop ears and bulging eyes. 2. Non-white color.	

Table 1. Japanese review standards for Akita-improved-variety rabbits.

REFERENCES

Abrantes J, van der Loo W, Le Pendu J, Esteves PJ. 2012. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. Veterinary Research 43:12. DOI: 10.1186/1297-9716-43-12

FAOSTAT [homepage on the Internet]. Food and Agriculture Organization of the United Nations. Statistics Division. URL: http://faostat3.fao.org/home/E.

Fujimoto G. 2003. The history of the jumbo rabbit "Akita improved variety". Nakasen-cho Farmland Policy Planning Division, Akita, Japan. [In Japanese].

Lebas F, Coudert P, de Rochambeau H, Thébault RG. 1997. The Rabbit: Husbandry, Health and Production 2nd Edition. FAO - Food and Agriculture Organization of the United Nations, Publications Division, Rome (Italy).

Maruyama O. 1786. Tokusa (scouring rush) & Rabbits. Shizuoka Prefectural Museum of Art. URL: http://spmoa.shizuoka.shizuoka.jp/japanese/collection/symphony/kosei/ pt1_ 03. php.

Matthee CA, van Vuuren BJ, Bell D, Robinson TJ. 2004. A Molecular Supermatrix of the Rabbits and Hares (Leporidae) Allows for the Identification of Five Intercontinental Exchanges During the Miocene. Systematic. Biology 53, 433–447.

McNitt JI, Lukefahr SD, Cheeke PR, Patton NM. 2013. Rabbit Production 9th Edition. CAB International, London (UK).

Nara S. 1889. Method for breeding meat rabbits. Chiba (Japan).

Steven et al. 1974. The Biology of the Laboratory Rabbit. Academic Press, INC., London, 2-5.

Raharjo YC, Bahar S. 2016. Rabbit breeding and research in Asia: perspectives and problems. Proceedings of 11th World Rabbit Congress. 353-394.

PO-06-1

BODY WEIGHT AND GENETIC COMPARISONS IN THAI NATIVE, BLACK-BONE HMONG AND BLACK-BONE CHINESE CHICKENS

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ABSTRACT

Consumption of chickens in term of Thai native and black-bone chickens in Thailand is increasing over time because Thai consumers pay attention to health and nutritive value. However, the limitation of Thai native and black-bone chickens is low growth rate, consequently, growth traits are of primary concern in breeding programs. The aims of this study were to compare body weights and to estimate their genetic parameters among Thai native (TN), black-bone Hmong (BH), and black-bone Chinese (BC) chickens. Data were obtained from the research and development network center for animal breeding (native chicken), Faculty of Agriculture, Khon Kaen University. A total of 68, 97, and 37 body weight records at 0, 4, 8, 12, and 14 weeks of age in each chicken breeds were analyzed using multivariate analysis. Fixed effects included hatch and sex as cross effects. Expectation maximization restricted maximum likelihood (EM-REML) and best linear unbiased prediction (BLUP) methods were used to estimate variance components and breeding values, respectively. The results showed that BC had the highest of body weight in both male and female ($P<0.05$) compared to TN and BH. According to heritability values of body weight, BC (0.59-0.94) had higher heritability value than TN (0.14-0.75) and BH (0.15-0.73). Meanwhile, genetic correlations between body weights at different weeks of age in every chicken breeds were positive. In conclusion, genetic evaluation could be use for improvement the growth performance in Thai native and black-bone chickens especially in black-bone Chinese chicken.

INTRODUCTION

Healthy food is becoming a new trend lifestyle for consumers around the world because people are concerning about their own health more and more. For this reason, food manufacturers in term of farmers need to produce their products in order to consistent with the requirements of the consumers. One of animal product, known as healthy food is the native chicken. The native chickens have several great features including good taste and soft meat tenderness (Jaturasitha et al., 2008). Also, within the meat has an essential substance that is important for the body such as melanin, (Tian et al., 2007) Currently, the Research and development network center for animal breeding (native chicken), Department of Animal Science, Khon Kaen University, Thailand has developed the new genetic crossbred chicken line from black-bone Chinese (BC), Thai native (TN), and black-bone Hmong (BH) chickens in order to satisfy consumers in terms of a healthy diet. However, before studying the genetic combining ability between breed and chemical composition within the meat of these chickens, we need to study the development potential of their growth rate because it is considered in relation to the production cost and potential production. Therefore, the objective of this research was to compare body weights and to estimate their genetic parameters among black-bone Chinese, Thai native, and black-bone Hmong chickens.

MATERIALS AND METHODS

Data

The data consisted of 68, 97, and 37 body weight records at 0, 4, 8, 12, and 14 weeks of age in BC, TN, and BH chickens, respectively were analyzed using multivariate analysis separated by breed. Records were obtained from the research and development network center for animal breeding (native chicken), Faculty of Agriculture, Khon Kaen University, Thailand. Fixed effects were hatch and sex which were classified as cross effects. Expectation-maximization restricted maximum likelihood (EM-REML) and best linear unbiased prediction (BLUP) methods were used to estimate variance components and breeding values, respectively by BLUPF90 Chicken PAK v 2.5 (Duangjinda et al., 2005). The model was as follows:

$$\begin{bmatrix} y_1 \\ \vdots \\ y_n \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & X_n \end{bmatrix} \begin{bmatrix} \beta_1 \\ \vdots \\ \beta_n \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & Z_n \end{bmatrix} \begin{bmatrix} a_1 \\ \vdots \\ a_n \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \vdots \\ \varepsilon_n \end{bmatrix}$$

Where y_1 to y_n are vectors of traits, β_1 to β_n are vectors of fixed effect (hatch and sex effects), a_1 to a_n are vectors of random additive genetic effects, ε_1 to ε_n are vectors of random residual effects, X, Z are incidence matrices relating y to β, a, respectively.

RESULTS AND DISCUSSIONS

In Figure 1a, the comparison of average body weights at different ages found BC had the highest growth and followed by TN and BH, respectively. When sex was considering separately (Figure 1b and Figure 1c), we found that BC also had the highest growth in both males and females. However, in the female group was found that TN at 12 and 14 weeks of age had growth rate closed to BC. So BC and TN are appropriate for developing as a hybrid native chicken to emphasize the growth characteristics primarily. The estimated heritability of body weights at different ages are shown in Table 1. The results showed that BC (0.59-0.94) had higher heritability value than TN (0.14-0.75) and BH (0.15-0.73)similarly to Sungkhapreecha et al. (2015) who reported that BC had the highest growth rate and genetic performance compared with other native chicken breeds. The heritability in our study showed that the genetic evaluation method is accuracy enough for genetic selection of chicken growth characteristic. In addition, genetic correlations between body weights at different weeks of age in every chicken breeds were positive. Thus, genetic selection could be conducted since day-0 chick (BW0). However, in practical, the selection of newborn chicks might be difficult because the expression of the growth is quite hard to evaluate. Therefore, the selection at the age of 4 or 8 weeks is probably more appropriate.

Keywords: Thai indigenous chicken, Genetic, Body weight, Growth performance

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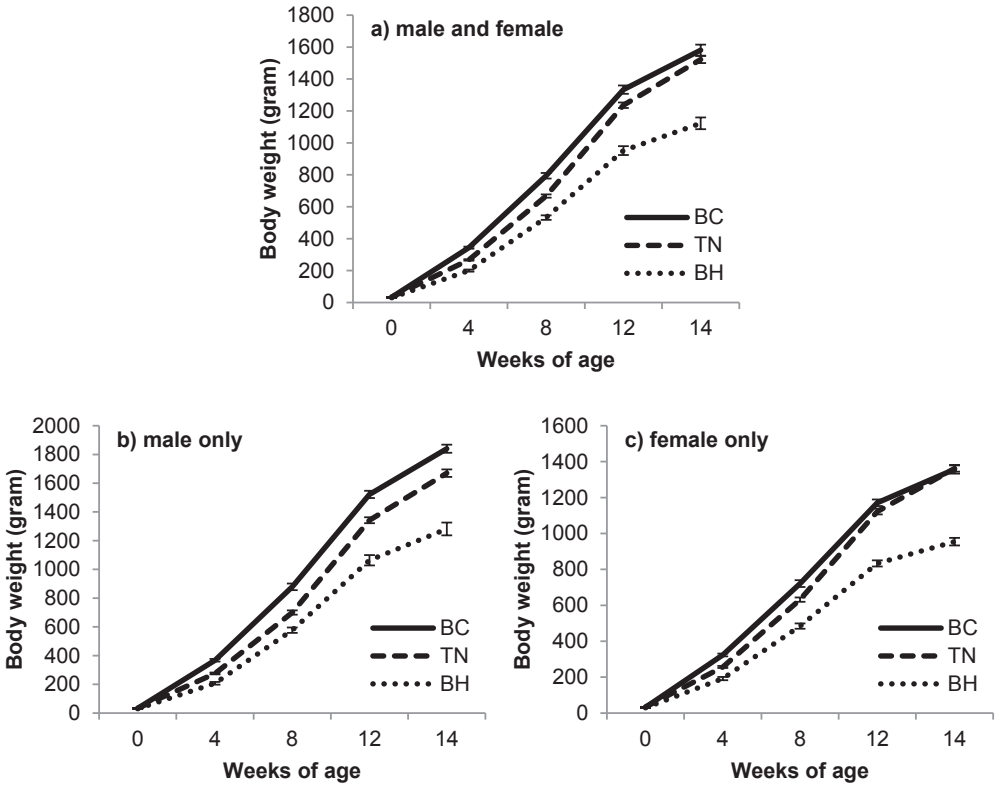

Figure 1. Mean of body weights (±Standard error) at 0, 4, 8, 12, and 14 weeks of age in Black-bone Chinese (BC), Thai native (TN), and Black-bone Hmong (BH) chickens.

Table 1. Heritability and genetic correlations of body weights at 0, 4, 8, 12, and 14 weeks of age in Black-bone Chinese (BC), Thai native (TN), and Black-bone Hmong (BH) chickens.

Breeds	Traits	No. of records	h^2	BW0	BW4	BW8	BW12	BW14
BC	BW0	68	0.94	1	0.49	0.35	0.46	0.44
	BW4	68	0.90		1	0.89	0.95	0.96
	BW8	68	0.77			1	0.95	0.88
	BW12	68	0.63				1	0.97
	BW14	68	0.59					1
TN	BW0	97	0.75	1	0.40	0.57	0.35	0.27
	BW4	97	0.37		1	0.84	0.57	0.49
	BW8	97	0.14			1	0.67	0.80
	BW12	97	0.43				1	0.96
	BW14	97	0.44					1
BH	BW0	37	0.72	1	0.11	0.11	0.47	0.42
	BW4	37	0.35		1	0.90	0.87	0.70
	BW8	37	0.73			1	0.92	0.73
	BW12	37	0.30				1	0.94
	BW14	37	0.15					1

REFERENCES

Duangjinda, M., I. Misztal, and S. Tsuruta. 2005. BLUPF90-ChickenPAK 2.5 [computer programs]. Khon Kaen University, Thailand.

Jaturasitha, S., T. Srikanchai, M. Kreuzer, and M. Wicke. 2008. Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red) Poult. Sci. 87:160-169.

Sungkhapreecha, P., M. Duangjinda, B. Laopaiboon, and W. Boonkum. 2015. Estimation of genetic parameters for growth performances in various black chicken lines and crossbred black chicken. KHON KAEN AGR. J. 43:309-318. (in Thai).

Tian, Y., M. Xie, W. Wang, H. Wu, Z. Fu, and L. Lin. 2007. Determination of carnosine in Black-Bone Silky Fowl (Gallus gallus domesticus Brisson) and common chicken by HPLC. Eur Food Res Technol. 226:311-.314

PO-06-3

Estimation of Genetic Parameters of Grey Brahman Cattle in Thailand Environment

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The objective of this study was to estimate genetic parameters of grey Brahman cattle raised in Thailand environment. Data were collected from livestock research and breeding stations under the Department of Livestock Development. The data analysis was based on 23,318 records collected from 1977 to 2016. Four weight traits used in the genetic analysis were birth weight (BW), weaning weight at 200 days of age (W2), yearling weight at 400 days of age (W4) and final weight at 600 days of age (W6). Multivariate animal mixed model was used to estimate genetic parameters and estimated breeding value. The results showed that estimates of heritabilities of BW, W2, W4 and W6 were 0.22 ± 0.02 0.20 ± 0.02 0.36 ± 0.12 and 0.50 ± 0.16, respectively. The genetic correlation estimates between BW and W2, BW and W4, BW and W6 were 0.27 ± 0.017 0.29 ± 0.05 and 0.07 ± 0.08 and between W2 and W4, W2 and W6, W4 and W6 were 0.54 ± 0.03 0.56 ± 0.05 and 0.47 ± 0.06, respectively. The genetic analysis showed that the genetic relationships between BW and weight traits measured later in live or post weaning growth were lower than the genetic relationships between W2 and later weight traits. Therefore, W2 is recommended for genetic selection to improve post weaning growth or post weaning weight traits in Grey Brahman cattle raised in Thailand environment.

INTRODUCTION

The Brahman is a tropical cattle breed (Bos indicus) developed from cattle of Indian origin, Guzerat Nellore or Ongole Gir Krishna and Valley. Thailand imported American Brahman cattle in 1954 by Bureau of Animal Husbandry and Genetic Improvement under Department of Livestock Development. Since then the cattle had been bred and selected for growth and fertility in Thailand environment. In 1995 the subsequence Brahman cattle were used for the establishment of Thai Brahman cattle with the ability to adapt to the environment in Thailand. The average birth weight was 28-30 kg, weaning weight at 200 days was 170 kg, adult males weigh 800-1,000 kg and females weigh 500-600 kg. Some characteristics of Thai Brahman cattle include adaptability to tropical environment, resistance to some diseases, ticks and insects. Thai Brahman cattle have been used in beef cattle development to increase growth and fertility efficiency in breeding programs, meat quality improvement in a synthetic breed of Kabinburi (crossing between Simmental and Thai grey Brahman). Two other synthetic breeds were developed from Thai Brahman namely Tak beef cattle (crossing between Charolais and Thai grey Brahman (Suwit, 2558)) and Kamphangsaen beef cattle (three breed crosses between Charolais, Thai Brahman and native cattle). Thai Brahman cattle are importance as a foundation of beef cattle development in Thailand. Therefore, a suitable breeding program is required for their genetic improvement. The genetic parameters specifically estimated from and for the herds are essential for the progress of the genetic selection for breeding improvement. Bourdon (2000) reported that heritability can be low (lower than 0.2), medium (0.2-0.4) and high (higher than 0.4). This study is aimed at estimation of genetic parameters of weight traits birth weight (BW), weaning weight at 200 days of age (W2), yearling weight at 400 days of age (W4) and body weight at 600 days of age (W6) of Thai grey Brahman cattle.

MATERIALS AND METHODS

The data used in this study were Thai grey Brahman beef cattle collected from livestock research and breeding stations under the Department of Livestock Development. The data analysis was based on 23,318 records collected from 1977 to 2016 stored in the database of animal breeding (e-Breeding) of Animal Breeding Information Center of Thailand. Four weight traits used in the genetic analysis were birth weight (BW) weaning weight at 200 days of age (W2) yearling weight at 400 days of age (W4) and body weight at 600 days of age (W6) of both males and females. Records available for analyses of traits are summarized in Table 1.

Table 1. Characteristics of weight traits data Grey Brahman Cattle in Thailand Environment.

Traits	N	Average	Std
Animal in pedigree	23,318	-	-
Birth weight records (BW) (kg)	16,599	28.49	3.48
Weaning weight records (W2) (kg)	12,174	163.03	31.11
400 day records (W4) (kg)	984	262.29	32.18
600 day records (W6) (kg)	592	375.93	71.06

Variance components were estimated using multivariate animal mixed model with restricted maximum likelihood average information (AI) algorithm (Patterson, and Thompson 1971) and estimation of breeding values method, BLUP (Henderson. 1984) by the computer program ASREML (Gilmour et al, 2002). The genetic parameters included heritability (H2 heritabilities); genetic and phenotypic correlations and breeding value (EBV; Estimated Breeding Value) of the birth weight (BW) the weaning weight at age 200 days (W2) the weight at the age of 400 day (W4) and the weight at the age of 600 day (W6) of beef cattle breed grey Brahman cattle in Thailand environment.

The model in the Matrix notation form

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 & 0 \\ 0 & X_2 & 0 & 0 \\ 0 & 0 & X_3 & 0 \\ 0 & 0 & 0 & X_4 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 & 0 \\ 0 & Z_2 & 0 & 0 \\ 0 & 0 & Z_3 & 0 \\ 0 & 0 & 0 & Z_4 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ a_4 \end{bmatrix} + \begin{bmatrix} S_1 & 0 & 0 & 0 \\ 0 & S_2 & 0 & 0 \\ 0 & 0 & S_3 & 0 \\ 0 & 0 & 0 & S_4 \end{bmatrix} \begin{bmatrix} pe_1 \\ pe_2 \\ pe_3 \\ pe_4 \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \\ \varepsilon_4 \end{bmatrix}$$

Where

y_i = the vector observations of traits (1 = birth weight (BW); 2= weaning weight at 200 days of age (W2); 3=yearling weight at 400 days of age (W4); 4=final weight at 600 days of age (w6)).

b_i = the vector of solution for the fixed effects (contemporary group, sex, age of dam and animal).

a_i = the vector of the direct additive genetic effects.

pe_i = the vector of the permanent environmental effects of the dams.

ε_i = the vector of the residual effects.

X_i, Z_i, S_i = incidence matrices of relating fixed effects, random direct additive genetic effects and permanent environmental effects of the dams to the observations.

Results and Discussions

Variance components and genetic parameters

The variance components and the heritabilities of weight traits; BW, W2, W4 and W6 of Thai grey Brahman cattle raising in the environment in Thailand are summarized in Table 2. The genetic variances of BW, W2, W4 and W6 were 2.1, 126.2, 216.7 and 615.3 respectively and phenotypic variances were estimated at 9.7, 628.6, 606.6 and 1238.0. The estimates of heritabilities were 0.22, 0.20, 0.36 and 0.50 respectively indicating that birth and weaning weights are moderately heritable whereas body weights measured at 400 and 600 days are highly heritable.

Table 2 Variance components and genetic parameters for BW, W2, W4 and W6

Traits	Variance components			Genetic parameters	
	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_p	h^2
BW	2.1 ± 0.2	0.2 ± 0.1	7.4 ± 0.2	9.7 ± 0.1	0.2 ± 0.02
W2	126.2 ± 14.2	55.3 ± 6.4	447.1 ± 11.4	628.6 ± 9.2	0.2 ± 0.02
W4	216.7 ± 81.6	-	389.9 ± 72.5	606.6 ± 43.4	0.4 ± 0.13
W6	615.3 ± 217.6	-	622.9 ± 189.9	1238.0 ± 123.2	0.5 ± 0.16

Traits:BW = Birth weight ;W2 = weaning weight at 200 days of age W4 = yearling weight at 400 days of age; W6 = final weight at 600 days of age

Variances:σ^2_a = direct additive ;σ^2_{pe}= permanent environment;σ^2_e = error;σ^2_p = phenotype;h^2 = heritability

Heritabilities of the 4 traits lied in medium - high levels consistent with the Bourdon (2000) who reported that heritability can be divided into 3 levels; a low level is less than 0.2 medium ranged between 0.2-0.4 and higher than 0.4. The results of this study are different from the report of Wuttipong et al (2010) who studied growth characteristics of Thai Brahman cattle and reported that heritability estimates of weights at BW, W2 and W6 were 0.32, 0.25 and 0.34. Thirachai (1996) reported that the estimates of heritability of Thai Brahman cattle weights at BW, W2 and W4 were 0.20, 0.36 and 0.21. Yaowaluck et al (2013) estimated that heritabilities of weights of BW, W2, W4 and W6 of Tak beef cattle at 0.29, 0.34, 0.26 and 0.33. In South African B.A. Pico (2004) reported heritability estimates of Brahman cattle for BW, W2, W4 and W6 at 0.28, 0.14, 0.14 and 0.18 respectively. From several studies of the heritability of animals of the same species, it can be seen that the results are different. This is due to the differences of the genetic structures of the populations, the managements, environment and the evaluation methods of variances. Genetic selection of the four studied weight traits in the Thai Brahman cattle can be obtained since the heritability estimates from this study were at the medium to high levels. The progress and efficiency of the genetic improvement for the Thai Brahman cattle is achievable through genetic selection. However, in breeding program attention should be paid to the environmental improvement as well.

Genetic and phenotypic correlations

Genetic and phenotypic correlations between BW, W2, W4 and W6 of Thai grey Brahman cattle raised in the environment of Thailand are shown in the Table 3.

Table 3 Genetic (below diagonal) and phenotypic (above diagonal) correlations estimates

Traits	BW	W2	W4	W6
BW	0	0.69 ± 0.05	0.62 ± 0.17	0.32 ± 0.20
W2	0.27 ± 0.01	0	0.54 ± 0.03	0.56 ± 0.05
W4	0.29 ± 0.05	0.74 ± 0.15	0	0.47 ± 0.06
W6	0.07 ± 0.08	0.80 ± 0.17	0.78 ± 0.25	0

In this study it was found that the correlation estimates of BW with W2, W4 and W6 were 0.27, 0.29 and 0.07; W2 with W4 and W6 were 0.74 and 0.80; and W4 with W6 was 0.78, respectively. The resulting correlation estimates conform to a report by Teerachai (1996) who also estimated genetic correlations in Thai Brahman cattle of BW with W2 and W4 of 0.44 and 0.18 and W2 with W4 of 0.43. Yaowaluck et al (2013) reported that the genetic correlations between BW with W2, W4 and W6 were 0.30 0.25 0.15; W2 with W4, and W6 were 0.62 and 0.31. W4 with W6 was 0.51, respectively. It can be seen that the correlation estimates between the four weight traits are all positive therefore improving by selecting weights at any ages; BW, W2, W4 and W6 would result in some progress of other weight traits in the same direction depending on the magnitude of the genetic correlations. Selection of calves with high BW and W2 would result in the increase in yearling weight W4 and W6 weight as the correlation coefficients are moderately positive. The longer the periods between body weight measurements tend to have the lower genetic and phenotypic correlations which is consistent with the Wuttipong et al (2010) who suggested selection for replacements of Thai Brahman in Thailand should be carried out at weaning (W2) approximately 20-25 % for sales to farmers and Thai Brahman cattle should be selected again at

W6.

Conclusion

Estimation of Genetic Parameters of Grey Brahman Cattle in Thailand Environment revealed that the genetic variances of BW, W2, W4 and W6 were 2.1, 126.2, 216.7 and 615.3 respectively and phenotypic variances were estimated at 9.7, 628.6, 606.6 and 1238.0 respectively. The estimates of heritabilities were 0.22, 0.20, 0.36 and 0.50 respectively and Correlation of BW with W2, W4 and W6 were 0.27, 0.29 and 0.07; W2 with W4 and W6 were 0.74 and 0.80 and W4 with W6 was 0.78 respectively. The genetic analysis showed that the genetic relationships between BW and weight traits measured later in live or post weaning growth were lower than the genetic relationships between W2 and later weight traits. Therefore, W2 is recommended for genetic selection to improve post weaning growth or post weaning weight traits in grey Brahman cattle raised in Thailand Environment. Genetic improvement of market weight of Thai Brahma cattle should include all the weight traits studied to form a suitable selection index using the appropriate genetic parameters specifically for the herds in Thailand.

Key words : Genetic parameter, Grey Brahman cattle**,** estimated breeding value of cattle, Beef cattle

Referance

B.A. Pico, F.W.C. Neser and J.B. van Wyk. 2004. Genetic parameters for growth traits in South African Brahman cattle. South African Journal of Animal Science 2004.

Bourdon, R.M. 2000. Understanding Animal Breeding. 2nd ed. Prentice-Hall, Inc., New Jesey.

Henderson, C.R. 1984. Applications of linear models in animal breeding. University of Guelp Press., Guelph.

Gilmour, A.R., B.R. Cullis, S.J. Welham and R. Thomson. 2002. ASReml Reference Manual. NSW Agriculture, Orange, Australia.

Patterson, H.D. and R. Thompson. 1971. Recovery of inter-block information when block sizes are unequal. Biometrica. 58:545-554.

Raciel J. Estrada-Leon-Juan G. Magana-Monforte-Jose C. Segura-Correa. 2014. Estimation of genetic parameters for preweaning growth traits of Brahman cattle in Southeastern Mexico. Trop Anim Health Prod46:771-776.

Yaowaluck Leepaijit, Ek Vitoonpong and Wutipong Intaratham. 2013. Estimated breeding value and index EBVs of growth traits in Tak beef cattle with genetic groups at various blood levels. e – Journal, Vol. 2 (Online 19 September 2013), Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok. Available Source: http://www.dld.go.th/Journal-2013. html. 20 May 2016.

Narumol Intra, Prasit Yimget and Ek Vitoonpong. 2012. Inbreeding Effect on Growth and Fertility Traits of Brahman Cattle Raising in Tabkwang Livestock Research and Breeding Center. e – Journal, Vol. 1 (Online: 20 July 2012), Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok. Available Source:http://www.dld.go.th/Journal-2012.html. 5 June 2016.

Wutipong Intaratham, Ek Vitoonpong and Yaowaluk Laepaijit. 2010. Estimated breeding value and genetic trends of growth traits in Brahman beef cattle from multivariate analysis. Available Source:http://www.dld.go.th/Journal-2010.html. 5 June 2016.

Suvit Boonprong. 2015. Manual for beef cattle for farmers. Division of Livestock Extension and Development, Department of Livestock Development, Ministry of Agriculture and Cooperatives. Available Source: http://breeding.dld.go.th/th/images/document/beef/.

5 June 2016.

PO-06-4

Improvement of Carcass Characteristics of Thai Pigs by Crossbreeding with Wild Boars

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INTRODUCTION

Thai pigs are well adapted to the hot and humid climate, tolerate low-quality feed and resistant to some diseases of Thailand (Na-Lampang, 1985; Rattanaronchart, 1994; Na-Lampang, 2002). This ability makes Thai pigs more appropriate to farmers in the rural areas of Thailand than the imported breed pigs. However, due to high fat deposition in the carcass makes Thai pig meat less favorable to the consumer. Thai wild boars are known for their low fat deposition in the carcass. Thus it was hypothesized that if Thai pigs were crossed with wild boar, the crossbred should have been leaner carcass than that of Thai pigs. The objective of this research was to improve carcass quality of Thai pigs by crossbreeding with wild boars.

MATERIAL AND METHODS

Experimental animals

Thirty wild boar crossbred (6 wild boars x 30 Thai gilts) litters were compared with 30 Thai pig litters (6 Thai boar x 30 Thai gilts) on litter size and pre-weaning growth rate. After weaning at 8 wk of age, 80 purebred Thai pigs (40 males and 40 females) and 80 wild boar crossbred pigs (40 males and 40 females) were reared in Suranaree University of Technology farm in the same housing condition with the same diet. All of the pigs were slaughtered at 32 wk old. The day before slaughter, the animals were weighed for determination of slaughter weight. The pigs were slaughtered according to Thai standards. Warm carcasses were measured for carcass weight, carcass length, back-far thickness, back-fat weight, abdominal and visceral fat weight, loin eye area, tender loin weight, loin weight, and jowl weight. Then, the longissimus muscle from left side between the 5th and 13th rib was removed and meat qualities were evaluated. Meat characteristics measured were L. dorsi muscle pH (measured at 1 and 24 hr post-mortem), meat color (brightness (L*), redness (a*) and yellowness (b*)), shear force, drip loss and cooking loss.

RESULTS AND CONCLUSION

Comparison of Litter Sizes and Performance of Piglets

Litter size at birth, litter size at weaning (8 wk of age), birth weight, 8 wk weight and pre-weaning average daily gain of wild boar crossbred pigs were not significantly different from Thai piglets.

Comparison of Growth Rate and Feed Efficiency during Fattening

Body weight at 32 wk of age, average daily gain and feed efficiency during 8 – 32 wk of age of wild boar crossbreds were lower ($P<0.05$) than those of Thai pigs.

Comparison of Carcass Characteristics

Wild boar crossbreds had lower body weight ($P<0.05$) than Thai pigs at 32 weeks of age due to the lower growth rate. This in turn caused them to have lower warm carcass weight ($P<0.05$). It was found that sex affected warm carcass weight in wild boar crossbred pigs, i.e. the value of male pigs was higher than that of female pigs, but not in Thai pigs. Dressing percentage of wild boar crossbred pigs and Thai pigs were not significantly different.

Wild boar crossbreds had shorter carcass and thinner average carcass back-fat than Thai pigs. Loin eye area of wild boar crossbreds was larger than that of Thai pigs.

Wild boar crossbreds had higher ($P<0.05$) loin weight and tender loin weight but had lower ($P<0.05$) neck meat weight, back-fat weight and abdominal fat weight than Thai pigs. Ham weight, shoulder weight and belly weight were not different between wild boar crossbreds and Thai pigs.

Comparison of Meat Quality Characteristics

No significances were observed in the pH values of L. dorsi muscle measured at 1 and 24 hr post mortem between the two types of pigs. Meat of wild boar crossbreds had lower brightness (L*) than Thai pigs, but did not differ in a* and b* values.

Shear force, drip lass and cooking loss values of wild boar crossbreds were not significantly different from those

of Thai pigs.

CONCLUSION

Crossbreeding Thai pigs with wild boar caused lower growth rate and feed efficiency. Crossbreeding Thai pigs with wild boar seems to be able to improve carcass quality of Thai pigs by reducing fat deposition in the carcass. However, due to their slower growth rate, the differences found in this study might be from the lower body weight of wild boar crossbreds at slaughter. So in order to confirm the result, there should be another study to compare the two types of pigs at the same slaughter weight. Moreover, total pork production and economic return per year of wild boar crossbreds should be compared with those of Thai pigs.

REFERENCE

Na-Lampang, P. 1985. Traditional pig raising systems in the Northeast. Farming System Research Project. Khon Kaen University. KKU/FSR, A/Te/No.1/85. Thailand.

Na-Lampang, P. 2002. Study on factors affecting conservation of genetic resource of Thai pigs in the Northeast. Research Final Report. Suranaree University of Technology. Thailand.

Rattanaronchart, S. 1994. Present situation of Thai native pigs. Project Report, 1994, Department of Animal Science, Chiang Mai University. Thailand.

PO-06-8

THE ESTIMATION OF HERITABILITY AND REPEATABILITY OF BIRTH WEIGHT AND WEANING WEIGHT OF HANWOO

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ABSTRACT

The birth weight and weaning weight of Hanwooare influenced by the abilities of cow.Birth weight receives uterus ability of cow, and weaning weight shows a difference according to lactation ability of cow. This study is conducted to estimate heritability and repeatability of birth weight and weaning weight strongly influenced by cow so to use excellent entity as basic information to be continuously used for breeding.The data used for the analysis was performed using the first parity and over second parities 16,697heads inbreeding farm.The birth weight is numerical value measured in birth, and weaning weight was used adjusted data at 90 days of age.In the statistical models, breeding farm, calving months, mating count, sex of calf and birth year-season effects were included as fixed effects, and the linear covariate term of first mating age were analyzed.The Calving months were the highest 9 months, and birth rate by the first fertilization is the highest mating count by 70.4%. In addition, first mating age is from 290 days to 625 days, so 446 days are the most first mating.In the first parity, heritability of birth weight, weaning weight are 0.20, 0.17, respectively. After the second parities, heritability of birth weight, weaning weight are 0.14, 0.12, respectively. The birth weight and weaning weight of repeatability were estimated to be 0.23, 0.30, respectively.A genetic correlation between birth weight in the first parity and birth weight after the second parities is 0.98, and a genetic correlation between weaning weight in the first parity and weaning weight after the second parities is 0.84.As a result of this study, moderate repeatability is showed because calves produced by a mother animal receive same factors from a mother animal and the maternal effect can act commonly on calves.
Key Words :Hanwoo, Birth weight, Weaning weight, Heritability, Repeatability

INTRODUCTION

The birth weight and weaning weight of Hanwoo are influenced by the abilities of cow. Birth weight receives uterus ability of cow, and weaning weight shows a difference according to lactation ability of cow. This study is conducted to estimate heritability and repeatability of birth weight and weaning weight strongly influenced by cow so to use excellent entity as basic information to be continuously used for breeding.

MATERIAL AND METHODS

With regard to materials used in this study, a total of 42,696 heads on calving according to parity for 16,697 cows breeding in 92 Hanwoo breeding farms from the year 2000 were used. And birth weight, trait used in the analysis, was value measured in calving. With regard to weaning weight, data adjusted to 90 days of age were used

90 Days weight = (weaning weight – birth weight)/(weaning age – birth day) * 90 age

With regard to environmental effect in estimating heritability and repeatability, a farm, parity, gender of calf, and calving year-season was considered to be a fixed effect. And calving age and first mating age was considered to be covariance. And an analysis was made by using REMLF90 program (Misztal, 2001).
A baseline data analysis of reproductive traits examined in this study was made by using SAS Ver9.1. And an analysis was made by using a multiple mixed model in order to estimate genetic parameter and breeding value for additive genetic effect of each trait.

The following medel was used to estimate genetic parameters within the population

$$y = X_b + Z_a + W_m + S_{pe} + e$$

Where **y** is a vector of birth weight and weaning weight observation, **b** is a vector of fixed effects, **a** is an unknown randow vector of additive genetic effects, **m** is vector of random maternal additive genetic effects, **pe** is a vector of random maternal permanent environmental effects, **S** is design matrix, **e** is an unknown random vector of residuals, **W** is design matrix, **X** and **Z** are known as incidence matrices relating obervation to **b** and **a**, reapecrively.

With regard to the estimation of genetic parameter, an analysis was made by using REMLF90 (Misztal, 2001) based on EM-REML algorism. An iterative estimation was made until the difference of each variance value converged to 10^{-11} or below. A total of 18,929 cows were used for pedigree data used in estimating and analyzing a genetic parameter.

Table 1 . Fixed effects, covariates and random effects in the models for traits analyzed

Traits[a]		Fixed effects[b]				Covariates[c]		Random effect	
		FARM	PARITY	SEX	YS	Age	1st Age	Animal	PE
1st Parity	BW	●		●	●	●	●	●	
	WW	●		●	●	●	●	●	
≥ 2nd Parity	BW	●	●	●	●	●		●	●
	WW	●	●	●	●	●		●	●

[a] BW : Birth Weight, WW : Weaning Weight. [b] FARM : Breeding Farm, SEX : Calf sex, YS : Calving year-season. Age : Age at the calving
[c] 1st Age : Age at the first mating

RESULTS AND DISCUSSION

Table 2. General means and standard deviation for traits.

Traits		N	Means	STD	Min	Max
1st Parity	1st Age	14,351	456.8	64.8	290	625
	BW	14,012	25.65	3.18	10	45
	WW	3,074	80.22	16.80	30.2	190.5
≥ 2nd Parity	BW	27,856	27.07	3.39	10	48
	WW	7,019	86.84	17.21	30.1	220.1

A change in birth weight and weaning weight according to parity was shown in Fig. 1.

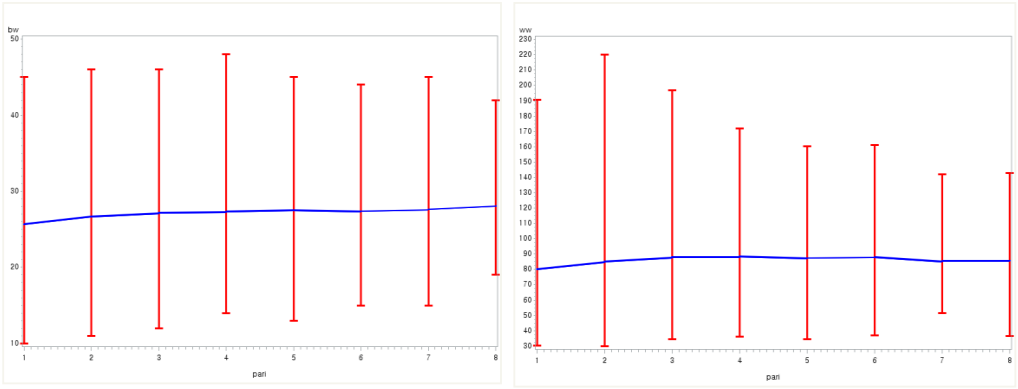

Figure 1. Plot of birth weight and weaning weight by parities.

It was shown that average birth weight was 25.6 kg in the 1st parity, 26.7 kg in the 2nd parity and 27.2 kg in the 3rd parity. And it was shown that average weaning weight was 80.2 kg in the 1st parity, 85.1 kg in the 2nd parity and 88.0kg in the 3rd parity. It was not shown that the level of increase in birth weight and weaning weight was significant according to parities.

The graph for the number of mating and month at the first mating was shown in Fig. 2

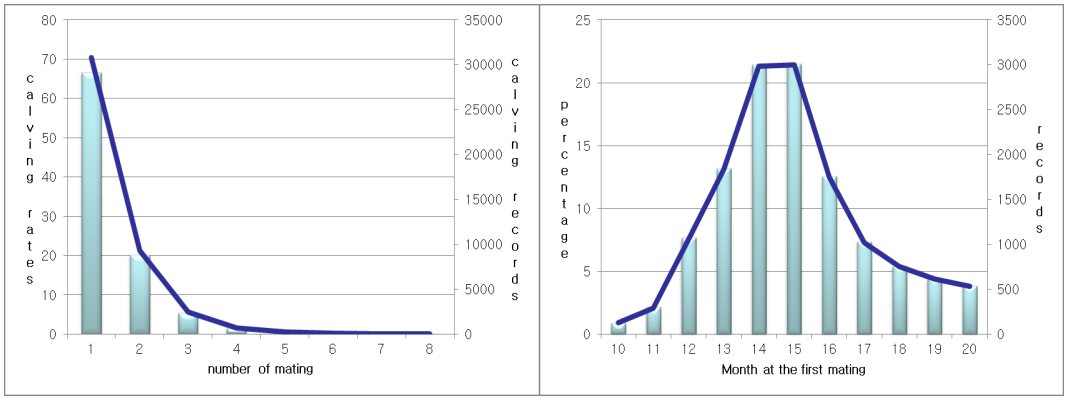

Figure 2. The graph for the number of mating and month at the first mating.

The 68% of cows were gestated in the 1st insemination, 20% of cows were gestated in the 2nd insemination and 5% of cows were gestated in the 3rd insemination for Hanwoo breeding farm, which made it possible to find that most of cows were gestated in the 1st~3rd insemination. With regard to first mating age, it was possible to find the results where most of cows were gestated at 14~15months of age. It is thought that this results from seasonal breeding-centered mating in the mating system implemented by the breeding farm.

Genotypic (co)variance regarding birth weight and weaning weight in the 1st and after 2nd parity was shown in Table 3, 4 and 5.

Table 3. Estimates of genetic (co)variance in birth weight, weaning weight.

Traits		1st Parity		2nd Parity	
		BW	WW	BW	WW
1st Parity	BW	0.843	1.255	0.654	1.303
	WW		29.55	1.047	18.45
≥ 2nd Parity	BW			0.563	1.148
	WW				17.69

Table 4. Estimates permanent environment (co)variance in birth weight, weaning weight

Traits		1st Parity		2nd Parity	
		BW	WW	BW	WW
1st Parity	BW	0	0	0	0
	WW		0	0	0
≥ 2nd Parity	BW			0.858	2.077
	WW				12.35

Table 5. Estimates of residual (co)variance in birth weight, weaning weight.

Traits		1st Parity		2nd Parity	
		BW	WW	BW	WW
1st Parity	BW	6.111	8.162	0	0
	WW		164.6	0	0
≥ 2nd Parity	BW			7.24	8.088
	WW				184.8

Phenotypic correlation and genetic correlation regarding birth weight and weaning weight in the 1st and after 2nd parity was shown in Table 6.

Table 6. Phenotypic correlations(below the diagonal) and genetic correlation(above the diagonal) among the birth weight, weaning weight.

Traits		1st Parity		2nd Parity	
		BW	WW	BW	WW
1st Parity	BW		0.35	0.98	0.45
	WW	0.31		0.35	0.84
≥ 2nd Parity	BW	-	-		0.47
	WW	-	-	0.30	

Phenotypic correlation and genetic correlation between birth weight and weaning weight in the 1st parity was estimated to be 0.31 and 0.35. And it was shown that genetic correlation regarding birth weight and weaning weight in the 1st and after 2nd parity was 0.98 and 0.84, which was high genetic correlation. And genetic correlation between birth weight and weaning weight was estimated to be 0.45. Phenotypic correlation and genetic correlation between birth weight and weaning weight was estimated to be 0.30 and 0.47 respectively.

The results of heritability, meteral heritability and repeatability regarding the birth weight and weaning weight in the 1st parity and after the 2nd parity were shown in Table 7.

Table 7. The heritability(h^2), maternal heritability(h_m^2), total heritability(h_T^2) and repeatability(r) in birth weight, weaning weight.

Traits		h^2	h_m^2	h_T^2	r
1st Parity	BW	0.12	-	-	-
	WW	0.15	-	-	-
≥ 2nd Parity	BW	0.07	0.10	0.23	0.20
	WW	0.09	0.06	0.24	0.11

Heritability regarding the birth weight and weaning weight in the 1st parity was estimated to be 0.12 and 0.15 respectively. And heritability, meteral heritability, total heritability and repeatability for the birth weight after the 2nd parity was estimated to be 0.07, 0.10, 0.23 and 0.20 respectively. And heritability, meteral heritability, total heritability and repeatability for the weaning weight was estimated to be 0.09, 0.06, 0.24 and 0.11 respectively

Park et al. (2001) published that the heritability for birth weight and weaning weight was 0.15 and 0.17 respectively, according to the results of analyzing the multiple-trait animal model regarding the birth weight and weaning weight in Hanwoo. HulyaAtil et al. (2005) showed that repeatability estimates for birth weight and weaning weight in Holstein Friesian were 0.75 and 0.15 respectively, which were a little lower resultant values than published data. VecihiAksakal et al. (2009) published that heritability and repeatability estimates for birth weight in the same breed were 0.23 and 0.20 respectively.

And Akhtar et al. (2012) published that heritability and repeatability estimates for birth weight and weaning weight in female Nili-Ravi Buffalo were 0.25, 0.17, 0.29 and 0.41 respectively, which were estimates higher than those in the results of this study.
According to the results of this study, it is thought that medium repeatability was shown because calves which a dam gave birth to were affected by the same factor from the dam, and because a meternal effect was produced on the calves in common.

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References

B.H.Park, J.H.Jung, Y.M.Cho, C.K.Park, Y.I.Park. 2001. Estimation of genetic parameters of birth weight and preweaning gain of Hanwoo using Multiple Trait Animal Model. J. Anim. Sci& Technol. (Kor.) 43(4) 445 ~ 450

Misztal, I. 2001. BLUPf90 family package. http://nce.ads.uga.edu.

P.Akhtar, U.Kalsoom, S.Ali, M.Yaqoob, K.Javed, M.E.Babar, M.I.Mustafa and J.I.Sultan, 2012, Genetic and Phenotypic Parameters for Growth Traits of Nili-Ravi Buffalo Heifers in Pakistan.

SAS Institute Inc. 2004. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.

VecihiAksakal and BahriBayram, 2009, Estimates of Genetic and Phenotypic Parameters for the Birth Weight of Calves of Holstein Friesian Cattle Reared Organically. Journal of Animal and Veterinary Advances 8(3):568~572

PO-06-9

Genetic and economic evaluation of Tak synthetic beef cattle breeding schemes

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Introduction

Tak synthetic beef breed was established at Tak livestock research and breeding center since 1986. They were being developed on the basis of 62.5 % Charolais and 37.5 % Brahman. The breeding objectives for this breed's establishment were to improve growth, carcass characteristics and fertility. As outlines by Department of Livestock Development (DLD) statistic (2014), Thailand had a total beef population of 4.3 million heads. These numbers of beef cattle were maintained by 745,408 households. The beef meat market could be typically classified into three different groups of consumers. The first group was the low market which contributed approximately 53 % of the whole market value. The second group was the medium market which emphasized on meat yield and tenderness of meat carcass. Brahman and European crossbred fattening cattle were mostly classified in this category. This group contributes 44% of the market value. The last group was the upper market which emphasized on both tenderness and intramuscular fat of carcass. European crossbred fattening cattle were mostly classified in this category. However, this group contributes only 3 % of the market value. According to three beef meat markets mentioned above, Tak beef breed played an important role for supplying both medium and high quality beef meat in Thailand. They were used in cow-calf production system. The mating systems were widely used both natural mating and artificial insemination. Bulls and cows were selected within herd and mated to produce male offspring for fattening at 500 to 550 kilogram for market weight and to produce female offspring for replacement. The current structure within Tak beef breed population requires some direct involvement of DLD livestock centers to maintain and improve a nucleus of this breed whose progress will be disseminated via a multiplier herd to the commercial village farmers.

Genetic improvement programs for Tak beef breed were business and involve more than one trait. Rate of improvement that were achieved in key economic traits typically were of the order 0.5 to 2 % of the mean per annum. To obtain these long-term changes, the relative economic importance of changes in all traits in the breeding objectives should be known in order to appropriately weight the genetic evaluations for those traits and achieve maximum response. Emphasis in breeding programs of Tak beef breed should be to evaluate and optimize gene flow principles to disseminate the improved genetics through the target industry. Therefore, the objectives of this article are to evaluate Tak beef breed breeding schemes base on profit per cow and annual genetic gains and on maximization of returns from investment in Tak beef breed population.

Materials and MethodsBreeding structures of Tak beef breed Information about the breeding structures of Tak beef breed population considered here was presented in Table 1. The breeding structures consisted of three tiers; nucleus, multiplier and commercial herds. Nucleus herd was genetically elite herds in three DLD's livestock station/center namely, Tak livestock research and breeding center, Phitsanulok and Nakorn Sawan livestock breeding stations. The primary functions of these nucleus herd were to produce elite animals according to the breeding objective and to distribute them to multiplier herd. Sire and dam replacement were selected only from nucleus herd. Multiplier herd was owned by progressive farmers or contract farmers under the guidance of DLD. The functions of the multiplier herd were to expand the genetic materials of the elite nucleus into greater numbers of animals to pass on to the commercial herd. Thus, the multiplier herd was a replication of the original nucleus which developed into two tiers, one tier being the true nucleus while the other tier involved satellites of the nucleus. Bulls for performance testing were selected from nucleus herd. Mating was conducted by natural mating. Dams mostly were the farmer's cows. Commercial herd was those raised by smallholder farmers throughout the country. They normally lacked good sires of their own and mating was by bulls from multiplier herd.

According to Table 1, the total number of population comprised 150,000 cows, 500 or 0.3 % of which were organized in nucleus herds, 7,500 or 5.0 % of which were in multiplier and the remaining, 142,000 or 94.7 % of which were in the commercial herd. There was natural mating, with a bull to cows ratio of 1:25, 1:50 and 1:50 in nucleus, multiplier and commercial herd, respectively.

Selection Group Ten selection groups, coded 1-10, Four selection groups were defined in the nucleus, where bulls

selection occurred both to improve the sire used (sires to breed sire, NS>NS) and the female retained (sire to breed dams, NS>ND). Selection of females in the nucleus herd also occurred to improve both sire (dams to breed sire, ND>NS) and female (dams to breed dams, ND>ND). The breeding structure was closed system due to replacement stocks for the nucleus herd was bred entirely from within the nucleus and genes can only move in one direction. The sires from nucleus herd were used to produce cows in multiplier herd (sire to breed dams, MS>MD) and female (dam to breed dam, MD>MD). The sires from multiplier herd were used to produce sires (sire to breed sire, MS>CS) and female to produce bulls (dam to breed sire, MD>CS) in commercial herd. The last two selection groups involve using of home-bred commercial sire to produce dam (CS>CD) and that of home-bred commercial dam to produce dam (CD>CD).

Production model

In this study a production model was developed to describe a Tak beef breed enterprise in DLD's livestock center/ station (DLD, 2009). The model allowed to compute economic weights for a number of traits related to the production system.

Selection criteria Selection criteria were designed to be 14 traits i.e. sale weight-direct component (SW-d), weaning weight (W2), yearling weigh (W4), cow weight (CoW), girth circumference (GIR4), hip high (HH4), body length (STP4), weaning rate (WR), scrotum circumference at yearling (SC4), Day to calving (DC), age at first calving (A1C), carcass weight (CW), back fat thickness (BF) and eye muscle area (EMA). There were 5 selection groups, for example, in selection group 1, all sires bred in the nucleus herd were considered to have the same information available. Therefore, selection groups for (1) NS>NS, (3) NS>ND, (5) MS>MD and (7) MS>CS were based on the same index. The information derived form 4 sources such as form individual, dam, paternal half sibs progeny of dam and paternal half sibs of dam with 9, 9, 7 and 2 selection criteria recorded. Selection group 2 consisted of (2) ND>NS and (4) ND>ND which the information derived form 4 sources with 7, 9, 7 and 2 selection criteria recorded. Likewise, selection group 3 for (6) MD>MD and (8) MD>CS and selection group 4 for (9) CS>CD, the information derive form 2 sources of individual and dam with 4, 5 and 5, 5 selection criteria recorded, respectively. The last one in selection group 5 for (10) CD>CD, the information derive only form one source of individual with 2 selection criteria recorded. All measurements for selection criteria traits were performed in nucleus herd. Nevertheless, in multiplier herd, body measurements such as HH4, GIR4, STP4 and SC4 were only taken.

Breeding objective

The breeding objective utilized addresses production from a pasture-base beef enterprise. The breeding objectives were to improve fertility, growth rate and carcass quality that integrated the cow-calf production system and feedlot segments. For fattening, the age was finished at 2.1 years, an averages live weight of 550 kilogram. Therefore, under this production system, the traits considered in the objective were WR, W4, SW-d, Cow and CW, respectively.

Derivation of economic weights

The function that best represented total herd profit was defined by Gron *et al*. (1989) as incomes minus costs.

Profit = (weaning rate × number of calves from birth to 600 days × survival rate from birth to 600 days × sale weight at 600 days × sale price at 600 days) + (weaning rate × number of calves from birth to sell at 2.1 years × survival rate from birth to 2.1 years × carcass price at 2.1 years) + (number of culled cows × sale weight of culled cows) – (weaning rate × number of calves from birth to 600 days × survival rate from birth to 600 days × total cost of 600 days calf) + (weaning rate × number of calves from birth to sell at 2.1 years × survival rate at sell at 2.1 years × total cost of sell at 2.1 years) + (number of wet cows × total cost of wet cows) + (number of dry cows × total cost of dry cows)

Biological and Technical Parameters

Estimates of productive lifetime, survival rate and age at first calving for various selection groups were vital for construction of the transmission matrix. The other values were required to calculate the proportion of selected animal and selection intensities.

Genetic and Phenotypic Parameters

Genetic and phenotypic parameters were derived from studies with Tak beef breed when
available (Intaratham, 2010; Laepaijit, 2013). When not available, they were derived from studies on other tropical beef breeds (Burrows, 2001).

Investment Parameter

The fixed costs included were those directly relating to management cost i.e. DLD's officer, center/station's officers and farm labor and material cost i.e. compensation, operational cost and feed cost. These costs occurred mainly in the nucleus herd. The estimates of these costs depended on fiscal year budget allocation for Tak beef breed in three livestock center/stations (DLD, 2016).

Evaluation of genetic and economic return

ZPLAN program (Karras *et al*., 1993) was used to calculation the return and annual genetic gains from one round of selection in a deterministic approach, employing selection index theory and gene flow methodology. The schemes evaluated herein were:

Scheme 1 basic run by natural mating for all herds

Scheme 2 scheme 1 plus varying 5 factor levels (5.0, 5.5, 6.0, 6.5 and 7.0%) of cow number in multiplier herd

Scheme 3 scheme 2 plus artificial insemination in multiplier herd for optimizing breeding program.

Results and Conclusion

Scheme 1 : Basic run by natural mating for all herds

The genetic gain per year, return, cost and profit per cow in population were presented in Table 2. Inserting the average generation interval (4.32 years) and the average genetic gain per generation of selection groups achieved in the nucleus herd, led to genetic gain per year for each trait. In this case the gene contributions of these four groups were equal at 25% for each group. The results revealed that genetic gain per year for growth (SW-d, W2, W4, CoW), fertility (SC4, WR, A1C, DC), carcass traits (CW, BF, EMA) and skeletal measurements (GIR4, HH4, STP4) were positive. In this studies agreed with Nitter et al., (1994); Graser et al., (1994); Waltl et al., (2002). These positive genetic gains were in the desirable direction to improve genetic of Tak beef breed.

For fertility, SC4 for male bulls and WR for female cows, trends were also positive and for DC and A1C the trends were negative. The genetic gains of fertility traits were all favorable because shorter DC and lower A1C and higher for WR led to an overall increase in profit. The genetic gain per year for breeding objective was 221.18 baht. This was the sum of the genetic gain per year of SW-d (1.92 kg), CoW (6.44 kg), WR (0.19%) and CW (0.46 kg), multiplied by economic weight of SW-d (26.55 baht), CoW (17.07 baht), WR (189.66 baht) and CW (50.75 baht). With regard to return and cost, the returns per cow for SW-d, CoW, WR and CW were 254.90, 513.70, 123.52 and 70.64 baht, respectively. The total return per cow in the population of 150,000 cows was obtained by summing the return per cow from breeding objective traits above which were 962.75 baht. The total costs of selection per cow were 72.88 baht. The total returns had more than the total costs gave the resulting profit which was to be 889.65 baht per cow. On the whole, the total costs in the population of 150,000 cows were 10,966,500 baht. The profits in the investment period (20 years) were 133,446,500 baht under this basic run analysis.

Scheme 2 : Varying 5 factor levels of various cow size in multiplier herd

The varying number of cows in multiplier herd which comprised 5 factor levels at 5.0, 5.5, 6.0, 6.5 and 7.0% of the total population was presented in Table 3. The results showed that the genetic gains per year and breeding objective were the same for all levels as indicated previously in table 2. However, of interest, the important issues were focused on the return, cost and profit per cow which varied according to the factor levels. The results indicated that the return, cost and profit per cow in multiplier herd at 7.0 % of the total population increased 2.05 %, 1.94 % and 2.06 % from the basic run, respectively. For the bottom line, the results showed that the total cost and profit increased 1.63 % and 2.06 % from basic run. The increase of total profit was greater than total cost due to performance recording in this study was mostly conducted in nucleus herd. Consequently, when more expanding the number of cow size in multiplier herd more receiving the total profit from investment on Tak breeding program.

Scheme 3 : Artificial insemination in multiplier herd

Used of AI in multiplier herd was presented in Table 4. The results showed that using AI in multiplier herd had higher total cost than using natural mating. The total cost for investment was to be 16,284,000 baht per year for this operation, compared to only 11,145,000 baht per year for natural mating in multiplier herd. However, used of AI in the multiplier herd influenced the breeding program outcomes in several ways. First, where AI was used, higher selection intensity could be applied when selecting breeding herd sires, as fewer sires were required. Second, selection accuracy was increased for all selection groups, as the altered population structure meant that information was available on a greater number of related animals. Both these factors act to increase genetic gain and profit was significantly increased even after accounting for the cost of AI. Importantly, under the 2-stage

selection structure modeled, the use of AI meant that profit was maximized with fewer bulls measured in the second stage, compared to breeding scheme using natural mating only. For the bottom line, the profit per cow of scheme 3 was higher than that of natural mating with an average of 1,115.02 baht. In conclusions, the optimizing breeding program of this study should to be scheme 3. The genetic gain per year for SW-d, W2, W4, CoW, GIR4, HH4, STP4, WR, SC4, DC, A1C, CW, BF and EMA were to be 1.92 kg., 1.44 kg, 1.89 kg, 6.44 kg, 0.29 cm, 0.50 cm, 1.05 cm, 0.19 %, 0.08 cm, -0.12 day, -0.004 year, 0.46 kg, -0.02 cm and 0.28 inch^2. The breeding objective, profit per cow and total profit were to be 221.63, 1,115.02, and 167,253,000 baht.

Table 1 Breeding structures of Tak beef breed population

Tiers	Amount (heads)
Number of cows	
nucleus herd	500
multiplier herd	7,500
commercial herd	142,000
Total number of cow	150,000
Bull per cows ratio	
nucleus herd	1 : 25
multiplier herd	1 : 50
commercial herd	1 : 50

Table 2 Genetic gain per year, return, cost and profit per cow in the population

Trait	Unit	Genetic gain per year	Cost	Return per cow
SW-d	kg	1.92		254.90
W2	kg	1.44		
W4	kg	1.89		
CoW	kg	6.44		513.70
GIR4	cm	0.29		
HH4	cm	0.50		
STP4	cm	1.05		
WR	%	0.19		123.52
SC4	cm	0.08		
DC	day	-0.12		
A1C	year	-0.004		
CW	kg	0.46		70.64
BF	cm	-0.02		
EMA	inch^2	0.28		
For breeding objective	baht	221.18		
Total return	baht			962.75
Total cost	baht		73.11	
Fixed cost	baht		67.98	
Variable cost	baht		4.90	
Dam	baht		0.24	
Profit	baht		889.65	

Table 3 Breeding objective, return, cost and profit per cow for varying number of cows in multiplier herd

Trait	Unit	Levels of various cow size (%)				
		5.0	5.5	6.0	6.5	7.0
Breeding objective	baht	221.18	221.18	221.18	221.18	221.18
Return per cow	baht	962.75	969.76	975.30	979.41	982.47
Cost per cow	baht	73.11	73.46	73.82	74.18	74.53
Profit per cow	baht	889.65	896.30	901.48	905.23	907.94
Total cost	baht	10,966,500	11,019,000	11,073,000	11,127,000	11,145,000
Total profit	baht	133,446,000	134,445,000	135,222,000	135,784,500	136,191,000

Table 4 Genetic gain per year, return, cost and profit per cow for using AI in multiplier herd

Trait	Unit	Mating in multiplier	
		Natural mating	Artificial insemination
SW-d	kg	1.92	1.92
W2	kg	1.44	1.44
W4	kg	1.89	1.89
CoW	kg	6.44	6.44
GIR4	cm	0.29	0.29
HH4	cm	0.50	0.50
STP4	cm	1.05	1.05
WR	%	0.19	0.19
SC4	cm	0.08	0.08
DC	day	-0.12	-0.12
A1C	year	-0.004	-0.004
CarW	kg	0.46	0.46
BF	cm	-0.02	-0.02
EMA	$inch^2$	0.28	0.28
Breeding objective	baht	221.18	221.63
Return per cow	baht	982.47	1,223.58
Cost per cow	baht	74.53	108.56
Profit per cow	baht	907.94	1,115.02
Total cost	baht	11,145,000	16,284,000
Total profit	baht	136,191,000	167,253,000

Reference

Archer, J.A., S.A. Barwick and H.U. Graser. 2004. Economic evaluation of beef cattle breeding schemes incorporating performance testing of bulls for feed intake. Aus. J. Ex. Agri. 44: 393-404.

Burrows, M.H. 2001. Variance and covariances between productive and adaptive traits and temperament in a composite breed of tropical beef cattle. Lives. Prod. Sci. 70:213-233.

Department of Livestock Development. 2014. Number of livestock inventory in part of Thailand 2014. Information and communication technology center. Ministry of Agriculture and Cooperative, Bangkok.

Department of Livestock Development. 2009. Manual for beef cattle production and research activities. Bureau of Animal Husbandry and Genetic Improvement, Ministry of Agriculture and Cooperative, Bangkok.

Department of Livestock Development. 2016. Plan for implementation and budget allocation in 2016 fiscal year. Bureau of Animal Husbandry and Genetic Improvement, Ministry of Agriculture and Cooperative, Bangkok.

Graser, H.U., G. Nitter and S.A. Barwick. 1994. Evaluation of advance industry breeding scheme for Australian beef cattle. II. Selection on combinations of growth, reproduction and carcass criteria. J. Agri. Res. 45: 1657-1669.

Grown, A.F. 1989. Cattle breeding goals and production circumferences. Ph.D. Thesis, Wangeningen Agriculture University, The Netherlands.

Intaratham, W., E. Witoonpong, and Y. Leephaijit. 2010. Estimated breeding value of growth traits for Tak beef cattle population. Available at: www.dld.go.th/breeding/journal /e_journal_2552.html.

Karras, K., E. Nieble, G. Nitter and H. Bartenschlager. 1993. ZPLAN – a PC computer program to optimize livestock selection program. Hohenheim University.

Laepaijit, Y., E. Vitoonpong and W. Intaratham. 2013. Estimated breeding value and index EBVs of growth traits in Tak beef cattle with genetic groups at various blood levels. Bureau of Animal Husbandry and Genetic Improvement, Department of Livestock Development, Bangkok.

Nitter, G., H.U. Graser and S.A. Barwick. 1994. Evaluation of advance industry breeding scheme for Australian beef cattle. I. Method of evaluation and analysis for an example population structure. J. Agri. Res. 45: 1641-1656.

Waltl, B.F., A. Willam and J. Solkner. 2002. Optimization of a specialized beef breeding program with a crossbreeding component. Arch. Tierz. Dummersoft. 45: 433-441.

PO-06-11 Genetic parameters and estimated breeding value of growth traits in Tak synthetic beef cattle

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INTRODUCTION

Tak synthetic beef breed was established at Tak livestock research and breeding center since 1986. They were being developed on the basis of 62.5 % Charolais and 37.5 % Brahman. The breeding objectives for this breed's establishment were to improve growth, carcass characteristics and fertility. Growth traits are of primary economic importance in cow-calf production system. They are known to be influenced by the direct genetic effect of the calf and the maternal genetic effect (Koch, 1972; Garrick, 1990; Meyer, 1992). The primary goal of animal breeders is to maximize the rate of genetic improvement. Knowledge of the nature and magnitude of population parameters (i.e. variance component and heritabilities) for a Tak synthetic beef cattle breed is needed to effectively design breeding programs and to estimate breeding values for traits of relevance to beef producers. In order to achieve optimum progress in the selection program for cow-calf production system genetic effect should be taken into account. Apart from this, genetic trends should be monitored over time to check the validity of the predictions made and to investigate direction of genetic change and whether the selection strategies implemented could reach a selection limit or have unexpected other effects. Therefore, the objectives of this study were to estimate genetic parameters, estimated breeding value and genetic trends of growth traits in Tak synthetic beef cattle.

MATERIALS AND METHODS

Data management

The data were collected from e-breeding database of three Tak synthetic beef cattle herds. The calves were born in years 1990 through 2015. The pedigree and data file were edited to standard protocol. Records outside three standard deviations from the overall means would be checked and eliminated. Other basic editing involved consistency checks for contemporary group, sex, blood fractions, age at weighing and age of dam. The contemporary groups were concatenated by herd-year-seasons of weighing. The final data set comprised 10,468 records for pedigree and 5,745, 4,994, 1,122 and 689 records with averages of 30.04 ± 4.80, 170.25 ± 37.07, 251.04 ± 33.36 and 326.67 ± 60.24 kg. for birth weight (BW), weaning (W2), 400 days weight (W4) and 600 days (W6), respectively.

Data analyses

Estimate (co) variance, genetic parameters and estimated breeding value.

Multivariate analysis was used for estimating variance and covariance components by the average information restricted maximum likelihood (AI-REML) and fitting animal model (Gilmour et al., 2002). The preliminary analyses were conducted for maternal genetic and maternal permanent environment effect *for birth weight and weaning weight*, 400 days and 600 days weight*, however, the results were closed to zero. Therefore these effects were ignored and considered only direct additive genetic effect for all traits and maternal permanent environment effect for birth weight and weaning weight.*

Genetic trends

Average estimated breeding values (EBV) of the year 1990 were set to be zero as a base group. The genetic trends were calculated for each trait by regressing the average EBV that deviated from the base group on birth year of calves.

RESULTS AND CONCLUTIONS

Variance component and Genetic parameter

Table 1 showed variance component and genetic parameter. The results revealed that direct genetic variances of birth weight, weaning, 400 days and 600 days weight were equal to be 4.42, 261.30, 391.10 and 617.80 kg^2. The phenotypic variances were equal to be 15.24, 766.40, 1,484.00 and 1,899.00 kg^2 and the direct heritabilities

were to be 0.29. 0.34, 0.26 and 0.33, respectively. However, for birth weight and weaning weight, the estimate of maternal permanent environmental effect played less an important role than those of direct genetic effect, explaining 4% and 10% of the phenotypic variance. The results of this study were higher than those reported by Intaratham et al. (2010) and Chokchareon (2003). The differences of estimation could be different among population due to data structure, the way of data editing, the magnitudes of contemporary group and model fitting. For our study, direct heritabilities were moderately to highly heritable, therefore, genetic improvement through selection could be achieved.

Genetic and Phenotypic correlations

Table 2 *showed that the genetic correlations of birth weight with weaning weight, 400 days and 600 days weight were 0.30, 0.25 and 0.15, between weaning weight with 400 days and 600 days weight were equal to be 0.62 and 0.31. The genetic correlation of 400 days with 600 days weight was to be 0.51. The genetic correlations among these four traits were low to moderate positive. The results of this study* agreed well with the study of Intaratham et al. (2010) *which found that the genetic correlation of birth weight with weaning weight and 400 days for Tak synthetic beef breed cattle were 0.43 and 0.36, respectively. The phenotypic correlations between birth weight and other weights were low to moderate positive which corresponding to the genetic correlation.* The results indicated that calves with high performance at weaning and 400 days weight tended to be heavier at birth. On the whole, levels of these correlations were comparable to other breeds (Kerin, 1999; Intaratham et al., 2008).

Estimated breeding value (EBV) and accuracies (ACC)

The average breeding value and the accuracy included all animal in pedigree were shown in Table 3.

EBV of *birth weight*

The averages for EBV and ACC of birth weight were 0.61 kg and 64%. The results were higher than Pairhot and Yodchai (2003) which reported that the average of EBV for birth weight in Tak synthetic beef cattle was to be 1.7 kg. This EBV used for selection to improve birth weight, however, in practice, Tak synthetic beef cattle would be selected for low birth weight. Therefore, this EBV should be zero or below from zero. This is particularly important in selection sires to mate with heifers.

EBV of *weaning weight*

The averages for EBV and ACC of weaning weight were 0.29 kg and 66%. The results were lower than Pairhot and Yodchai (2003) which reported that the average of EBVs for weaning weight in Tak synthetic beef cattle was to be 8.8 kg. This EBV used for selection to improve early growth traits. Therefore, selection should emphasize on high EBV to improve weaning weight of the next calves.

EBV of *400 days weight*

The averages for EBV and ACC of 400 days weight were 0.65 kg and 56%. The results were lower than Pairhot and Yodchai (2003) which reported that the average of EBVs for 400 days weight was to be 11.31 kg. This EBV indicated the additive direct genetic of animal and used for selection to improve yearling weight. Therefore, selection should emphasize on high EBV to improve yearling weight of the next calves.

EBV of *600 days weight*

The averages for EBV and ACC of 600 days weight were 1.06 kg and 44%. The results were lower than Pairhot and Yodchai (2003) which reported that the average of EBVs for 600 days weight was to be 13.91 kg. This EBV indicated the additive direct genetic of animal and used for selection to improve 1.5 years weight. Therefore, selection should emphasize on high EBV to improve 1.5 years weight for fattening.

Genetic trends of growth traits

Figure 1 *showed genetic* trends of EBV birth and weaning weight. The results revealed that the trends of both traits were positively increased. However, the genetic trend for birth weight was slightly increased at 0.02 kg per year. The genetic trend for weaning weight was improved to increase at 0.33 kg per year. The trends of both traits were in consistence with breeding objective to get low birth weight but hight weaning weight of calves.

Figure 2 showed genetic trends of EBV 400 days and 600 days weight. The results revealed that the trend of EBV 400 days weight was positively increased with an average trend of 4.46 kg per year. EBV of 400 days weight

ranged from -0.49 to 10.33 kg. For EBV 600 days weight ranged from -6.23 to 9.19 kg with an average trend of 1.17 kg per year. The results of this study indicated that the genetic gain for growth traits of Tak synthetic beef cattle were improved continuously over the past 26 years.

Table 1 Estimate and standard error of variance component and genetic parameters of Tak beef cattle

Parameter	BW	W2	W4	W6
Vp	15.24±0.33	766.40±18.63	1,484.00±65.70	1,899.00±114.70
Va	4.42±0.53	261.30±30.02	391.10±86.33	617.80±187.80
Vpe	0.66±0.20	80.77±11.48	-	-
*V*e	10.16±0.40	424.40±20.81	1,093.00±80.75	1,281.00±163.30
h^2	0.29±0.03	0.34±0.03	0.26±0.05	0.33±0.09
pe^2	0.04±0.01	0.10±0.01	-	-

Vp= phenotypic variance
Va= direct additive genetic variance
Vpe= maternal permanent environment variance,
*V*e= residual variance
h^2 = direct heritability
pe^2= Maternal permanent environment variance as proportion of the phenotypic variance

Table 2 Estimates and standard errors of phenotypic correlations (above diagonal) and direct additive genetic correlations (below diagonal) of Tak synthetic beef cattle

Traits	BW	W2	W4	W6
BW		0.30±0.01	0.25±0.03	0.15±0.04
W2	0.48±0.07		0.62±0.02	0.31±0.04
W4	0.30±0.12	0.69±0.08		0.51±0.04
W6	0.24±0.14	0.36±0.14	0.66±0.14	

Table 3 Estimate breeding value and accuracies in Tak synthetic beef cattle

Traits		Average	Min	Max
BW	EBV	0.61	-5.01	4.36
	ACC	0.64	0.32	0.96
W2	EBV	0.29	-43.67	39.21
	ACC	0.66	0.27	0.97
W4	EBV	0.65	-46.65	41.55
	ACC	0.56	0.30	0.90
W6	EBV	1.06	-40.73	43.44
	ACC	0.44	0.24	0.84
Index EBVs		0.46	-27.84	27.69

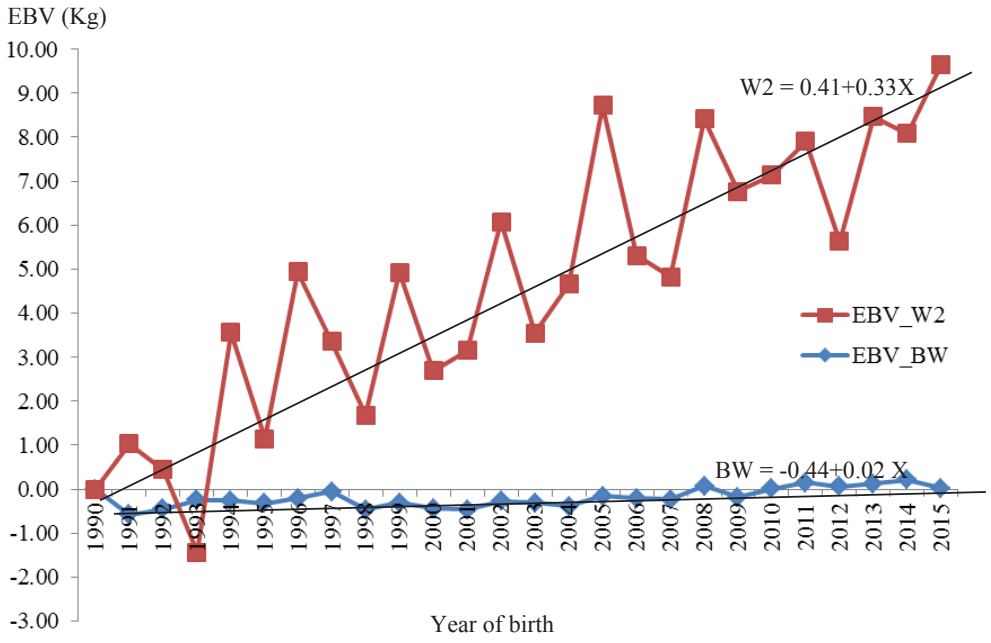

Figure 1 Genetic trends of birth weight and weaning weight of Tak synthetic beef cattle over years 1990 to 2015

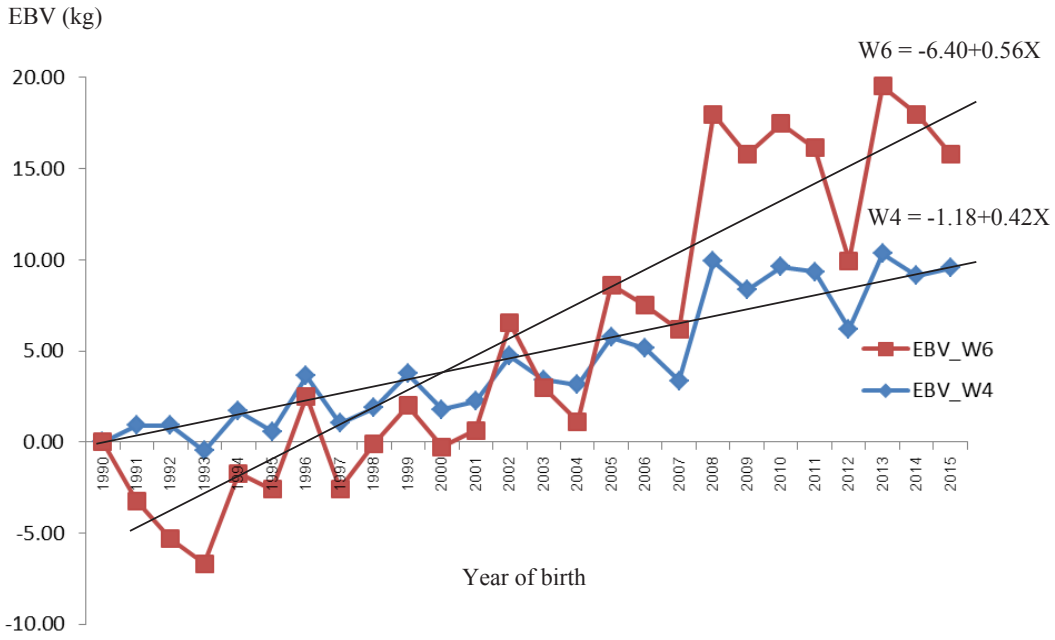

Figure 2 Genetic trends of 400 days and 600 days weight of Tak synthetic beef cattle over years 1990 to 2015

REFERENCES

Chockchareon, S. 2003. Breeding value prediction on growth traits and milking ability in Brahman using single trait and multiple traits BLUP. M.S. thesis, Khon Kaen University.

Garrick, D. J. 1990. Maternal effects on growth in beef cattle. In: Proceedings of the 8th Conference of the Australia Association of Animal Breeding and Genetics. New Zealand. pp. 397-400.

Gilmour, A.R., B.R. Cullis, S.J. Welham and R. Thomson. 2002. ASReml Reference Manual. NSW Agriculture, Orange, Australia.

Intaratham, W., E. Witoonpong, and Y. Leephaijit. 2010. Estimated breeding value of growth traits for Tak beef cattle population. Available at: www.dld.go.th/breeding/journal/e_journal_2552.html

Intaratham, W., S. Koonawootrittriron, P. Sopannarath, H-U. Graser and S. Tumwasorn. 2008. Genetic parameters and annual trends for birth and weaning weights of a Northeastern Thai indigenous cattle line. Asian-Aust. J. Anim. Sci. 4:478-483.

Kerin, M. 1999. Report on the analysis of Thailand Buffalo and Brahman data. ACIAR Project PN9311, Animal Genetics and Breeding Unit, University of New England, Australia.

Koch, R. M. 1972. The role of maternal effects in animal breeding: VI. Maternal effects in beef cattle. J. Anim. Sci. 35:1316-1323.

Meyer, K. 1992. Variance components due to direct and maternal effects for growth traits of Australian beef cattle. Livest. Prod. Sci. 31:179-204.

Sirisom, P. and Y. Tongthainan. 2003. Developing of Tak Beef Cattle in Thailand EBV Estimates of Growth Traits of Tak Beef at Various Charolais Blood Levels. Available at: www.dld.go.th/breeding/journal/e_journal_2549.html

PO-06-13

Association of WUR SNP with Production Traits in Duroc pigs

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Porcine reproductive and respiratory syndrome virus (PRRS) is one of the most important infectious disease threat to pig production worldwide (Lyoo, 2015). This infectious disease costs the U.S. pork industry $560 million annually (Neumann et al., 2005). It is also the most significant disease impacting the pig production in Europe and Asia (Rowland et al., 2012). The single nucleotide polymorphism WUR10000125 (WUR) in chromosome 4 in pig has been previously shown to be associated with host response to PRRS infection based on weight gain and viremia levels. (Gol et al., 2015; Niu et al., 2015). Nowadays, international efforts are underway to assess resistance and susceptibility to PRRS using tools such as gene arrays, single nucleotide polymorphisms (SNPs) chips, genome-wide association studies (GWAS), proteomics, and advanced bioinformatics (Lunney and Chen, 2010). The evaluation of SNPs and their association with production traits helps in computation of correlations between SNPs and production traits necessary to enable use of the SNPs in animal selection schemes (Manichaikul et al., 2012).

There is need to proof the association of WUR SNP with pig production traits to enable possible use of the SNP in pig production performance improvement programs. Therefore, the objective of this study is to analyzed the relationship of these genotypes to the production traits including average daily gain (ADG), backfat thickness (BF), loin muscle area (EMA), lean percentage (LP), and age at 90 kilograms (D90). So, we could further study the genetic effect of WUR SNP.

Materials and Methods

Samples and traits

A total of 670 tissue samples were collected from a population of Duroc pigs. About 40.0g of loin tissue were collected from each pig. Genomic DNA samples were extracted from tissue sample of each animal using the standard phenol/chloroform method, and then DNA was diluted to 100? after quality evaluation. Five production traits were evaluated including average daily gain (ADG), backfat thickness (BF), loin muscle area (LMA), lean percentage (LP), and days at 90kg (D90). The mean and standard deviation of production traits are shown in Table 1.

Statistical analysis

Statistical analysis of data was done using SAS version 9.2. Initial computations were performed using SAS Proc GLM to evaluate non-genetic factors for inclusion in the model. The following general mixed animal model was applied for analysis:

$$Y_{ijkl} = \mu_i + p_{ij} + hyw_{ik} + a_{ijkl} + \beta\ wt_{ijkl}(cov) + e_{ijkl}$$

where Y_{ijkl} = observation for a traits *i;* μ_i = overall mean of i^{th}; p_{ij} = j^{th} parity effect of i^{th} trait; hyw_{ik} = k^{th} herd-year-week effect of i^{th} trait; a_{ijkl} = animal random effect; β = covariate of number of final weight; wt_{ijkl} = final weight; e_{ijkl} = random error. The pedigree file contained all tested animals and their ancestors traced ten generations back.

Results and Discussion

Genotype and allele frequency of WUR polymorphism

The genotypic and allele frequencies of WUR SNP are shown in Table 2. Among the 670 samples, the number of AA, AG, and GG genotypes in WUR SNP were 375, 241 and 54, respectively. Their corresponding genotype frequencies were 0.56, 0.36 and 0.08. the allele frequency for disease resistant gene A was 0.74, while the allele frequency for sensitive gene G was 0.26. Abella et al. (2016) also detected three genotypes in Duroc population. The Hardy-Weinberg (H-W) P-value (0.09) generated for WUR SNP in the study indicates that WUR SNP was not specific to the pig population in the study and has genetic continuity in the entire pig breed.

Association between WUR SNP polymorphism and production traits

All pig genotypes in the study had similar ADG, LMA, and D90 values that were not significantly different (P<0.05) as shown in Table 3. This meant that there were no significant differences detected in the polymorphisms of WUR SNP with these traits. Abella et al. (2016) showed that WUR SNP was associated to ADG in vaccinated pigs There were significant differences in polymorphisms of WUR SNP (P<0.05) with BF and LP between pig genotypes. Significantly (P<0.05) lower and similar BF were recorded in pigs with AA and AG genotypes in comparison to pigs with GG genotypes. AA and AG genotypes showed significantly higher LP than GG genotype (P<0.05). Hess et al. (2014) reported that WUR SNP was correlated in weight gain in pigs.The results indicated that WUR SNP was associated with BF and LP. The polymorphisms at WUR SNP may play roles in the production performance improvement programs for pigs through its associations with BF and LP in pigs.

Conclusion

The WUR SNP was associated with BF and LP in this study and this may play roles in the production performance improvement programs for pigs. However, effect of WUR SNP on these traits need to be established in the more complex disease conditions that animals are exposed in the field.

Table 1. Mean and standard deviation for pig production traits

Traits	N	Mean	Min	Max	SD
ADG (g/day)	670	644.06	510.34	828.77	51.63
BF (mm)	670	14.89	7.90	24.92	2.62
LMA (cm^2)	578	27.27	19.74	38.78	3.04
LP (%)	578	55.31	37.00	62.90	3.01
D90 (days)	670	141.93	117	166	8.39

Table 2. Genotypic and allele frequencies of WUR10000125 SNP

N	Genotype frequency			Allele frequency		H-W
	AA	AG	GG	A	G	p-value
670	0.56(375)	0.36(241)	0.08(54)	0.74	0.26	0.09

Table 3. Association of polymorphisms in WUR10000125 SNP with production traits

Traits	Genotype		
	AA (375)	AG (241)	GG (54)
ADG (g/day)	629.60±9.07	628.06±9.42	622.18±11.14
BF (mm)	$15.25±0.43^b$	$15.50±0.44^b$	$16.36±0.52^a$
LMA (cm^2)*	28.23±2.05	27.96±2.03	27.57±2.07
LP (%)*	$55.05±1.94^a$	$54.22±1.93^b$	$53.70±1.96^c$
D90 (days)	144.96±1.49	145.18±1.54	145.95±1.83

* For LMA and LP, numbers of animals are 314, 212 and 52 corresponding to genotypes AA, AG and GG, respectively.

REFERENCES

Abella, G. et al. 2016. A WUR SNP is associated with European Porcine Reproductive and Respiratory Virus Syndrome resistance and growth performance in pigs. Research in veterinary science 104: 117-122.

Gol, S., J. Estany, L. Fraile, and R. Pena. 2015. Expression profiling of the GBP1 gene as a candidate gene for porcine reproductive and respiratory syndrome resistance. Animal Genetics 46: 599-606.

Hess, A. et al. 2014. Genetic Parameters and Effects for a Major QTL of Piglets Experimentally Infected with a Second Porcine Reproductive and Respiratory Syndrome Virus. In: Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Lunney, J. K., and H. Chen. 2010. Genetic control of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection. Virus Research 154: 161-169.

Lyoo, Y. S. 2015. Porcine reproductive and respiratory syndrome virus vaccine does not fit in classical vaccinology. Clinical and experimental vaccine research 4: 159-165.

Manichaikul, A. W., C. K. Williams, Q. Wong, M. Sale, J. Pankow, M. Tsai, J. Rotter, S. Rich, and J. Mychaleckj. 2012. Analysis of family and population-based samples in cohort genome-wide association studies. Human genetics 131: 275-287.

Neumann, E. J. et al. 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. Journal of the American Veterinary Medical Association 227: 385-392.

Niu, P., S. W. Kim, W. I. Kim, and K. S. Kim. 2015. Association analyses of DNA polymorphisms in immune-related candidate genes GBP1, GBP2, CD163, and CD169 with porcine growth and meat quality traits. Journal of Biomedical Research 16: 40-46.

Rowland, R. R., J. Lunney, and J. Dekkers. 2012. Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance. Should we aim for genetic improvement in host resistance or tolerance to infectious disease?, 78.

PO-06-15

Factors Associated with Mummified Fetuses in Yorkshire and Landrace Sows

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Introduction

The number of piglets produced per sow is one of the main factors affecting the economic returns in pig breeding industry. Improvement of litter size is an increasingly important breeding goal in swine (Lawlor and Lynch, 2007). The heritability estimates of litter size in pig is low (0.10) and several factors must be considered to get improve (Jung et al., 2008). Fetal losses such as mummified fetuses and still births and pre-weaning mortality are among the most important causes of losses in commercial swine herds (Dial et al., 1992). The incidence of mummified fetuses has been linked with litter size, parity, infectious diseases, uterine capacity, environmental temperature and mycotoxins (Dial et al 1992). However, risk factors on mortality rates may vary from one farm to another. Identification of the risk factors associated with the occurrence of mummified fetuses can aid in improving herd reproductive efficiency.This study was conducted to evaluate the potential risk factors on the number of mummified piglets in highly prolific sows in commercial farms of South Korea.

Materials and Methods

Data

Reproductive data for this study were from 18,941 Landrace and 50,285 Yorkshire litters for a period of 15 years, from 2001 to 2015. Data included the litter size, number of born alive, date of parity, parity number, mortality and mummified fetuses. Litter size comprised number of born alive and mortality. Mortality included still birth, stillborn and mummified.

Analysis

Frequency distributions and descriptive statistics using univariate procedure were created to characterized mummified occurrence and potential risk factors. The dependence or independence of the target traits was tested with the chi-square test. The association of the risk factors with sows having at least one mummified fetus in a given litter (yes or no) was determined by binary logistic regression analysis. Potential risk factors included in the models as categories were farm, season and parity. Parities were categorized as 1, 2, 3, 4, 5, 6 and ≥7, while season were classified as spring, summer, fall and winter. Litter size, range 1 to 25 was included as a continuous covariate. Analysis were conducted using SAS version 9.2. Probabilities were calculated fixing one of the farm in each breed, fall season and sows from parity 3.

Results and Discussion

The average litter size was 11.55 ± 3.12 and 12.33 ± 3.29 for Landrace and Yorkshire breeds, respectively. The incidence rate of mummies in Landrace was 2.86% while 2.68% in Yorkshire. The frequencies of litters with 0, 1, 2 and ≥3 mummies in Landrace were 79.55%, 13.77%, 4.21% and 2.47%, respectively. On the other hand, the values in Yorkshire were 78.33%, 14.55%, 4.89% and 2.23%, correspondingly.

The regression coefficients, odds ratios, 95% confidence intervals and probabilities in Landrace and Yorkshire breeds are shown in Table 1 and 2, respectively. Farm ($P<0.0001$), season ($P<0.0001$) and parity ($P<0.001$). were risk factors for fetal mummification in logistic regression analysis in both breeds.

The odds of litters having mummified fetus in Landrace breed were higher in L2 farm ($P<0.0001$) compared to two farms. In Yorkshire, sows in farms YF2 and YF3 had the highest odds of mummification ($P<0.0001$). The lowest odds were in YF5. The odds of mummification were higher during summer in Landrace ($P<0.05$) and Yorkshire ($P<0.0001$). Increased embryonic mortality are seen in pigs bred during the summer (Merck Veterinary Manual, 2015). Chu (2005) and Segura-Correa et al., (2007) reported differences between seasons while Segura-Correa and Solorio-Rivera (2013) did not found any significant differences. Sows of parity ≥7 had higher odds of mummification in Landrace ($P<0.001$) and Yorkshire ($P<0.0001$). In Landrace, higher odds of mummification were

also observed in parity 1 (*P<0.01*) and 6 (*P<0.001*). The lowest odds occurred in parity 3 in Landrace while parity 2 in Yorkshire. Borges et al. (2005) also reported an increased probability of having a mummified fetus in older sows.

Conclusion

Prolific sows in late parities (≥7 in this study) had higher risk of mummification. Moreover, the risk of mummified fetus was higher during summer season. Therefore, more attention should be given in those factors to reduce the rate of mummified fetuses.

Table 1. Risk factors associated with litters with at least one mummified piglets in Landrace

Factor	Number of litters	Estimate	SE	OR	95% CI		*P*
					Lower	Upper	
Farm							
L1	3878	0	-	1	-	-	-
L2	8530	0.414	0.050	1.513	1.371	1.670	<0.0001
L3	6083	-0.012	0.055	0.988	0.887	1.102	0.831
Season							
Winter	4356	-0.127	0.055	0.881	0.791	0.981	0.021
Spring	4608	-0.047	0.054	0.954	0.859	1.059	0.375
Summer	4872	0.136	0.052	1.145	1.035	1.268	0.009
Fall	4655	0	-	1	-	-	-
Parity							
1	5256	0.061	0.062	1.062	0.941	1.199	0.327
2	3416	0.055	0.066	1.057	0.928	1.203	0.405
3	2809	0	-	1	-	-	-
4	2264	0.133	0.071	1.143	0.995	1.312	0.059
5	1783	0.121	0.076	1.129	0.974	1.309	0.109
6	1286	0.105	0.084	1.111	0.942	1.310	0.212
≥7	1677	0.247	0.077	1.280	1.102	1.488	0.001

SE = standard error; OR = odds ratio; CI = confidence interval; *P* = probability.

Table 2. Risk factors associated with litters with at least one mummified piglets in Yorkshire

Factor	Number of litters	Estimate	SE	OR	95% CI		*P*
					Lower	Upper	
Farm							
Y1	9484	0	-	1	-	-	-
Y2	16709	0.310	0.033	1.364	1.278	1.456	<0.0001
Y3	5460	0.314	0.043	1.369	1.257	1.490	<0.0001
Y4	11426	0.031	0.037	1.031	0.960	1.108	0.397
Y5	7206	-0.078	0.042	0.925	0.852	1.005	0.065
Season							
Winter	12020	-0.122	0.033	0.885	0.830	0.944	0.001
Spring	12462	-0.050	0.032	0.951	0.893	1.013	0.121
Summer	13265	0.126	0.031	1.135	1.068	1.206	<0.0001
Fall	12538	0	-	1	-	-	-
Parity							
1	11920	0.119	0.038	1.127	1.047	1.213	0.002
2	9469	-0.037	0.039	0.964	0.893	1.040	0.343
3	8187	0	-	1	-	-	-
4	6524	0.079	0.041	1.082	0.999	1.173	0.053
5	5186	0.036	0.044	1.037	0.951	1.130	0.412
6	4044	0.160	0.047	1.173	1.070	1.286	0.001

References

Borges, V. F., M. L. Bernardi, F. P. Bortolozzo and I. Wentz. 2005. Risk factors for stillbirth and foetal mummification in four Brazilian swine herds. J. Prevetmed. 70:165-176

Chu, M. X. 2005. Statistical Analysis of Stillbirths in Different Genotypes of Sows. Asian Australas. J. Anim. Sci 18: 1475-1478.

Dial, G. D., Marsh, W.E., Polson, D. D., Vaillancourt, J. P., 1992. Reproductive failure: differential diagnosis. In: Leman, A. D., Straw, B. E., Mengeling, W. L., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of Swine, seventh ed. Iowa State University Press, Ames, IA, pp. 88–137.

Jung, D. J., B. W. Kim, S. H. Ro, H. S. Kim, W. K. Moon, H. Y. Kim, H. G. Jang, L. S. Choi, J. T. Jeon and J. G. Lee. 2008. Estimation of environmental effect and genetic parameter on reproduction traits for on-farm test records. J. Anim. Sci. 50(1):33-44.

Lawlor, P. G. and P. B. Lynch. 2007. A review of factors influencing litter size in Irish sows. Irish Veterinary Journal. 60:359–366.

Le Cozler, Y. L., C. Guyomarc'h, X. Pichodo, P. Y. Quinio and H. Pellois. 2002. Factors associated with stillborn and mummified piglets in high-prolific sows. Anim. Res. 51:261-268.

Merck Veterinary Manual: 11th Edition. The Merck Veterinary Manual Whitehouse Station, NJ: Merck & Co., Inc.

Segura-Correa J C, Alzina-López A and Solorio-Rivera J L 2007 Evaluation of three models and risk factors associated with stillborn piglets in Yucatan, Mexico. Técnica Pecuaria en México 45(2): 227-236.

Segura-Correa, J., and J. Solorio-Rivera. 2013. Risk factors for stillborn pigs and mummified fetuses in two swine farms in southeastern Mexico. Livestock Research for Rural Development.

PO-06-19

Genetic Parameter Estimation for Weight Traits of Goat Populations in Thailand

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ABSTRACT

The objective of this study was to estimate genetic parameters for weight traits of goat populations in Thailand. The traits included Birth Weight (BW), weaning weight (W3) and weight at 6 months of age (W6) of 4,813 Thai native purebred and crossbred goat records comprising 3,807 Boer, 1,713 Anglo Nubian and 3,006 crossbred individual weight and pedigree records collected from year 1995 to 2016 in the Government livestock breeding stations in Thailand. The multivariate animal mixed model was used in the genetic analysis of the traits and included fixed effects of breed group, birth type (single or multiple born), dam age at giving birth, contemporary group of herd-year-season and random effects of direct and maternal additive genetic effects of the animals considering the direct additive-maternal covariance. It was found that male goats were consistently heavier than females across breed groups and birth types. The average BW of male and female were 2.69 ± 0.03 and 2.55 ± 0.03 kg; W3 were 12.02 ± 0.20 and 10.93 ± 0.20 kg and W6 were 17.89 ± 0.32 and 16.35 ± 0.32kg, respectively. The average weaning weights by breed groups of Thai Native, Boer, Anglo Nubian and crossbred were; 8.22 ± 0.27, 12.46 ± 0.22, 13.07 ± 0.26, and 11.53 ± 0.28 kg; and for W6 were 11.86 ± 0.44, 18.02 ± 0.34, 19.26 ± 0.45 and 17.86 ± 0.46 kg, respectively. Single birth kids were heavier at weaning than twins being 12.97 ± 0.14 and 11.58 ± 0.15 kg and for W6 being 18.22 ± 0.20 and 16.88 ± 0.21 kg. The direct heritability estimates for BW, W3 and W6 were 0.30 ± 0.04, 0.33 ± 0.04 and 0.31 ± 0.05 and maternal heritability were 0.29 ± 0.03, 0.13 ± 0.02 and 0.07 ± 0.03. Antagonistic effects between direct and maternal genetic were found in all weight traits studied with genetic correlation estimates of -0.64 ± 0.06, -0.49 ± 0.07 and -0.40 ± 0.13, respectively. It is recommended from this study to consider birth type, breed group, sex, age of dam and negative genetic relationships between direct and maternal effects to be included for the unbiased prediction of goat weight breeding improvement.

INTRODUCTION

Livestock improvement adds capacity to the production of animal a way to improve the genetic by improving the process of genetic selection and breeding (Nudcha, 2011). In general, the reproductive characteristics have low heritability value. The selection must take a long period and slow response. Genetic parameters estimate with accuracy, it is necessary to apply to the breeding value (Kongcharoen *et al.,*2011). The goat population in the country at present year is 539,583 with the number of 43,118 farmers raising goats (Department of Livestock Development, 2015).The objective of a breeding program for livestock is to maximize the rate of genetic progress for economically important traits. The heritability and genetic relationship between traits are needed for planning an efficient breeding system and development of effective genetic evaluation (Anothaisinthawee *et al.,* 2012) Thus, the objective of this study was to estimate variance components and genetic parameters for weight traits; Birth weight (BW), weaning weight (W3) and weight at 6 months of age using a data set from Thai Government research stations.

MATERIALS AND METHODS

Animal and management

Goat data containing weights and pedigree records were obtained from 1995 to 2016 from 11 Government Breeding and Research Stations, Bureau of Animal Husbandry and Genetic Improvement (BAHGI), Department of Livestock Development (DLD). Goats were generally pastured on pastures and supplemented concentrates (16% crude protein and 2600 kcal/kg ME) at 1% of body weight. The goats were treated for internal parasites and vaccinated against FMD twice annually against the general epidemic diseases. A total of 13,339 records (6,788 Female and 6,551 Male) of goat weights of three purebred groups included 3,807 Boer (BB), 1,713 Anglo-Nubian (AA) and 4,813 Thai native, two-way crosses 1,327 Anglo Nubian-Thai native (AN), 811 Boer-Anglo Nubian (BA) and 868 three-way crosses (BAN). Characteristics of data of goat studied traits Birth weight (BW), weaning weight (W3) and weight at 6 months of age are shown Table 1.

Table1 Characteristics of the pedigree and data structure of goat

Data structure	BW	W3	W6
No of records	9,461	9,006	3,354
Average weight[1/] (in kg)	2.83±0.63	12.32±4.27	17.24±5.77

[1/]Mean ± standard deviation

Statistical analysis

Preliminary analysis of fixed effects on weight and growth rate traits were performed using the Generalized Linear Model (GLM) procedure of a Statistical Analysis System (SAS, 1996) to identify non-genetic factors to be included in the final model. Fixed effects were sex, contemporary group of herd-year-season of birth, breed group, age of dam when giving birth and birth type. Fixed effects were obtained by LSMEANS statement of the SAS program (SAS, 1996). Multi-trait animal mixed model include random effects of direct additive and maternal genetic for variance components and genetic parameter estimates as shown below (Nakavisut *et al.,* 2012). y =Xb + Za + Zm + e with cov (a,m) ≠ 0 where y is a n x 1 vector of records, b the fixed effects in the model with association matrix X, a is the vector of direct genetic effects with association matrix Za, m the vector of maternal genetic effects with association matrix Zm and e the vector of residual (error) effects.

RESULTS AND DISCUSSIONS

Genetic parameter estimates

The estimates of direct heritability for BW, W3 and W6 were 0.30 ± 0.04, 0.33 ± 0.04 and 0.31 ± 0.05 with estimates of maternal heritability of 0.29 ± 0.03, 0.13 ± 0.02 and 0.07 ± 0.03, respectively (Table 2). The maternal effects were important determinants of estimated genetic parameters for birth traits (Zhang *et al.,* 2008). Antagonistic direct-maternal genetic correlations were found in all weight traits being -0.64 ± 0.06, -0.49 ± 0.07 and -0.40 ± 0.13 respectively. For all the weight traits, estimates of the correlations between direct additive and maternal genetic (r_{am}) were negative. Most estimates of additive genetic and phenotypic correlations between weight traits were slightly to moderately positive except for the negative phenotypic correlation of -0.31 between BW and W4 (Table 3) suggesting that phenotypically birth weight is not a good predictor of goat weight at an adult age, this might be different from other species because goats mostly have both single and multiple births that significantly affect birth weight while the genetic effects can express much more in the adult goats such as W4 in this study. This might result in the negative estimate of the phenotypic correlation found in this study. Medium and positive environmental correlations indicated the important effects of environmental factors on early growth traits reported by Zhang et al. (2008).

Table 2 Genetic parameter and variance component of random effect of goat weight and growth traits

Model	h^2_a	h^2_m	r_{am}	V_p	V_a	V_m
BW	0.30±0.04	0.29±0.03	-0.64±0.06	0.23±0.00	0.07±0.01	0.07±0.01
W3	0.33±0.04	0.13±0.02	-0.49±0.07	8.63±0.16	2.86±0.36	1.12±0.19
W6	0.31±0.05	0.07±0.03	-0.40±0.13	14.59±0.39	4.46±0.77	0.98±0.43

H^2_a: heritability, h^2_m: maternal heritability, r_{am}: direct-maternal genetic correlation, V_p: phenotypic variance, V_a: direct additive genetic variance, V_m: maternal genetic variance

Table 3 Phenotypic (below diagonal) and genetic correlations (above diagonal) in goat weight

	BW	W3	W4
BW		0.33±0.06	0.26±0.08
W3	0.18±0.01		0.74±0.06
W4	-0.31±0.12	0.63±0.04	

Mean ± standard deviation

Description of the growth traits in all fixed effects

The Least-squares means of body weights (Table 4) showed that male goats were consistently heavier and grew faster than females across breed groups and birth types. The Birth weight of male and female were 2.69 ± 0.03 and 2.55 ± 0.03 kg and W3 weights were 12.02 ± 0.20 and 10.93 ± 0.20 kg and W6 weights were 17.89 ± 0.32 and 16.35 ± 0.32 kg. The effects of sex in this study agree well with those found in literatures Anothaisinthawee et al. (2012). Single birth goats were heavier than twins being 2.84 ± 0.01 and 2.66 ± 0.01 kg for BW and 12.97 ± 0.14 and 11.58 ± 0.15 kg for W3 and 18.22 ± 0.20 and 16.88 ± 0.21 kg for W6. The results of this research showed that age of dams significantly affect the weight traits. Least-squares means of BW, W3 and W6 showed a peak values in the 3 to 5 years of age, and then tend to decline. Single births and male kids had the heaviest live weight and the largest body size at birth (Zhang *et al.,* 2008). Because the single birth has no competition for nutrition supply of dam in gestation period as of the multiple birth ones.

Table 4 Least–squares means (LSM) and standard errors (SE) of fixed effects of goat weight and growth traits

Effect	BW (kg)				W3 (kg)				W6 (kg)			
	N	LSM±SE			N	LSM±SE			N	LSM±SE		
		Male	Female	Total		Male	Female	Total		Male	Female	Total
Breeds												
(purebred)												
BB	3,743	2.95±0.03	2.82±0.03	2.88±0.03	2,814	13.01±0.23	11.92±0.23	12.46±0.22	1,201	18.79±0.35	17.25±0.35	18.02±0.34
AA	1,534	2.86±0.04	2.73±0.04	2.80±0.04	1,129	13.61±0.27	12.53±0.27	13.07±0.26	346	20.03±0.45	18.49±0.45	19.26±0.45
NN	1,510	2.14±0.04	2.03±0.04	2.10±0.04	2,850	8.76±0.27	7.67±0.27	8.22±0.27	1,040	12.62±0.44	11.09±0.44	11.86±0.44
(2-way crosses)												
AN	1,038	2.29±0.04	2.15±0.04	2.22±0.04	881	10.01±0.30	8.93±0.30	9.47±0.29	197	15.71±0.50	14.17±0.49	14.94±0.49
BA	796	2.96±0.04	2.83±0.04	2.89±0.04	618	13.59±0.27	12.50±0.27	13.05±0.27	177	19.93±0.45	18.40±0.45	19.16±0.45
(3-way crosses)												
BAN	840	2.89±0.04	2.76±0.04	2.83±0.04	714	13.14±0.29	12.06±0.29	12.60±0.28	393	20.25±0.44	18.71±0.44	19.48±0.43
Total		2.69±0.03	2.55±0.03			12.02±0.20	10.93±0.20			17.89±0.32	16.35±0.32	

Effect	BW (kg)		W3 (kg)		W6 (kg)	
	N	LSM±SE	N	LSM±SE	N	LSM±SE
Age of dams						
1 year old		2.68±0.02		11.87±0.18		17.37±0.27
2 years old		2.76±0.01		12.31±0.16		17.51±0.23
3 years old		2.78±0.01		12.08±0.16		17.92±0.24
4 years old		2.74±0.02		12.28±0.17		18.02±0.25
5 years old		2.74±0.02		12.24±0.18		17.26±0.25
≥6 years old		2.71±0.02		11.86±0.17		17.40±0.25
Birth Type						
1	4,979	2.84±0.01	4,093	12.97±0.14	1,636	18.22±0.20
2	4,092	2.66±0.01	4,390	11.58±0.15	1,530	16.88±0.21
3	354	2.48±0.03	484	11.09±0.20	172	17.10±0.33
4	36	2.50±0.09	39	10.28±0.53	16	16.29±0.96

BB: Bore, AA: Anglo-Nubian, NN: Thai Native, AN: Anglo-Nubian-Thai Native, BA: Bore-Anglo-Nubian and BAN: Bore-Anglo-Nubian-Thai Native; BW: Birth weight, W3: weaning weight and W6: weight at 6 months of age

CONCLUSIONS

This study showed important effects of environmental factors on growth traits, which should be taken into consideration in genetic evaluations. The fixed effects of breed group, birth type, dam age at giving birth are important to growth traits of goat population in Thailand, and should be fitted in the animal models. It is evident that random effects of direct and maternal additive genetic effects significantly affect pre-weaning growth, and should be taken into consideration in genetic progress. High and negative correlation coefficients between direct additive and maternal genetic (r_{am}) effects are also obtained for pre-weaning weights.

Key Words: Goat, Weight, Genetic Parameters

REFERENCES

Nudcha s. 2011. Animal Breeding . (Revised in 2554) Avaliable at: http://seekun.net/a-im-1-4-1.pdf

Kongcharoen A., Chaiwatanasin w. and Chomchai S. Estimates of Genetic Parameter for Litter Birth Weight at Different Parities in Swine. The 23rd National Graduate Research Conference at Rajamangala University of Technology Isan :228 – 234

Department of Livestock Development. 2015 Avaliable at: http://ict.dld.go.th/th2/images/stories/stat_web/yearly/2558/province/7.goatsheep_province.pdf

Anothaisinthawee S., Nakavisut S., Wiriyasombat C., Amano T. and Nomura K. 2012.
Genetic parameter estimates of growth traits in Thai meat goats. The 15th AAAP Animal Science Congress,Thailand, 2012.
Nakavisut S., Roisri P., Buakaew P. and Anothaisinthawee S. 2012. Genetic Parameter Estimates for
Weight and Growth Rate Traits of Sheep Populations in Thailand. The 15th AAAP Animal Science Congress,Thailand, 2012.
Zhang, C.Y., Yang, L.G., Shen, Z., 2008. Variance components and genetic parameters for weight and size at birth in the Boer goat. Livestock Science 115:73–79.

PO-06-20

Genetic Variation of MSTN, TGF- β 3 and PIT1 Gene in Thai Native Chicken

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Introduction

Muscle growth is an important trait in Thai native chicken since increase muscle growth resulting an increasing of meat production. In Thailand, native chicken breeds; Pradoo Hang Dam morkho55 and Chee KKU 12 have been developing by 9 generations for improving of meat production. Thai native chicken is also favored for healthy meat and hence it is selected to have a high meat yield and quality. To improve meat production, a balancing of growth and meat quality is required. Recently, genetic variations related to growth were studied.

Myostatin (*MSTN*) is a member of the transforming growth factor-β family with a key role in inhibition of muscle growth by negative regulation of both myoblast proliferation and differentiation. Hence, myostatin acts to limit skeletal muscle mass by regulating both the number and growth of muscle fibers. In chickens, Gu et al. (2003) found out that the myostatin gene does not only regulate the skeletal muscle development, but also participate in the fat metabolism and disposition. Ye et al. (2007) evaluated the effects of several polymorphisms of the myostatin gene in three elite commercial broiler chicken lines on performance and mortality traits and suggested that the myostatin gene had pleiotropic effects on broiler performance**.** Transforming growth factor beta 3 (TGF-β3) is a type of protein, known as a cytokine, which is involved in cell differentiation, embryogenesis and development. It belongs to a large family of cytokines called the transforming growth factor beta superfamily, which includes the TGF-β family, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) as well as the inhibins and activins (Piek et al., 1999). In chicken, TGF-β3 is mapped to chromosome 5 (Groenen et al., 2000). The biological activities of chicken TGF-β isoforms appear to be similar to those of mammals (Cogburn et al., 2000). The chicken *PIT1* gene has been shown to play important roles in regulation of growth performance and carcass traits by controlling the expression of chicken growth hormone, prolactin, and thyroid-stimulating hormone-β genes. Recently, it has been indicated that polymorphisms of *PIT1* gene were associated with chicken growth and carcass traits (Nie et al., 2008). Therefore, it is necessary to study variation of *MSTN*, *TGF-ß*3 and *PIT1* as a candidate gene for growth and carcass traits in chickens. The aim of this work was to investigate variation of *MSTN*, *TGF-ß*3 and *PIT1* in Thai native chicken.

Materials and methods

A total of seventy-three blood samples were collected from Chee KKU12 and fifty-five samples from Pradoo Hang Dam 55. Genomic DNA was extracted from the whole blood using the Guanidine-HCL methods. DNA qualification and concentration were evaluated by spectrophotometer (Nano-Drop2000, Delaware USA). PCR-restriction fragment length polymorphism (RFLP) was used to identify DNA patterns of *MSTN*, *TGF-β*3 and *PIT1*. Three restriction fragment length polymorphism (RFLP) were typed on the *MSTN* (*MSTN*/B*bv*I), *TGF-β*3 (*TGF-β*3/B*sl*I) and *PIT1* (*PIT1*/Tsp509I) which was described by Zhang et al. (2011) and Amirinia et al. (2011). The *MSTN*, *TGF-ß3* and *PIT1* allele frequencies were calculated by simple allele counting (Falconer and Mackay, 1996).

Results and discussion

Three genotypes of *MSTN* (AA, AG and GG genotypes), *TGF-β3* (AA, AB and BB genotypes) and *PIT1* (AA, AB and BB genotypes) were observed in chee KKU 12 and Pradoo Hang Dam 55 (Figure 1). Variation of homologous *AA* in *MSTN* in Pradoo Hand Dam morkho55 was found to be greater than in Chee KKU12. While, a high frequency of *AA* (0.76) was identified in Pradoo Hang Dam morkho55, a low frequency (0.14) observed in Chee KKU12. A frequency of *GG* (0.24) was also present in Pradoo Hang Dam morkho55, but it was present at a high frequency (0.49) in Chee KKU12. There was no heterozygous *AG* in Pradoo Hang Dam morkho55. Furthermore, variation of heterozygous *AB* in *PIT1* and *TGF-β3* was found to be greater than homozygous variants in Pradoo Hang Dam morkho55 and Chee KKU12 (Table 1).

Conclusions

The present study provides evidence that there are various patterns of *MSTN, TGF-β3* and *PIT1* gene in Thai native chicken. The variation detected in this study might underpin the development of gene markers for improved muscle growth in Thai native breeding, the genes warrant further investigation.

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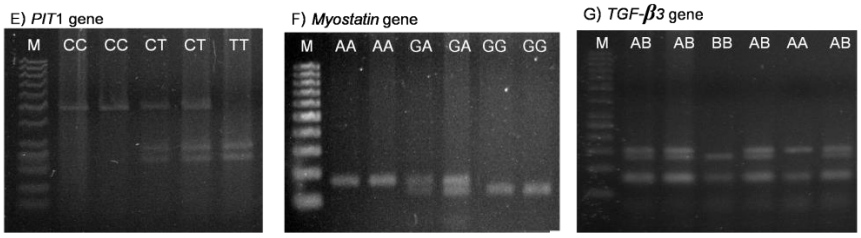

Figure 1 PCR-RFLP patterns of *cGH* (A), *IGF*-1(B) *ApoB* (C), *FASN* (D), *PIT*1(E) *Myostatin* (F) and *TGF-β*3 (G) gene in Chee KKU12 and Pradoo Hang Dam morkho55 (M = 100 bp Marker Ladder)

Table 1 Genotype and allele frequency of *MSTN*, *TGF-β* and *PIT1* in Chee KKU12 and Pradoo Hang Dam 55.

Gene	Genotypes	Frequency (N)		Allele	Frequency (N)	
		Chee	Pradoo Hang Dam 55		Chee	Pradoo Hang Dam 55
MSTN	AA	0.14(10)	0.76(42)	A	0.325	0.760
	AG	0.37(27)	0.00(0)	G	0.675	0.240
	GG	0.49(36)	0.24(12)			
TGF-β3	AA	0.30(22)	0.54(30)	A	0.56	0.75
	AB	0.52(38)	0.42(23)	B	0.44	0.25
	BB	0.18(13)	0.04(2)			
PIT1	AA	0.26(19)	0.24(13)	A	0.54	0.55
	AB	0.56(41)	0.62(34)	B	0.46	0.45
	BB	0.18(13)	0.14(8)			

References

Amirinia, C., H.R. Seyedabadi, N. Amirmozafari, R.V. Torshizi, M. Chamani, A.J. Aliabad and M.A. Abbasi. 2011. Association of transforming growth factor-ß3 gene polymorphism with growth and body composition traits in Iranian commercial broiler lines. African Journal of Biotechnology. 10(10) : 1784-1788

Cogburn, L.A., J. Burnside and C.G. Scanes. 2000. Physiology of growth and development. in Sturkie's Avian Physiology. 5th ed. G. C. Whittow, ed. Academic Press, San Diego. 635-656.

Groenen, M.A., H.H Cheng, N. Bumstead, B.F Benkel, W.E. Briles, T. Burke, D.W Burt, L.B Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. Ponce de Leon, M. Soller, H. Takahashi and A. Vignal. 2000. A consensus linkage map of the chicken genome. Genome Res. 10: 137-147.

Gu, Z.L., D.H. Zhu, N. Li, H. Li, X.M. Deng and C.X. Wu. 2003. Polymorphisms of myostatin gene and its relationship with the development of skeletal muscle and fat in chickens. Sci. China Life Sci., 33(3): 273-280.

Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to Quantitative genetics. Ed 4th, Longmans Green, Harlow. Essex, UK.

Nie, Q., Fang, M. Xie, L. Zhou, M. Liang, Z. Luo, Z. Wang, G. Bi, W. Liang, C. Zhang, W. Zhang, X. 2008. The PIT1 gene polymorphisms were associated with chicken growth traits. BMC genetics 9: 20.

Piek, E., C.H. Heldin and P. Ten Dijke. 1999. Specificity, diversity, and regulation in TGF-β superfamily signaling. FASEB J. 13: 2105-2124.

Ye, X.H, S.R. Brown, K. Nones, L.L. Coutinho, J.C.M. Dekkers and S.J. Lamont. 2007. Associations of myostatin gene polymorphisms with performance and mortality traits in broiler chickens. Genet. Sel. Evol., 39(1): 73-89.

Zhang, G. X., X.H. Zhao, J.Y. Wang, F.X. Ding and L. Zhang. 2011. Effect of an exon 1 mutation in the myostatin gene on the growth traits of the Bian chicken. Animal Genetics, 43, 458–459.

PO-06-22

Most Probable Producing Ability of Bali Cows for Calving Interval and Calf Growth Performance

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INTRODUCTION

Most Probable Producing Ability (MPPA) is a means of comparing the maternal performance potential of cows based on the records of their offspring. An MPPA value can be considered as a deviation in maternal performance from the average value for the herd (Itulya, 1980). Maternal performance refers to a composite of characteristics of the cow such as the ability to raise a calf with a high weaning weight. In this sense, MPPA can be used by the individual livestock producer as the basis for culling cows within a herd. It cannot be used for comparison among cows in different herds since it is estimated as the deviation of the individual performance from its herd average. The aim of the study is to compare the performance potential of Bali cows based on their reproductive performance and the records of their offspring. Calving interval is an important reproduction trait in selecting cows to be retained in a herd as it is an indicator of lifetime maternal ability of the cow.

MATERIAL AND METHODS

Data

Data on 735 registered Bali cattle owned and managed by Livestock Center of Excellence (Balai Pembibitan Ternak Unggul, BPTU) were used to study cow productivity in terms of Most Probable Producing Ability. The data consisted of 296 weaning weight data derived from 99 dams with 2-7 records per dam, 245 yearling weight data derived from 86 dams with 2-6 records per dam, and 194 calving interval data came from 63 dams with 2-7 records per dams. A summary of the data structure from 1994 to 2006 for each trait is presented in Table 1. Completeness of records for the traits considered in the study is presented in Table 2. The data were used to estimate the repeatability and most probable producing ability (MPPA) values of weaning weight, yearling weight and calving interval for the Bali cows.

Management of animals

The project on the genetic improvement of growth performance of the Bali breed was started in 1976 by the Agriculture Ministry of Indonesia. In this project, bulls were selected at 1 year of age from village breeding centers (Tabanan and Karang Asem). Then, the bulls were assigned to herds in Pulukan to participate in performance test under the supervision of Bali Breeding Center. In these herds pedigree information and other information related to growth traits were collected and recorded in the database of Bali Breeding Center for the purpose of investigating the success of the Bali project. The mating period for the Bali breed was from July to December and mating was by artificial insemination (AI) and natural mating. Calving commenced from April to October of the following year. The calves were weighed and ear tagged within 12 hours of birth. The identities of the newborns and of their parents, date of birth, sex, and birth weight were recorded. The calves were outdoors together with their dams until weaning. The length of the suckling period was not the same for all calves. During the suckling period, calves were additionally fed with king grass and commercial concentrate. Most of the calves were weaned in May when they were 210 ± 15 days of age. After weaning, the calves were separated from their dams and put in different herds. From 18 months of age, the animals were managed similarly for one year in order to evaluate their performance. All young cattle were fed the same grasses and put in the same paddock.

Data Analysis

All weaning weights and yearling weights were adjusted to the average age of 205 days and 365 days, respectively. Since sex has been determined to be a significant source of variation in weaning weight in cattle, it was decided to adjust all the females to males. The repeatability estimates for weaning weight, yearling weight and calving interval were determined by analysis of intra-class correlation coefficient based on formula: .

Estimates of the variance components were obtained by equating the mean squares to the expected mean squares in the model as follows:

Sources

E (MS)	
Between dam	$s^2_e + k_1 s^2_W$
Within dam	s^2_e

Only dams having two or more offspring were included in the analysis.
The estimated repeatability for weaning weight, yearling weight and calving interval were used to calculate the MPPA of the cows for the respective traits, using the following formula (Hardjosubroto, 1994).
MPPA = (nr/(1 + (n-1)r))(P-P)
Where:
MPPA = Most Probable Producing Ability
n = number of data
r = repeatability
P = average of trait estimated
P = average of trait population

RESULT AND DISCUSSION

The results in Table 3 show the weaning weight, yearling weight and calving interval by year. The mean of weaning weights and yearling weights for males and females were 95.56 ± 17.25, 87.57 ± 18.45, 143.39 ± 25.78 and 136.90 ± 22.01 kg, respectively. The mean calving interval was 391.62 ± 22.59 days.
Both weaning weights and yearling weights were higher for males than for females. This is in agreement with a number of studies on Bali cattle and other breed (Jan, 2000; Echternkamp et al, 2007; Casas et al, 2011; McHugh et al, 2014). The mean calving interval indicated that reproduction efficiency of the cows was in accordance to other studies for Bali breed, and other beef and dairy cattle breeds (Komariah et al, 1982; McHugh et al, 2014; Hare et al, 2006; Löf et al, 2007). Hafez (2000) stated that calving interval depends on the efficiency of heat detection (in the case of AI) and fertility of the males and females.
In addition, weaning age, days open, body condition, pregnancy diagnosis and diseases control are some factors which can influence calving interval. Mating system adopted for the herd may also affect calving interval. Most of the cows (90%) were mated using AI with the rate of services per conception (S/C) of about 1.2 ± 0.06. The other ten percent of the cows were mated naturally using the best bull from the performance test. The bulls were progeny tested for certain traits, which included weaning weight and yearling weight.
The repeatability estimates for weaning weight, yearling weight and calving interval are given in Table 4.
The values of repeatability are somewhat lower than the estimates reported in the literatures (Arango et al, 2002; Suhada et al, 2009). The low repeatability estimate would limit the usefulness of MPPA as an estimate of future cow productivity. Other sources of environmental and non-additive genetic variation, such as age of dam, year of birth and season of birth may have to be accounted for in the estimation of repeatability. Then the value of repeatability may be higher and more accurate.
Of the 99 cows tested on weaning weights, 45.45% had MPPA values above the herd average. Percent cows with MPPA above herd average for yearling weights and calving interval were 50% and 36.51%, respectively. Cows with low MPPA for weaning weight, yearling weight and calving interval should be culled from the herd in order to improve future cow productivity of the herd. The best ten cows with the highest MPPA estimates for weaning weight, yearling weight and calving interval are listed in Table 5.
The dam code of the top 10 cows for each trait was different; meaning cows predicted to have the best performance for one trait need not be among the top for the others. There is no dams in the top 10 cows for all three traits, but there are three dams (506.90, 505.90 and 766.88) which had the same ranking for weaning weight and yearling weight. MPPA estimate depends on the value of repeatability for the trait, the performance of the animal for the trait and the number of records available for the animal for the trait. Based on the formula for MPPA, higher repeatability values and higher performance of the animal concerned would result the higher MPPA estimates. In terms of maternal performance, MPPA does not provide a complete evaluation of the cow as each estimate is for a single trait. Furthermore, the economic value of the individual traits is not considered. It was

believed that culling cows on the basis of calving interval could improve average reproductive performance of herds (Itulya, 1980).

CONCLUSION

From the findings of the present study it may be concluded that the repeatability estimates for weaning weight, yearling weight and calving interval in the Bali cattle are rather low. In general, reproduction traits are heavily influenced not only by the environment but also by management decisions (e.g. voluntary waiting period for mating of animals). In addition, in developing countries such as Indonesia, the data often includes missing records that might bias the findings. The repeatability values have to be corrected for known fixed effects, other than just sex, such as year of calving, season of calving, dam age, etc. Corrections for these factors would improve the repeatability values and, therefore, give more reliable estimates of MPPA for the traits. This is important if cows are to be culled based on estimated MPPA values.

Table 1. Mean, standard deviation (SD), and coefficient of variation (CV) of weaning weight, yearling weight and calving interval

Items	Traits		
	Weaning weight	Yearling weight	Calving interval
Number of records	296	245	194
Total number of dams	99	86	63
Number of dams with			
2 records	46	44	23
3 records	26	21	23
4 records	16	12	9
5 records	5	6	6
6 records	5	3	1
7 records	1	0	1
Mean	88.59	120.09	560.65
SD	15.78	22.01	255.02
CV	17.81	18.33	45.49
Min.	55.68	68.68	347.67
Max.	163.38	183.13	1475

Table 2. Completeness of the 735 records for the three traits

No. of records of data	Weaning weight	Yearling weight	Calving interval
579	x	x	x
6	-	-	x
13	x	-	x
105	x	x	-
26	x	-	-
6	-	x	-

Note: x indicates trait data was available for the records.

Table 3. Mean of weaning weight, yearling weight and calving interval

Year	Weaning weight		Yearling weight		Calving interval (days)
	Male (kg)	Female (kg)	Male (kg)	Female (kg)	
1	93.45±16.09	83.56±17.78	142.46±23.76	134.45±16.26	400.00±15.67
2	93.52±13.68	83.13±14.22	142.36±28.12	134.34±20.16	396.00±21.18
3	94.56±18.70	84.33±16.52	143.53±23.30	135.30±21.21	396.00±20.22
4	92.51±12.14	89.93±10.61	143.16±25.46	138.68±14.15	385.78±17.81
5	94.50±38.70	82.30±16.50	144.30±36.00	135.30±26.20	396.00±16.34
6	93.45±15.12	89.75±15.60	143.66±20.36	138.70±15.43	385.78±19.92
7	93.65±10.15	89.83±12.50	143.75±15.45	138.98±17.23	395.78±18.98
8	93.26±12.14	90.50±10.61	145.26±25.46	139.68±14.15	388.00±20.79
9	91.10±19.60	85.88±26.12	142.20±20.14	132.51±16.20	385.98±22.16
10	115.60±19.60	96.50±14.45	143.24±24.43	141.05±29.67	386.83±21.56

Table 4. Repeatability estimates for characters measured

Traits	Repeatability
Weaning Weight	0.006±0.059
Yearling Weight	0.022±0.068
Calving Interval	0.115±0.078

Table 5. The best ten cows based on MPPA for weaning weight, yearling weight and calving interval.

Weaning weights		Yearling weights		Calving interval	
N	Dam code	N	Dam code	N	Dam code
3	716.87	5	701.90	2	745.93
4	833.85	2	724.87	3	512.90
3	506.90	3	506.90	2	859.92
3	505.90	3	505.90	2	716.87
6	702.90	2	790.89	2	809.91
7	718.90	3	833.85	3	701.93
3	766.88	3	766.88	3	741.91
2	790.89	3	840.90	3	746.88
3	724.87	2	798.90	2	833.90
3	725.87	6	718.90	5	742.88

N: number of records

REFERENCES

Arango, J.A., L. V. Cundiff & L. D. Van Vleck. 2002. Genetic parameters for weight, weight adjusted for body condition score, height, and body condition score in beef cows. J. Anim. Sci. 2002. 80:3112–3122

Casas, E., R. M. Thallman, & L. V. Cundiff. 2011. Birth and weaning traits in crossbred cattle from Hereford, Angus, Brahman, Boran, Tuli, and Belgian Blue sires. J. Anim. Sci. 89:979–987

Echternkamp, S.E., R.M. Thallman, R.A. Cushman, M.F. Allan, & K.E. Gregory. 2007. Increased Calf Production in Cattle Selected for Twin Ovulations. J. Anim. Sci. 85: 3239-3248.

Hardjosubroto, W. 1994. Aplikasi Pemuliabiakan Ternak di Lapangan. PT. Gramedia Widiasarana Indonesia, Jakarta.

Hare, E., H. D. Norman, & J. R. Wright. 2006. Trends in Calving Ages and Calving Intervals for Dairy Cattle Breeds in the United States. J. Dairy Sci. 89: 1:365-370.

Itulya, S.B. 1980. Most Probable Producing Ability, Fertility and Related Selection Criteria for Hereford Cows. Dissertation. The University of Arizona.

Jan, R. 2000. Penampilan Sapi Bali di Wilayah Proyek Pembibitan dan Pengembangan Sapi Bali di Daerah Tingkat I Bali. Tesis PPS-UGM, Yogyakarta.

Komariah, S.S. Mansjoer & Pangestu. 1982. Beberapa Sifat Reproduksi Induk Sapi Bali di Pulau Bali. Abstrak pada Buku Panduan Seminar Nasional Sehari Bersama Pemuliaan Ternak. Bogor.

Löf. E., H. Gustafsson, & U. Emanuelson. 2007. Associations Between Herd Characteristics and Reproductive Efficiency in Dairy Herds. J. Dairy Sci. 90:4897–4907.

McHugh, N., A. R. Cromie, R. D. Evans, & D. P. Berry. 2014. Validation of national genetic evaluations for maternal beef cattle traits using Irish field data. J. Anim. Sci. 2014.92:1423–1432

Suhada, H, Sumadi, & N. Ngadiyono. 2009. Estimation of Genetic Parameters of Production Characteristics on Simmental Cattle at Balai Pembibitan Ternak Unggul Sapi Potong Padang Mengatas, West Sumatera. Buletin Peternakan 33(1): 1-7.

PO-06-24

Number of sire and dam on genetic response of growth trait in Thai native chicken

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INTRODUCTION

Currently, Thai consumers prefer meat from Thai native chickens more and more because they have good meat texture led to a good taste and low cholesterol content compared to commercial broilers (Wattanachant et al., 2004; Jaturasitha et al., 2008). However, one of major limitation of Thai native is low growth rate and low numbers of egg production. From this reason, animal breeders need to improve these traits via the selection and mating plan. In theory, the number of parents in based population (G0) related with an effective population size (*Ne*) (Falconer and Mackay, 1996). Theoretically, the *Ne* is related to maximum response which explained in Falconer and Mackay (1996). The objective of this study was to compare genetic response of growth and egg production traits in Thai native chicken under index selection based on the difference number of cocks and hens and selected short term (5 generations) and long term (10 generations).

MATERRIALS AND METHODS

Simulation for scenarios

The traits are body weight at 16 weeks of age (WT16) and egg production from age at first egg to age at 300 days (EA300) were analyzed. The number of hens at 200,250, 300, 350, 400, 450 and 500 birds were combined with number of cocks at 30, 50, and 70 birds, so there are 21 scenarios for testing effect number of parents to select. The traits were generated by using SIMF90 which developed by Duangjinda et al. (2005). Primary parameters were used simulation to generate phenotype of traits (data not showed). The regression responses of WT16 in each scenario were used for plotting 3D-diagram, when selected over five generations. Only 350 birds of hen are frequently used for the based population (G0) and 30, 50, and 70 birds of cocks were used for mating and measuring of genetic response over five generations based on index selection as follow; . The long term selection of WT16 was selected over ten generations. Ten replications were conducted for each selection scenarios.

Statistical analysis and estimated selection response

Data and pedigrees from six generations of simulation were used to estimate variance components under the bivariate animal model by AIREML. The breeding values were estimated using the BLUPF90 PigPak 2.5 software (Duangjinda et al., 2005). The averages EBV across generations were plotted to measure genetic trend. The slope linear regressions of responses in each scenario were plotted by 3D-plot diagram.

RESULTS AND DISCUSSIONS

The number of cocks and hens on selection of WT16 showed in a 3D-plot diagram (Figure 1). The result showed that 250-300 birds of hens were appropriates number for small number of cocks (10-30 cocks). Based on recent population with 350 hens, the simulation of this study showed that a decreasing number of cocks from 70 birds had reduced the genetic response. This result is confirmed in Figure 2 (a). These results are described by the lower effective population size (*Ne*) had effected on the diminishing of genetic response (Falconer and Mackay, 1996). In addition, the lower *Ne* is a one of cause on increased inbreeding depression (Nomura et al., 2001; and Muir, 2000) The genetic response of WT16 and EA300 base on index selection were showed in Figure 2. Fitting number of hens at 350 birds, the 70 birds of cock has the greatest impact on genetic response of WT16 when compared to 30 and 50 birds of cock. It is possible that number of cocks (ranged from 30-70 birds) were not significantly responded on low heritability trait i.e. EA300 (h^2 = 0.14). Selection for WT16 and EA300 based on index selection were found that the genetic response increase in both traits.

The long term selection based on small number of cocks and hens (10 cocks and 50 hens), the genetic response was slightly increased in the first of 3rd generation and the 8th generation into selection limit (Figure 3). It is possible that a reducing of genetic variation after selection and an increasing of inbreeding rate led to slowly

diminish of response in several selections which are described by Falconer and Mackay, (1996) and Muir, (2000).

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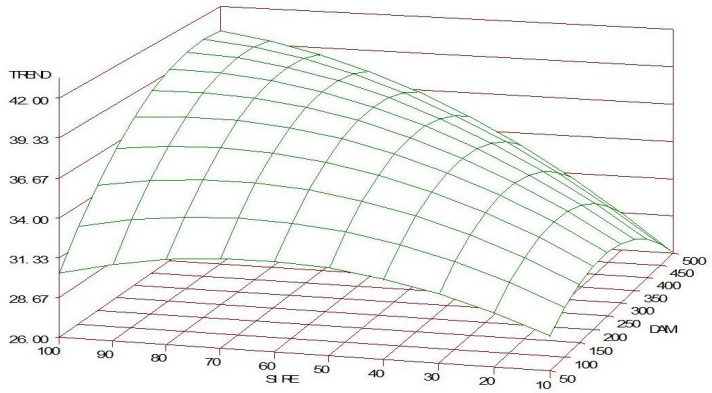

Figure 1 the effect of number of cocks and hens to select on genetic response of body weight at 16 weeks (WT16) of Thai native chickens (Chee)

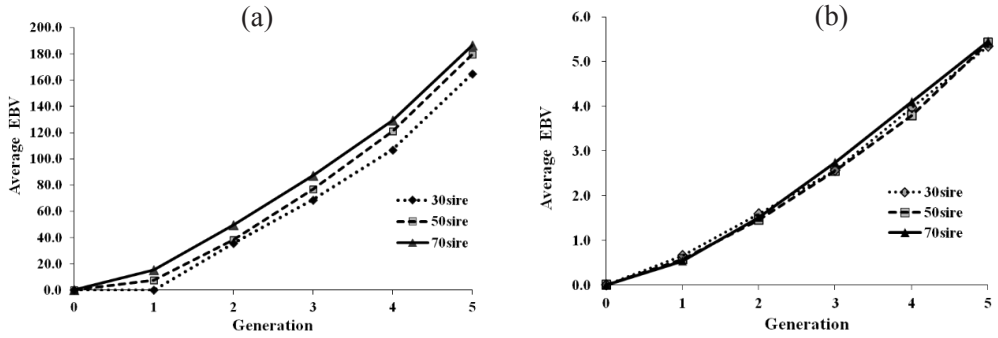

Figure 2 genetic trend of (a) body weight at 16 weeks of age (WT16) and (b) egg production from age at first egg to age at 300 days (EA300) of Thai native chickens (Chee)

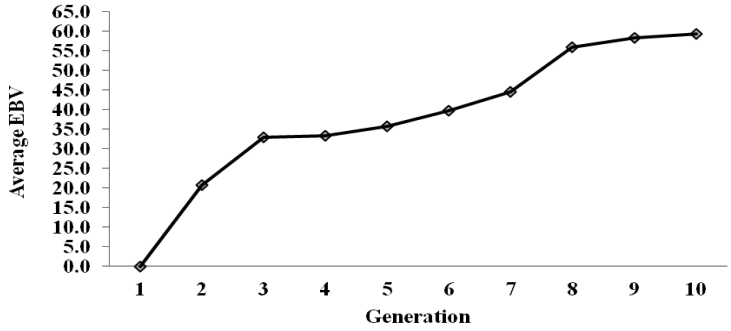

Figure 3 genetic trend of market weight (WT16) in long term selection of Thai native chickens (Chee) based on the effective population size small.

REFERENCES

Duangjinda, M., I. Misztal, S.T. Surata. 2005. BLUPF90 ChickenPak 2.5 Genetic Evaluation and Simulation Program. http://ag2.kku.ac.th/monchai/blup/bin/. Accessed 16 May 2008.

Falconer, D. S., and T. F. C. Mackey, 1996. Introduction to quantitative genetics. 4th edition. Longman, Oxford.

Jaturasitha, S., T. Srikanchai, M. Kreuzer, and M. Wicke. 2008. Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-boned and Thai native) and imported extensive breeds (Bresse and Rhode Island Red). Poult. Sci. 87:160-169.

Muir, W. M., 2000. The interaction of selection intensity, inbreeding depression, and random genetic drift on short- and long-term response to selection: Results using finite locus and finite population size models incorporating directional dominance. J. Anim. Sci.79:1-11.

Nomura, T., T. Honda, and F. Mukai. 2001. Inbreeding and effective population size of Japanese black cattle. J. Anim. Sci. 79:366-370.

Wattanachant, S., S. Benjakul, and D. A. Ledward. 2004. Composition, color, and texture of Thai indigenous and broiler chicken muscles. Poult. Sci. 83:123-128.

PO-06-28

The effect of Work Safety, Animal Welfare Criteria on Profitability in Dairy

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Introduction

Injuries is very common problem at livestock farms, sometimes worker can disabled because of these problem. After injuries, there is nothing to do accept treatment costs and healing effort of the people.

A casual assessment of any group of farm or ranch workers will often detect missing digits and limbs, impaired mobility, or a wide range of scars from accidents with both animals and machine. The most animal injuries are caused by large animals, and horses and cows cause the most hospitalizations (Busch et al. 1986; Conrad, 1994).

Dairy farming is known to be associated with a high risk of occupational injury and dairy cattle are repeatedly cited as one of the major sources of injuries on dairy farms. There are few occupations in which the evidence of disabling injuries is more common than agriculture. Large animal-related injury is common among farming communities, with 24% of farmers in one survey reporting livestock related injuries, 10 and 1.7% large-livestock farmers reporting animal-related injury in one year (Sprince et al. 2003). In cattle-related accidents, fatalities have been shown to be related to aggressive behavior of the animal (Ornehult et al. 1989).

Many task has injury risk during daily activity at farm. Tagging newborn calves and clipping cattle, feeding and herding were the most commonly cited situation in which the injury occurred (Lindsay et al. 2004). Moving cows to hoof trimming involved much higher injury risk exposure to the handler than moving cows to milking. When moving cows to milking, risk situations were primarily associated with **facility design** and the perceived **energy level of the handler**. The more aversive hoof trimming procedure involved higher frequencies of **fear responses by the cows**, more forceful interactions by the handler and higher rates of incidents and risk situations. The correlations reported significant between specific human-cow interactions and facility characteristics and incidents (Lindahl, 2014). MAny people who work with animals including farmers, veterinarians, butchers, and workers in zoos and circuses are all at risk (Wiggins et al., 1989). People at greatest risk for these injuries are those whose occupation or livelihood involves large animals (Nogalski et al., 2007). Langley et al. (2001) reported that large animals (cows and horses) caused the majority of deaths (67%) among workers in farms. Farmers and farm workers can easily be injured by livestock. Farm animals are often subjected to aversive handling, which can result in them becoming fearful of humans. Fear of people can reduce animal well-being and possibly milk production, and increase the risk of injury to both animals and handlers. Research on the topic of human-animal interaction and its relationship to productivity and health is limited. Because of this, this review will focused on the sources of risk and the perception level of risk strategies are investigated in the dairy farms aspect of the effect of work safety, animal welfare criteria on profitability. A variety of strategies have been revealed for risk sources strategies which have been classified. These factors have been investigated in detail and presented.

Barn environment and facility design

The cattle often detect and perceive their environments quite differently from humans. There are a variety of configurations of structures. Researchers reported that livestock farms tended to be high injury risk increased with increasing number of hours worked on the farm (Elkington 1990; Brison & Pickett 1992; Pratt et al., 1992; Zhou & Roseman, 1994; Nordstrom et al., 1995). The farm environment also main risk factor because of capacity, worker, and high annual production (Zhou & Roseman, 1994; Pickett et al., 1995). The patterns of injury have been fairly consistently reported across these studies, with farm machinery, accidental falls, and animal related injuries being the three major external causes of injury (Brison & Pickett, 1992; Zhou & Roseman, 1994; Nordstrom et al., 1995). The common elements of farm are walking and working surfaces, holes and floor openings, handrails and railings, stairs and fixed ladders and cages structures to plan and identifying hazards on farm.

Floor type enad material and specifications is very important for slipping of animals and humans. Also opening for water or waste pipe can cause slippery floor. Properly located gates can block off a travel lane and direct the cow into a desire area. Gates may also be used within pens to form a funnel to direct a reluctant cow into a stanchion or other lockup. They can also be part of a smaller confinement area for breeding or rectal examination. Over 75% of the animal-related human injuries are due to insufficient restraining equipment and facilities on most

dairy farms. Proper application and the right choice of restraining equipment and facilities are very important consideration for reducing potential injuries to the dairy farmer. Use a rope halter, squeeze chute, and headgate when you engage in major animal handling activities such as hoof trimming, breeding, and applying medication. Use a squeeze chute with a headgate to protect yourself from the animal's violent movements. Use a tail holder to prevent eye injuries when milking or examining the animal. Maintain a treatment stall on your farm to reduce the risk of injuries to yourself as well as the veterinarian during activities such as pregnancy examination, vaccination, medication, deworming, and artificial insemination.

Eliminate obstacles that may be in a milker's way such as unwound hoses or extra buckets. Falls can cause injuries and substantial loss of productivity. Milking parlors, upright silos, gravity flow feed bins, grain storage bins, and pump reception pits are a few agricultural structures that have fixed ladders, cages and landing platforms. There are special requirements for agricultural structure related to grain bins and external wall ladders and chute ladders for silos. An additional hazard within animal handling structures involves crowd gates. Ensure that crowd gates have the appropriate stop measures installed. Install the gate to allow a gap between the furthest wall to prevent a person from becoming entrapped or crushed. Ensure there is a gap large enough from the bottom of the gate to the floor to prevent a person from being crushed or tangled.

Dairy cattle are commonly restrained in squeeze chutes for various procedures. Cows will remain calmer in chutes that have covered sides that prevent the animal from seeing human movement. Close the chute slowly and steadily to apply even pressure to the animal. Barn enviroment has specifik micro climate such as air quality, light intensity, voices and air velocity. The proper design, construction and operation of a cattle handling facility is important to ensure safe working conditions for animals and humans. Understanding the inherent behavior of cattle, plus working them slowly and quietly, will reduce injuries and help make an operation run more smoothly and efficiently (Hubert et al., 2012).

Farm worker

The farm worker is management of animals with a safe, effective, and low-stress. Farm animals are often subjected to aversive handling, which can result in them becoming fearful of humans. Fear of people can reduce animal well-being and possibly milk production, and increase the risk of injury to both animals and handlers. Pickett et al. (1995) conclude that young adult male farmers have the highest rates of injury and warrant targeting by injury control programs.Breuer et al (2000) investigated the relationships between the behavioral response to humans and the milk production of cows at 31 commercial dairy farms. They found that several cow behaviors that are indicative of fear of humans were moderately or highly correlated with milk yield and composition. In farms where milk yield was low, cows showed less approach to the researcher in the standard fear test than at farms where milk yield was higher. Observations from the study indicated that where restlessness was high, productivity was low. Restlessness, which they measured by the number of flinch, step and kick responses, is indicative of stress. Negative treatment by handlers include hits, slaps, tail twist, shouting, and fast speed of movement, whereas positive behaviors include stroking, rubbing, hand resting on the animals back or flank, and slow and deliberate movement and talking. Hemsworth et al. (2000) reported that the significant correlations between stockperson attitudes and behavior and cow behavior and productivity, although not evidence of causal relationships, indicate the possibility of targeting these human characteristics to reduce fear responses of dairy cows to humans and improve the cows' productivity. Many farmers sustain minor injuries while working with cattle. Common injuries include cuts, bruises, fractures, sprains and strains). Although the animals are implicated in such injuries, in most cases the incidents occur due to inappropriate behaviors of people or a lack of control of the animals (Nogalski et al., 2007). There was a significant difference in the animals' respond to the handler. Handler style was also different between the animals; two main components contributed to this variation: "attitude towards the animals" and "knowledge and experience" of the animals. Cattle remember "bad" experiences and create associations from fear memories. For example, if a man with a beard caused a cow pain, she may exhibit fear towards all men with beards. This makes calm and respectful handling at all times even more important. Lindahl et al. (2016), reported that the some interactions (such as forceful tactile interactions with an object and pulling a neck strap or halter) appeared to be related to potentially dangerous incidents where the handler was being kicked, head-butted, or run over by a cow. These errors in judgement and action are due to a variety of reasons, but occur most when when people are tired, hurried, upset, preoccupied, or careless. Because human has specific physical, psychological, and physiological characteristics which affect the occurrence of life threatening accidents. Personal protective equipment must be provided to the worker.

Handler position, voices and body movement contains very important signals for cattle. Handler using this messages positively manage animal safe and healthy. Because of these handler must be get training about animal handling, identify and implement proper animal handling techniques during all management procedures. Training and work description is very important activities. But many farm cn not use these type tarinng course. But new and young workers must be tarin about how to work safely with farm animals. During training sessions, emphasize all known animal behavior problems. Also demonstrate the use and effectiveness of all animal restraining equipment and facilities available. There must be a escape route before beginning work with animals. Excited animals are harder to handle. If cattle become nervous or excited when being worked, stop and allow the animal 30 minutes for their heart rates to return to normal

Cattle senses

An understanding of livestock behavior will facilitate handling, reduce stress for the animal and handler, and improve animal welfare and handler safety. Handlers who understand livestock behavior can reduce animal stress. The positive human contact, starting at a young age is very important role in cow aggressive behavior. The two fundamental rules for working with cows are slow and quiet. Using these rules, the job will be done faster with the least amount of stress to the cows and less stress and aggressive behaviour to worker.

When handling cattle, it is important to remember that cows have different senses from the all others. They has specific sights and sounds and live than humans. Loud noises, especially high pitched noises, frighten cattle. Cows commonly kick forward and out to the side. They also have a tendency to kick toward the side where they have pain from inflammation or injuries. Therefore, if a cow is suffering from mastitis of only one quarter, you may want to consider approaching her from the side of the non-affected udder when examining or milking. When cattle are moved quietly they remain more calm and easier to handle. Studies have shown that cattle have panoramic vision of 330 degrees and have a blind spot directly in the back of their heads. However, they have poor depth perception and cannot focus quickly on close objects. Cows usually lower their heads to look at something because their vertical vision is only about 60 degrees (compared to 140 degrees in humans). They will also walk slowly in unfamiliar environments. Cows should be given enough time to move and walk at their own pace without being rushed. Cows can hear well and don't like very high, screeching sounds (hitting or yelling) can create a lot of fear and stress. Cows are prey animals and feel safer in a crowd, so they can be nervous when alone. Lindahl et al (2016) observed that the milking, cows were quite easily moved using few interactions. As expected, the cows showed no behavioral signs of stress, fear, or resistance and their heart rate only rose slightly from the baseline (i.e., the average heart rate during an undisturbed period before handling). Moving cows to hoof trimming involved more forceful and gentle interactions compared with moving cows to milking. Furthermore, the cows showed much higher frequencies of behaviors indicative of aversion and fear (e.g., freezing, balking, and resistance), as well as a higher increase in heart rate. Cattle are generally color blind and have poor depth perception, thus they are very sensitive to contrast. Eliminate blind turns, dark shadows and swinging/dangling items in their path to enable easier movement.The risk of injury to which handlers were exposed also increased when moving cows to hoof trimming rather than to routine milking. Dairy bulls are known to be more aggressive than other bulls. Research has shown that this may be due to the difference in calf raising methods; beef bulls are typically reared on the mother cow whereas dairy bulls are bottle/bucket fed by people. This causes dairy bulls to direct their challenges to people rather than towards other cattle. The risk of bull attacks may be lessened by raising dairy bull calves together starting at 6 weeks of age. The treatment of a bull as a working animal rather than a pet may also make him easier to handle. If an escaped cow or horse is located and they are not an immediate threat to people, allow them to be alone for 30 minutes so they will calm down. Twenty minutes is required for the animal's heart rate to return to normal. When the escaped animal has calmed down, it can be quietly moved. Interestingly, a lone animal often returns of its own volition to other horses and cattl

Results

Research results indicate a need for changes in the way aversive routine procedures are performed on dairy farms so as to increase handler safety, but also improve animal welfare, ease of handling and efficiency. The risks associated with animal injury can be minimized by training employees on proper animal handling as well as worker positioning. Many injuries occur when a person is forced into contact with a structure by an animal.

Literature

Breuer K, Hemsworth PH, Barnett JL, Matthews LR, Coleman GJ., 2000. Behavioural response to humans and the productivity of commercial dairy cows. Appl Anim Behav Sci. 2000 Mar 1;66(4):273-288.

Brison, R.J. & Pickett, C.W. (1992). Nonfatal injuries in 117 eastern Ontario beef and dairy farms: A one-year study. Am J Ind Med, Vol. 21, pp. 623-636

Busch Jr HM, Cogbill TH, Landercasper J, 1986. Blunt bovine and equine trauma. J Trauma 1986;26(June (6)):559–60.

Conrad L. 1994. The maul of the wild: animal attacks can produce significant trauma. Emerg Med Serv 1994;23(March (3)):71-2. 76.

Elkington, J.M. (1990), ''A case-control study of farmwork-related injuries in Olmstead County, Minnesota'' [Dissertation], The University of Minnesota, Minneapolis

Hemsworth P.H., Coleman G.J., Barnett J.L., Borg S., 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. J Anim Sci. 2000 Nov;78(11):2821-31.

Hubert, D.J., Huhnke, R.L. & Harp, S.L. (2003). Cattle handling safety in working facilities. Available from: http://pods.dasnr.okstate.edu/docushare/dsweb/Get/ Document- 4821/BAE-1738web.pdf

Langley, R.L. & Hunter, J.L. (2001). Occupational fatalities due to animal-related events. Wilderness Environ Med, Vol. 12, pp. 168-174

Lindahl, C., Pinzke, S., Herlin, A., Keeling L.J., 2016. Human-animal interactions and safety during dairy cattle handling—Comparing moving cows to milking and hoof trimming. Journal of Dairy Science, Volume 99, Issue 3, March 2016, Pages 2131–2141

Lindsay S, Selvaraj S, Macdonald JW, 2004. Injuries to Scottish farmers while tagging and clipping cattle: a cross-sectional survey. Occup Med (Lond) 2004;54(March (2)):86–91.

Murphy, C. G., McGuire, C.M., O'Malley, N., Harrington, P., 2016. Cow-related trauma: A 10-year review of injuries admitted to a single institution. Injury, Int. J. Care Injured 41 (2010) 548–550

Nogalski, A., Jankiewicz, L., Cwik, G., Karski, J. & Matuszewski, L. (2007). Animal related injuries treated at the Department of Trauma and Emergency Medicine, Medical University of Lublin. Ann Agric Environ Med, Vol. 14, pp. 57-61

Nordstrom, D.L., Layde, P.M., Olson, K.A., Stueland, D.T., Brand, L. & Follen, M.A. (1995). Incidence of farm-work-related acute injury in a defined population. Am J Ind Med, Vol. 28, pp. 551-554

Ornehult L, Eriksson A, Bjo¨rnstig U. 1989. Fatalities caused by nonvenomous animals: a ten-year summary from Sweden. Accid Anal Prev 1989;21(August (4)):377–81.

Pickett W, Brison RJ, Niezgoda H, Chipman ML., Nonfatal farm injuries in Ontario: a population-based survey.Accid Anal Prev. 1995 Aug;27(4):425-33.

Pratt, D.S., Marvel, L., Darrow, D., Stallones, L., May, J. & Jenkin, P. (1992). The dangers of dairy farming: The experience of 600 workers followed for two years. Am J Ind Med, Vol. 21, pp. 637-650

Sprince NL, Park H, Zwerling C, 2003. Risk factors for animal-related injury among Iowa large-livestock farmers: a case–control study nested in the Agricultural Health Study. J Rural Health 2003;19(Spring (2)):165–73.

Wiggins, P., Schenker, M.B., Green, R. & Samuels, S. (1989). Prevalence of hazardous exposures in veterinary practice. Am J Ind Med, Vol. 16, pp. 55-66

Zhou, C. & Roseman, J.M. (1994). Agricultural injuries among a population- based sample of farm operators in Alabama. Am J Ind Med, Vol. 25, pp. 385-402

PO-06-31

Effect of Days Postpartum and Locality of Hanwoo Cows by Parity

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Introduction

The maternal nutrient and energy source are lots of important resource to produce and breed calves. Hence, cows' body composition and reproductive performances are very crucial factors. Recently, the researchers have been studied to improve genetic and phenotypic traits (Lee et al, 2003a; Lee et al, 2003b; Lee et al, 2006; Lee et al, 2014) and to investigate reproductive performances(Choi et al, 2004; Ha et al, 2009) of Hanwoo cows in Korea. Nevertheless, the data for change of body condition of Hanwoo cow at carving are lacking. Therefore, the objective of this study was to investigate the variation of ultrasound measures on rib eye area of cows by days postpartum and by localities of cows.

MATERIALS AND METHODS

Data

A total of 740 cows and heifers born form 2009 to 2013 and raised on 5 regions were scanned with ultrasound machines owned and operated by local agents. Scanning position was common at vertical application on longissimus dorsi muscle area between 13th thoracic vertebrae and first lumbar vertebrae. And the ultrasound scan images were analyzed to measure backfat thickness (UBF), eye muscle area (UEMA) and visually appraised score of marbling (UMS, 1 to 27 points, higher the score higher the marbling). Table 1 shows the number of cows by birth-year, location and age groups of days to parturition.

Statistical analyses

Data were fitted go general linear models to check the significances of fixed effects on the traits under study (SAS Institute Inc., Cary, NC, 2002). For ultrasound measure traits, effect birth-year, location and age group at ultrasound measurements were all significant sources of variation.

Result

The analysis of variance for ultrasound measurements of Hanwoo cows for parity is given in Table 2. Six age groups of days postpartum by 15 days interval were shown to affect ultrasound measures after first parturition ($p < 0.01$). But the effects became insignificant at the second or at the third parity. Locality of cow farm was a significant source of variation for all ultrasound scan measures of all parity except UMS at the second parity. Least square means of ultrasound measurements by birth year are given in Table 3. Except for UBF, UEMA and UMS of second-parity, and UBF of third-parity, all of measurements had a significant effect ($p < 0.05$). The result of least square means of ultrasound measurements by localtity is reported in Table 4. It was showed a significant difference in each region ($p < 0.01$). Table 5 provides least square means of ultrasound measurements by age groups of days postpartum. There was no significant difference in the UBF, UEMA and UMS of all of age groups for second- and third- parity, except first-parity.

Table1. Number of cows by birth-year, location and age groups of days to parturition

Items	Group	Parity		
		1st	2nd	3rd
Brith-year	2010			76
	2011		136	178
	2012	169	391	15
	2013	557	46	
	2014	28		
Locality	A	69	68	35
	B	52	42	16
	C	185	62	14
	D	33	34	12
	E	64	51	32
	F	16	19	8
	G	90	72	43
	H	128	124	57
	I	117	101	52
Age groups of days to parturition	1(1~15days)	152	76	30
	2(16~30days)	116	86	46
	3(31~45days)	132	79	49
	4(46~60days)	99	79	36
	5(61~75days)	68	48	25
	6(76~90days)	75	79	22
	7(91~105days)	47	58	27
	8(106~120days)	38	44	19
	9(121~135days)	27	24	15
Total		754	573	269

Table 2. Analysis of variance for ultrasound measurements of hanwoo cows for parity

Parity	Source	df	UBF	UEMA	UMS
1st	Birth-year	2	2.357	79.992	8.602
	Locality	8	21.395**	558.701**	48.966**
	Age groups of days to parturition	8	4.616	125.228	4.130
	error	735	3.785	86.828	1.899
	RMSE		1.945	9.318	3.606
2nd	Birth-year	2	31.138**	345.420*	7.884
	Locality	8	46.380**	813.951**	25.697**
	Age groups of days to parturition	8	8.644	91.815	2.923
	error	554	5.307	92.197	3.006
	RMSE		2.304	9.602	1.734
3rd	Birth-year	2	5.383	357.951*	4.636
	Locality	8	23.484**	593.674**	10.795**
	Age groups of days to parturition	8	4.947	106.704	3.138
	error	250	5.797	95.788	3.640
	RMSE		2.408	9.787	1.908

UBF : ultrasound back fat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
RMSE : root mean-square error
* $p<0.05$, ** $p<0.01$

Table 3. Least square means of ultrasound measurements by year at birth for parity

Year at Birth	UBF	UEMA	UMS
	---------- 1st parity ----------		
	*	**	**
2010	5.02	64.68	5.80
2011	4.20	63.40	6.13
2012	3.08	54.50	4.53
2013	2.91	49.78	3.55
	---------- 2nd parity ----------		
	ns	ns	ns
2010	2.89	53.66	4.76
2011	3.13	53.88	4.15
2012	2.71	54.81	4.55
	---------- 3rd parity ----------		
	*	*	ns
2009	4.35	64.20	5.21
2010	2.97	54.98	4.39
2011	4.43	58.63	4.66

UBF: ultrasound backfat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score

* p<0.05, **p<0.01

Table 4. Least square means of ultrasound measurements by locality for parity

Year at Birth	UBF	UEMA	UMS
	---------- 1st parity ----------		
	**	**	**
A	4.65	60.15	5.24
B	3.16	53.10	4.45
C	4.14	63.35	5.88
D	3.93	54.52	4.05
E	3.13	59.32	5.39
	---------- 2nd parity ----------		
	**	**	**
A	4.02	55.15	4.94
B	2.52	53.56	4.58
C	2.89	58.70	4.28
D	3.11	51.08	3.66
E	2.02	52.08	4.83
	---------- 3rd parity ----------		
	**	**	**
A	6.28	68.78	5.92
B	4.39	62.85	5.37
C	4.24	58.19	5.15
D	2.30	48.55	2.65
E	2.37	57.99	4.69

UBF: ultrasound backfat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score

* p<0.05, **p<0.01

Table 5. Least square means of ultrasound measurements by age groups of days postpartum for parity

Group[1]	UBF	UEMA	UMS
	---------- 1st parity ----------		
	**	**	**
1	3.39	55.21	5.04
2	3.22	56.22	4.83
3	3.17	55.27	4.50
4	3.39	55.36	4.07
5	4.27	60.83	5.47
6	5.36	65.64	6.10
	---------- 2nd parity ----------		
	ns	ns	ns
1	2.71	54.17	4.75
2	2.63	53.48	4.73
3	2.35	52.40	4.04
4	2.78	55.10	4.75
5	2.95	52.64	4.45
6	4.04	56.91	4.92
	---------- 3rd parity ----------		
	ns	ns	ns
1	4.33	59.96	5.21
2	4.97	63.67	5.34
3	4.11	59.93	4.81
4	3.58	57.52	4.50
5	3.23	60.35	4.19
6	3.27	54.19	4.47

UBF: ultrasound backfat thickness, UEMA : ultrasound eye muscle area, UMS : ultrasound marbling score
[1]Group : age groups of days postpartum
* $p<0.05$, ** $p<0.01$

Reference

Choi, S. B., Y. H. Choy, J. W. Lee, K. S. Baek, Y. K. Kim, S. K. Son and N. S. Kim. Body condition score of Hanwoo cows and reproductive performances. 2004. J. Anim. Sci & Technol. (Kor.) 46(1):31~38

Ha, J. J., J. B. Kim, C. W. Lee and Y. H. Song. 2009. Studies on live-carcass trait of parity and region in Hanwoo cows measured by real-time ultrasound. J. Anim. Sci. & Technol(Kor.) 51:3255~262.

Lee, C. W., J. G. Choi, K. J. Jeon, K. J. Na, C. Lee, B. K. Yang, and J. B. Kim. 2003a. Estimation of growth curve for evaluation of growth characteristics for Hanwoo cows. J. Anim. Sci. Technol. (Kor.) 45:509-516.

Lee, C. W., J. G. Choi, G. J. Jeon, K. J. Na, C. Lee, J. M. Hwang, B. W. Kim, and J. B. Kim. 2003b. Estimation of parameters for individual growth curves of cows in *Bos taurus Coreanae*. J. Anim. Sci. Technol. (Kor.) 45:689-694.

Lee, D. H., V. Choudhary, G. H. Lee. 2006. Genetic parameter estimates for ultrasonic meat qualities in Hanwoo cows. Aisa-Aus. J. Anim. Sci. 19(4): 468~474

Lee, J. H., S. H. Oh, Y. M. Lee, Y. S. Kim, H. J. Son, D. J. Jeong, N. C. Whitley, and J. J. Kim. 2014. Study on growth curves of longissimus dorsi muscle area, backfat thickness and body conformation for Hanwoo (Korea Native) cows. Asian-Aust. J. Anim. Sci. 27(9): 1250-1253.

SAS. 2002. SAS/STAT User's Guide. V. 9.1 SAS Institute Inc., Cary, NC, USA

PO-06-32 Estimation of genetic parameters for milk production and lactation length for the Murrah buffalo in Thailand

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Objective

The world population of domestic buffaloes, *Bubalus bubalis,* is actually about 168 million head: 161 million can be found in Asia (95.83%); 3.71 million are in Africa and 3.3 million in South America (Borghese and Mazzi, 2005). Two subspecies of domestic buffalo are riverine and swamp types; river buffalo has 50 chromosomes and swamp buffalo has 48 chromosomes (Pasha and Hayat, 2012). The swamp type is characterized by slow growth and low milk production but river buffalo, especially Murrah breed is the finest genetic of milk production.
In a breeding improvement program, knowing the heritability, phenotypic and genetic correlation of the economic traits that are used as selection planning is significant. The objective of the present study was to estimate heritability and correlation on total milk yield, lactation length, milk per day and milk peak yield in Murrah and crossbred buffaloes that can be used for breeding selection to improve the dairy buffalo population in Thailand.

Methodology

Data on 314 records of Murrah (292) and crossbred (22) buffaloes, collected from 2001 – 2013 by Murrah farm is located in Chachoengsoa province, Thailand, which is the only one intensive buffalo dairy farm from Murrah and crossbred buffaloes were included only if the buffaloes produce milk for minimum of 90 days. Data were used to estimated variance and covariance component by Restricted Maximum Likelihood (REML). Heritability, phenotypic, and genetic correlation were estimated from the component of variance and covariance for total milk yield, lactation length, milk per day and milk peak yield. All traits were analyzed using the same animal model.

Y = Vector of observations for each trait
X = The matrix that associates b with y
b = Vector for fixed effects of herd – year – season,
Z = The matrix that associates a with y
a = Vector for direct additive genetic effect
e = Vector of residual error term not explained by other parts of the model

Results and Conclusion

The overall means of total milk yield, lactation length, milk per day, and milk peak yield were 1024 ± 563.59, 603.43 ± 282.49 kg, 247.45 ± 110.42, 212.09 ± 85.06 day, 4.02 ± 1.22, 2.86 ± 0.70 kg, and 8.08 ± 2.47, 5.53 ± 1.36 kg, respectively, in Murrah and crossbred buffaloes (Table 1).
Estimated of heritability for total milk yield, lactation length, milk per day, and milk peak yield are shown in Table 2. Heritability of total milk yield seem to be lower than estimate obtained by Tonhati et al. (2000); Ibrahim et al. (2012), which were 0.38, 0.40 respectively. Heritability of lactation length to be lower than estimated by Malhado et al. (2013); Ibrahim et al. (2012) which were 0.15, 0.30 respectively. Rosati and Van Vleck (2002) found heritability was low by buffaloes have not been intensive selected in the past, and the model can only partially account for management variability. The variation in milk production and other traits link to milk producing can be assigned mainly to environmental effects (non-genetic effect) causing low heritability estimated.
Heritability of milk peak yield was medium (0.373). A similar estimate of heritability (0.48) was reported by Pareek and Narang (2014). Which represented that 37.3% of variation in milk peak yield was caused by genetic. Therefore, emphasis should be given to selection plan and breeding program for improvement of this trait.
Phenotypic correlations between total milk yield and lactation length, milk per day, and milk peak yield were 0.917, 0.702, and 0.685 while the genetic correlations were 0.990, 0.996, and 0.997, respectively (Table 2). Estimates of phenotypic and genetic correlation between traits are similar can be found in the dairy cattle reported. The phenotypic and genetic correlation between the trait total milk yield and other traits were high and positive. Therefore, a selection purpose for milk production can be reached by increasing of lactation length, milk per day,

and milk peak yield. Malhado et al. (2009) and Barros et al. (2014) report a similar result.

Table 1 The means of total milk yield, lactation length, milk per day, and milk peak in buffaloes

Traits	Means ± SE	
	Murrah	Crossbred
Total milk yield (kg)	1024.26±563.59	603.43±282.49
Lactation length (day)	247.45±110.42	212.09±85.06
Milk per day (kg)	4.02±1.22	2.86±0.70
Milk peak yield (kg)	8.08±2.47	5.53±1.36

Table 2 Estimates of heritability (diagonal), phenotypic correlation (above diagonal), and genetic correlation among traits

	TMY	LL	AVM	Peak
TMY	**0.111**	0.917	0.702	0.685
LL	0.990	**0.015**	0.467	0.519
AVM	0.996	0.998	**0.467**	0.818
Peak	0.995	0.997	0.995	**0.373**

References

Barros, C.C., D.P. Oliveira, N.A. Hurtdo-Lugo, R.R. Aspilcueta-Borquis and H. Tonhati. 2014. Estimates of genetic parameters for economic traits in dairy buffalo. Proceeding, 10th Congress of genetic applied to livestock production.

Borghese, A. and M. Mazzi. 2005. Buffalo population and strategies in the world. Buffalo. production and research. REU Technical series 67. Food and Agriculture Organization of the United Nations, Rome.

Ibrahim, M.A. A.S. Khattab, Set El-Habaeib, S. Awad, J. Tozser. 2012. Genetic parameters for buffalo milk yield and milk quality traits using animal model. Animal Welfare, Ethology and Housing Systems. 8(2): 175-182.

Malhado, C.H.M., A.A. Ramos, P.L.S. Carneiro, D.M.M.R. Azevedo, P.R.A.M. Affonso, D.G. Pereira and J.C. Souza. 2009. Genetic parameters of reproductive and productive traits in cross-breed water buffaloes in Brazil. Rev. Bras. Saude Prod. An. 10: 830-839.

Malhado, C.H.M., A.C.M. Malhado, A.de A. Ramos, P.L. Carneir, J.C. de Souza and A. Pala. 2013. Genetic parameters for milk yield, lactation length and calving intervals of Murrah buffaloes from Brazil. R. Bras. Zootec. 42(8).

Pareek, N.K. and R. Nagang. 2014. Genetic analysis of first lactation persistency and milk production traits in graded Murrah buffaloes. Buffalo Bulletin. 33(4): 432-436.

Pasha, T.N. and Z. Hayat. 2012. Present situation and future perspective of buffalo production in Asia. J. Anim Plant Sci. 22(Sup 3): 250-256.

Rosati, A. and L.D. Van Vleck. 2002. Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo *Bubalus bubalis* population. Livest. Prod. Sci. 74: 185-190.

Tonhati, H., F.B. Vasconcellos and L.G. Albuquerque. 2000. Genetic aspects of productive and reproductive traits in a Murrah buffalo herd in São Paulo, Brazil. Journal of Animal Breeding Genetics 117:331-336.

PO-06-33 Effects of Metabolizable Energy and Protein Levels on Egg Production Performences and Egg Quality of Laying Hens

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ABSTRACT

A 3x2 factorial experiment with 3 dietary ME levels (2,600; 2,700and 2,800 kcal/kg) and 2CP levels (16.5 and 17.5%) was conducted to evaluate the effect of ME and CP on egg production performances and egg quality of laying hens as rearing in tropical climates. A total of 360 commercial Isa Brown laying hens, 24 wk of age, were randomly assigned into 6 treatments (5 replicates with 12 birds per replicate). Feed and water were provided *ad libitum* throughout the experiment (12 wk). There was an interaction between ME and CP on egg production performances (EP), egg mass (EM) and feed conversion ratio (FRC), ($P<0.05$). The highest EP and EM were observed with 16.5% and 2,700 kcal ME/kg in the diet. Diets with high CP (17.5%) and low energy (2,600 kcalME/kg) showed the poorest EP, EM, eggshell guality (ESQ). From the results of this experiment, it could be conclusion that moderate ME (2,700 kcalME/kg) and moderate balance protein level (16.5%CP) was the optimum levels for EP, EM and FRC of brown laying hens as rearing in tropical climates.

INTRODUCTION

Dietary ME and CP are the major nutritional parameters of the diets of laying hens. There are very limited studies, however, on the optimum combination of ME and balanced protein as rearing in tropical climates. Higher environmental temperatures and humidity are known to reduce feed intake (FI),body weight (BW), EP, egg weight (EW), EM and ESQ (Mashaly et al, 2004).Therefore, it is more important for the present study to evaluate the effect of metabolizable energy and protein levels on egg production performances and egg quality of laying hens as rearing in tropical climates.

MATERIALS AND METHODS

All procedures used in this study were approved by the Animal Care Committee of Rajamagala University of Technology Srivijaya. Six diets were formulated in 3x2 factorial design with 3 levels of ME (2,600; 2,700 and 2,800 kcal/kg) and 2 levels of protein levels of protein levels (16.5 and 17.5%). The ideal digestible amino acid pattern, calcium, non-phytate phosphorus were suggested by NRC (1994). A total of 360 commercial Isa Brown laying hens, 24 wk of age, were randomly assigned into 6 treatments (5 replicates with 12 birds per replicate). Birds reared in open-side house and lighting (16h/d) throughout the experimental periods from 24 to 36 wk. All hens were supplied with feed and water *ad libitum.* Eggproduction (EP), egg weight (EW), and cracked egg were recorded daily. The mortality was determined daily and the feed intake was adjusted accordingly, feed intake was determined weekly by substracting the ending feed weight of each trough (each replicate) from the beginning feed weight. Egg mass (EM) was then calculated as: EM=EWxEP.Body weigh was obtained by weighing 9 hens per replicate at the end of the experiment. The FCR was expressed as the ratio of FI to EM. Mortality data was transformed into arc sine before analysis. Data were analyzed by the general linear modle procedures as suggested dyStell and Torrie (1997) as 3x2 factorial arrangement with CP and ME as the main effects. One-way ANOVA was also used to analyze the difference among all treatments. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was used to separate means. All statements of significance are based on $P<0.05$.

RESULTS AND DISCUSSION

The results of the experiment for egg production performances and egg quality was shown in Table1. The EP, EM and FCR were affected by the ME and CP levels as well as the ME x CP interaction ($P<0.05$). At low CP levels (16.5%) and increased ME from 2,600 to 2,800 kcal/kg improved EP and EM but decreased FCR ($P<0.05$).The FI and FCR decreased withincreasing ME from 2,600 to 2,800 kcal/kg ($P<0.05$). The ME had no effect on EW ($P>0.05$) while EW increased with CP level increased from 16.5 to 17.5% ($P<0.05$) It was shown that the poorest egg production performance was observed with 17.5% CP and 2,600 kcalME/kg in the diet. This result were aggremented with

the reported of Wu et al., (2007) who described that layers as reared in the tropical climates may perform better when fed diet contained 2,700 kcalME/kg compared with feeding 2,350 kcalME/kg. It was may be dued to energy had effect on the accumulation of eggyolk in ovary and had effect on the often of evulation of laying hens. This resuts was similar with the report of khajali et al., (2007) who reported that layers can perform well on diets containing approximately 14 to 15% balanced protein compared with those fed a diet with 17% CP.

Table1. Composition and nutrient levels of the experimental diets

Item	Treatment					
	16.5%CP			17.5%CP		
	2,600kcal ME/Kg	2,700kcal ME/Kg	2,800kcal ME/Kg	2,600kcal ME/Kg	2,700kcal ME/Kg	2,800kcal ME/Kg
Ingreclient,%						
Corn	54.75	51.65	48.75	51.94	47.84	44.24
Rice bran	15	15	15	14	14	14
Soybean meal, (48%CP)	21.0	22.0	23.0	25.0	27.0	28.0
Limestone	7.80	7.80	7.70	7.70	7.70	7.70
Dicalcium phosphate(P_{24})	0.50	0.60	0.60	0.40	0.50	0.50
Palm oil	-	2.0	4.0	-	2.0	4.60
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
DL-methionine	0.10	0.10	0.10	0.11	0.11	0.11
Premix[1/]	0.50	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated nutrient Composition (%)						
CP	16.47	16.49	16.46	17.48	17.50	17.49
ME (kgcal/kg)	2,604	2,702	2,800	2,601	2,703	2,803
Ca	3.51	3.47	3.52	3.50	3.51	3.53
Non-phytate phosphorus	0.45	0.44	0.45	0.45	0.44	0.45
Digestible amino acids						
Lysine	0.66	0.67	0.66	0.72	0.71	0.72
Methionine	0.33	0.32	0.32	0.36	0.37	0.36
Threonine	0.46	0.47	0.47	0.50	0.51	0.51
Tryptophan	0.14	0.15	0.14	0.15	0.15	0.14

[1/]The premix provided the following (mg per kg of diet) thiamine, 1; pyridoxine, 2; cyanocobalamin,0.01; niacin, 15; panthothenic acid, 10; 2-tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2, retinol acetate, 2.75; cholesterol, 0.06; choline, 650; copper, 8; iron, 45; managanese, 80; zinc, 60; selenium, 0.18; hydrated sodium calclumaluminosilicates, 800.

Among the egg quality parameters in this experiment, only YC increased ($P<0.05$) with dietary ME levels. EST and YP decreased and AP increased with dietary CP levels ($P<0.05$). There was no significant interation between ME and CP on YP and YC ($P>0.05$). The YC increased and AP decreased with increased CP levels. This result was in agreement with previous study (Penz and Jensen; 1991). YC increased with ME from 2,600 to 2,800 kcal/kg. The YC is known to be controlled by xanthophyll pigments, including lutein and zeaxanthin (Lesson and Summers,2005), which are usually obtained from corn.

Table2 Dietary ME and CP levels on egg production performances and egg quality of layinghens as rearing in tropical climates.

Item		Parameters								
		EP(%)	EW(g)	EM(g/b)	FI(g/d)	FRC	EST (mm)	AP(%)	YP(%)	YC
Treatments										
16.5%CP	2,600ME kcal/kg	92.50^{ab}	58.81	54.52^{abc}	132.01^{a}	2.30^{ab}	0.65	65.24^{ab}	22.96	7.34^{ab}
	2,700ME kcal/kg	93.81^{ab}	59.13	53.47^{bc}	122.74^{ab}	2.29^{bc}	0.349	63.02^{bc}	24.74	7.8^{a}
	2,800ME kcal/kg	92.10^{ab}	59.43	52.68^{c}	120.65^{bc}	2.31^{ab}	0.351	63.11^{bc}	24.98	7.99^{a}
17.5%CP	2,600ME kcal/kg	71.41^{c}	58.60	54.54^{abc}	127.44^{ab}	2.95^{a}	0.344	65.01^{ab}	23.97	7.81^{a}
	2,700ME kcal/kg	90.02^{b}	59.04	55.61^{ab}	121.30^{bc}	2.29^{bc}	0.343	66.09^{a}	23.99	7.86^{a}
	2,800ME kcal/kg	93.91^{a}	59.02	55.10^{abc}	120.02^{bc}	2.29^{bc}	0.339	63.34	24.01	7.92^{a}
Pooled SE		6.12	1.01	6.40	3.90	0.21	0.031	1.72	1.34	0.91
Main effect										
16.5%CP		91.10^{A}	59.17	54.37^{A}	124.3	2.30^{B}	0.350	36.70^{B}	24.09^{A}	7.61
17.5%CP		88.04^{A}	59.41	51.22^{B}	123.20	2.49^{A}	0.347	65.01^{A}	23.62^{B}	7.64
2,600kcalME/kg		91.75^{X}	57.92	54.30^{y}	126.31^{X}	2.34^{y}	0.339	64.30	23.81	7.38^{xy}
2,700kcalME/kg		91.04^{X}	59.82	52.60^{x}	121.02^{y}	2.26^{z}	0.343	64.19	24.32	7.81^{x}
2,800kcalME/kg		91.12^{X}	59.06	53.80^{x}	120.01^{y}	2.24^{z}	0.345	63.82	24.11	7.83^{x}
ANOVA*(P-value)*										
Treatment		<0.054	NS	<0.05	<0.05	<0.052	NS	<0.05	<0.05	<0.05
CP		<0.051	NS	<0.051	NS	<0.05	NS	<0.05	<0.05	<0.05
ME		<0.05	NS	<0.052	<0.051	<0.05	NS	NS	NS	<0.05
CPxME		<0.01	NS	<0.05	NS	<0.05	NS	<.05	<0.05	NS

a-e Mean in a column having no common superscripts differ significantly (P<0.05)

A-C Mean in a column having no common superscripts differ significantly (P<0.05)

X-Z Mean in a column having no common superscripts differ significantly (P<0.05

CONCLUSIONS

Based on the data under this experimental condition. It was fount that the moderate metablelizable energy and crude protein levels at the levels of 2,700 kcalME/kg and 16.5%, respectively are the optimum levels for egg production performances and egg quality of laying hens as rearing in tropical climates.

Keywords :metabolizable energy, protein levels, brown laying hens, tropical climates

REFERENCES

Khajali, F., M. Faraji, and S.K. Dehkordi.2007. Effects of reducted-protein diets at constant total sulfur amino acid .lysine ratio on pullet development and subsequent laying hen. performance. Am. J. Anim Vet.Sci.2:89-92.

Lesson, S and J.D Summers. 2005. Commercial poultry Nutrition. Nothingham University Press, Nottingham, UK

Mashaly, M.M., G.L.Hendricks, M.A.Kalama, A. E. Gehad, A.O Abbas, and P.H. Patterson.2004. Effect of heat stress on production paameters and immune responses of commercial laying hens. Poult. Sci. 83:889-894

NRC 1994 Nutrient Reguirements of poultry. 9 threv.ed. Natural Academic Press, Washington, DC

Olomu, J.M., and S.A. Offiong.1983. The performance of brown egg-type layers fed different protein and energy levels in tropics. Poultry Sci. 62:345-352

Penz, A.M., and L.S. Jensen. 1991. Influence of protein concentration, amino acid supplementation, and daily time of access to high-or low-protein diets on egg weight and components in laying hens. Poult.Sci.70:2460-2466.

Rao, S.V.R., V. Ravindran, T. Srilatha, A.K. Panda and M.V.L.N. Raju. 2011. Effect of dietry concentrations of energy, crude protein, lysine, and methionine on the performance of white Leghorn layers in the tropics. J. Appl. Poolt. Res. 20:528-541.

Stell R.G.D.J.H. Torrie, and D.A. Dickey. 1997. Principles and procedures of Statistcs:A Biometrical Approach. 3rd.ed. McGraw-Hill Bock. Co., New York.

Wu,G., M.M Bryant, P. Gunawardna, and D.A. Roland. 2007. Effect of nutrient density on performance, egg cemponents, egg solids, egg quality, and profit in eight commercial Leghorn strains during phaseone. Poult. Sci. 86:6911-697.

PO-06-34 Effect of enzyme supplementation from tomato pomace by Aspergillus niger on live performance of broiler chickens

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Introduction

Broiler feed ingredients are based on corn and soybean meal. When birds are fed those feedstuff which contain high levels of non-starch polysaccharides, digesta viscosity increase and nutrient digestibility reduce. That's why poultry industries have been using enzymes to improve nutrient digestibility and increase the energy value of diets. The feed enzyme is beneficial reducing the excretion of those nutrients to the environment (Avila et al., 2010). Currently, solid state bioprocessing is a large interest in the production of enzyme such as cellulase, amylase, pectinase, protease, and lipase. Solid state fermentation is one of the simple and inexpensive process to produce enzyme in which microbial growth and product formation occur on the surfaces of solid substrates. Various agricultural products are often used as the traditional solid state substrates. One of them is tomato pomace which is a left over product from the tomato processing plant in Thailand. Tomato pomace contains various mineral and trace elements which needed for microorganism development. The objective of this study was to evaluate live performance, carcass and parts yields and blood parameters of broilers fed enzyme supplementation from tomato pomace by *Aspergillus niger* at 49 d of age.

Materials and Methods

A total of 264 mixed-sex broilers were raised in 24 pens to 49 d of age (4 pens/diet; 11 birds/pen using a two-stage feeding program. Birds were fed *ad libitum* with six dietary treatments from tomato pomace treated by *Aspergillus niger* (TPT): 1) Basal diet (control); 2) 0.1% TPT; 3) 0.2% TPT; 4) 0.3% TPT; 5) 0.4% TPT, and 6) 0.5% TPT. Xylanase and cellulase from TPT contains 490,011 and 29,413 unit/g dried weight, respectively. All birds were weighed on a per pen basis at 14, 21 and 49 d of age and body weights (BW), average dairy gain (ADG), adjusted feed conversion ratio (FCR) and mortality were determined. At 49 d of age, five birds per pen were processed to evaluate the effect of treatments on carcass quality and yield parameters. Carcasses were chilled for 4 h in static slush-ice. Whole carcass, abdominal fat, parts (wings, drumsticks, thighs) and deboned breast (fillets and tenders) weights and yields were determined. Blood samples (5 birds/pen) were analyzed for cholesterol, triglyceride, HDL and LDL. The data were analyzed by the GLM procedure of SAS program. The Tukey's test was used to compare and separate means when main effects were significant ($P<0.05$).

Results

No differences in live performance (BW, ADG and FCR) were detected ($P>0.05$) due to dietary treatments in this study (Table 1). Whole carcass, abdominal fat and parts (wings, leg quarters, drumsticks, thighs, breast fillets and tenders) weights and yields did not differ ($P>0.05$) because of dietary treatments (Table 2). However, abdominal fat yield was reduced ($P<0.05$) when birds fed 0.1% TPT enzyme dietary treatment in the study. Cholesterol, triglyceride, HDL and LDL in serum of broiler chickens were not influenced by the enzyme dietary treatments at 49 d of age (Table 3). However, there was a numerical trend of improvement in cholesterol, triglyceride, and HDL with enzyme supplementation from tomato pomace by *Aspergillus niger*. Birds fed 0.1% TPT tended to have lower cholesterol and triglyceride concentration compared to the control diet.

Conclusions

Adding 0.1% of TPT tended to increase HDL and reduce cholesterol and triglyceride. The improvement is achieved through the lowest inclusion level of enzyme on abdominal fat deposition and blood parameters.

Table 1 Effect of enzyme supplementation from tomato pomace by *Aspergillus niger* on live performance of broiler chickens at 49 d of age.

Treatments	Initial	1-21 d			22-49 d			1-49 d		
	BW[1]	BW	ADG	FCR	BW	ADG	FCR	BW	ADG	FCR
	NS[2]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control	165	929	44	1.89	1420	51	2.10	2349	84	1.70
0.1% TPT	165	956	46	1.74	1469	52	1.94	2425	87	1.71
0.2% TPT	163	1001	48	1.69	1331	48	2.06	2332	83	1.71
0.3% TPT	164	927	44	1.68	1254	45	2.02	2181	78	1.73
0.4% TPT	164	996	48	1.67	988	35	2.40	1985	70	1.73
0.5% TPT	165	946	45	1.63	1372	49	1.71	2318	82	1.73
SEM[3]	7.57	60.07	2.84	0.11	121.45	4.34	0.19	124.35	4.44	0.01

[1]BW = Body weight (g), ADG = Average daily gain (g/d), ADFI = Average daily feed intake (g/bird/d), FCR = Feed conversion ratio (adjusted for mortality), [2]NS = Not significant (P>0.05), [3]SEM = Standard error of the mean

Table 2 Effect of enzyme supplementation from tomato pomace by *Aspergillus niger* on carcass weight and yield of broiler chickens at 49 d of age.

Factors	Control	0.1% TPT	0.2% TPT	0.3% TPT	0.4% TPT	0.5% TPT	SEM[3]
Pre slaughter weight (g)	2,349	2,425	2,333	2,181	1,985	2,319	105.32
Chilled carcass							
Weight (g)	1,789	1,842	1,759	1,625	1,648	1,793	107.36
Yield (%)[1]	76.6	76.1	75.9	74.7	83.7	77.6	4.28
Abdominal Fat							
Weight (g)	31	30	36	41	44	35	5.07
Yield (%)	1.3[ab]	1.2[b]	1.6[ab]	1.9[ab]	2.2[a]	1.5[ab]	0.20
Wings							
Weight (g)	168	181	180	159	161	173	10.61
Yield (%)	7.2	7.5	7.8	7.3	8.2	7.5	0.45
Drumsticks							
Weight (g)	222	228	206	204	212	207	15.73
Yield (%)	9.5	9.4	8.9	9.4	10.7	9.0	0.62
Thighs							
Weight (g)	272	295	245	224	232	278	20.9
Yield (%)	11.6	12.1	10.5	10.3	11.7	12.1	0.80
Fillets							
Weight (g)	324	326	323	299	282	291	17.85
Yield (%)	13.9	13.5	13.9	13.7	14.5	12.6	0.80
Tenders							
Weight (g)	83	83	85	80	71	75	4.24
Yield (%)	3.6	3.4	3.6	3.7	3.7	3.2	0.19

[1]As percent of pre-slaughter weight, [2]Not significant (P>0.05), [3]SEM = Standard Error of the Mean, [abc]Means within a row with difference superscripts differ significantly (P<0.05).

Table 3 Effect of enzyme supplementation from tomato pomace by *Aspergillus niger* on cholesterol, triglyceride, HDL and LDL of broiler chickens at 49 d of age.

Treatments	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	NS[1]	NS	NS	-
Control	123.50	135.13	44.25	<30
0.1% TPT	112.38	92.50	54.63	<30
0.2% TPT	117.38	117.25	50.75	<30
0.3% TPT	116.25	121.75	51.88	<30
0.4% TPT	121.88	124.50	46.25	<30
0.5% TPT	119.63	124.00	47.50	<30
SEM[2]	5.01	23.53	6.74	-

[1]Not significant (P>0.05), [2]SEM = Standard Error of the Mean.

References

Berovič, M., and H. Ostroveršnik. 1997. Production of *Aspergillus niger* pectolytic enzymes by solid state bioprocessing of apple pomace. J. Biotechnol. 53(1): 47-53.

Douglas, M. W., C. M. Parsons, and M. R. Bedford. 2000. Effect of various soybean meal sources and Avizyme on chick growth performance and ileal digestible energy. J. Applied Poult. Res. 9: 74-80.

Sornlake, W., P., Matetaviparee, N. Rattanaphan, S. Tanapongpipat, and L. Eurwilaichitr. 2013. β-Mannanase production by *Aspergillus niger* BCC4525 and its efficacy on broiler performance. J. Sci. Food Agric. 93(13): 3345-51.

PO-06-38

Lipoteichoic acid and muramyl dipeptide do not affect food and water intake in chicks

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Objective

Bacterial infections are associated with non-specific symptoms including fever, somnolence, anorexia and adipsia in animals (Kent et al., 1992; Kanra et al., 2006). These symptoms are triggered by bacterial components such as lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria (Kanra et al., 2006). In addition, other bacterial components are also thought to be related to the symptoms induced by bacterial infections in mammals. For example, muramyl dipeptide (MDP), a component of cell walls of both Gram-negative and Gram-positive bacteria has been demonstrated to inhibit feeding behavior and gastric emptying in rodents (Langhans et al., 1990; Plata-Salamán, 1999).

Similar to mammals, several studies revealed that LPS induces hyperthermia, anorexia, anti-dipsogenic effect, somnolent activity and retardation of crop emptying in chickens when administered centrally or peripherally (Johnson et al., 1993a;1993b; Cheng et al., 2004; Tachibana et al., 2016), as has reported in rodents. This suggests that other bacterial components may also affect feeding behavior in chickens although there is paucity of information about the effect of other bacterial components including MDP. The effect of lipoteichoic acid (LTA), which is a component of Gram-positive bacteria, also has not been investigated yet in chickens.

Thus, the purpose of the present study was to investigate whether intraperitoneal (IP) and intracerebroventricular (ICV) injection of LTA and MDP affects feeding and drinking behavior in chicks. The effect of LPS was also determined as the positive control.

Methodology

Day-old male layer chicks (*Gallus gallus*, Julia) were purchased from a local hatchery (Nihon Layer, Gifu, Japan) and reared in a room kept at 30°C with continuous lighting. A commercial diet (crude protein: 24%, metabolizable energy: 3050 kcal/kg, Toyohashi Feed Mills Co. Ltd, Aichi, Japan) and water were available ad libitum to the chicks. Chicks were transferred to their individual cages 1 day before each experiment. They were weighed and distributed into experimental groups so that the average body weight was as uniform as possible between treatment groups. This study was approved by the Committee of Animal Care and Use in Ehime University (No. 08-o3-10).

All injections were made between 0600 and 0800. LPS (*Escherichia coli*, O-127:H6, Wako Pure Chemical Industries, Osaka, Japan), MDP (Bachem, Bubendorf, Switzerland) and LTA (Sigma-Aldrich Co. LLC, MO, USA) were dissolved in filtered phosphate buffered saline (PBS, pH 7.4) for the IP injection. The vehicle alone was used for the control treatment. These solutions were injected via the IP route at a volume of 0.2 ml per chick. In ICV injection study, these compounds were dissolved in PBS containing 0.1% Evans Blue dye and this vehicle alone was used for the control treatment. The ICV injections were performed according to a previously reported method (Davis et al., 1979). Briefly, the head of the chick was inserted into an acrylic box which had a hole at the top plate. The injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 3 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. The solution was injected through the hole using a micro-syringe at a volume of 10 µl. The injection procedure is rapid and does not result in additional stress to neonatal chicks judging from food intake and corticosterone release data (Furuse et al., 1999; Saito et al., 20005). At the end of each experiment, the chicks were euthanized with an overdose of pentobarbital. The brain was then removed to confirm the accuracy of injection. Any chicks that did not show the presence of Evans Blue dye in the lateral ventricle were not used for analyses.

IP injection study, 6-day-old male layer chicks were injected with 0 (vehicle only) or 200 µg (4 mg/kg body weight) LTA, MDP or LPS under an ad libitum feeding condition. Then pre-weighed feeder was given to the chicks and then the food intake was measured at 1, 3, 6 and 24 h after the injection. Water intake was also measured

at 3 and 24 h after the injection with correction of the evaporation. Food and water intake were measured at an accuracy of 0.001 g with a digital balance.

In an ICV injection study, 5- or 6-day-old chicks were injected with 0 (vehicle only) or 5 μg LTA, MDP or LPS under an ad libitum feeding condition. Then food and water intake were measured as noted above.

Data were statistically analyzed with two-way repeated measures analysis of variance with respect to each bacterial components and time. When a significant interaction was observed, t-test was performed in order to compere between groups at each time. Data are expressed as means ± SEM, and statistical significance was set at P.

Results

Figure 1 shows the effect of IP injection of LPS, MDP and LTA on food intake in chicks. IP injection of MDP had no effect on food intake [$F(1,18)=0.05$, $P=0.83$] and no significant interaction between MDP and time [$F(3,54)=0.05$, $P=0.99$] was observed. LTA also did not affect food intake [$F(1,18)=1.11$, $P=0.31$] and there was no significant interaction [$F(3,54)=0.52$, $P=0.67$]. On the other hand, the injection of LPS significantly decreased food intake [$F(1,18)=34.30$, $P<0.01$] at all times determined (Fig. 1).

Figure 1 also shows the effect of IP injection of LPS, MDP and LTA on water intake in chicks. Neither IP injection of MDP [$F(1,18)=1.68$, $P=0.83$] nor LTA [$F(1,18)=0.05$, $P=0.83$] affected water intake in chicks while LPS significantly decreased it [$F(1,18)=37.98$, $P<0.01$].

The anorexigenic and anti-dipsogenic effect of LPS was similar to previous studies (Johnson et al., 1993a;1993b; Cheng et al., 2004), suggesting that our experimental procedures were suitable to examine the change in food and water intake. Nevertheless, the injection of MDP and LTA did not affect feeding and drinking behavior. In rats, IP injection of MDP (1.6 mg/kg body weight) has been demonstrated to suppress feeding behavior (Langhans et al., 1990) while the injection of 200 μg (4 mg/kg body weight) MDP had no effect in the present study. Our preliminary experiment also showed that IP injection of 2 mg/kg body weight MDP and LTA did not affect food and water intake in chicks (data not shown), suggesting that these bacterial components in the peripheral tissues would not affect feeding and drinking behavior in chicks.

In the subsequent experiment, the ICV injection of bacterial components were examined in order to clarify whether bacterial components directly affect the brain and modified feeding and drinking behavior. Figure 2 shows the ICV injection of LPS, MDP and LTA on food intake in chicks. The ICV injection of MDP [$F(1,17)=0.01$, $P=0.93$] and LTA [$F(1,18)=1.17$, $P=0.29$] did not affect food intake. The interactions between MDP and time [$F(3,51)=0.30$, $P=0.82$] or LTA and time [$F(3,54)=0.65$, $P=0.58$] were not also significant. The effect of LPS was not also significant [$F(1,15)=3.78$, $P=0.07$] but the significant interaction between LPS and time [$F(3,45)=4.42$, $P<0.01$] was observed. Chicks treated with LPS significantly ate more diet than the control at 1 h after the injection, but the food intake in LPS group became decreasing at 6 h and reached to significance at 24 h.

The effect of ICV injection of LPS, MDP and LTA on water intake is shown in Fig. 2. Water intake was not affected by the injection of MDP [$F(1,17)=1.14$, $P=0.30$] and LTA [$F(1,18)=1.01$, $P=0.61$]. Although the effect of LPS was not also significant [$F(1,15)=3.35$, $P=0.09$], the interaction between LPS and time was significant [$F(3,45)=6.49$, $P<0.05$]. Water intake of chicks treated with LPS tended to be more than that of the control group at 3 h after the injection, but it significantly decreased at 24 h.

Similar to IP injection, the ICV injection of MDP and LTA did not affect food and water intake. In mammals, ICV injection of MDP has been demonstrated to inhibit feeding behavior (Plata-Salamán, 1999). To our knowledge, on the other hand, the effect of LTA on feeding drinking behavior has not been demonstrated yet in both mammals. Anyway, it is possible that MDP and LTA did not directly affect brain in order to affect feeding and drinking behavior in chicks. ICV injection of LPS significantly decreased food intake at 24 h after the injection as reported previously (Johnson et al., 1993a;1993b), but it significantly increased food intake at 1 h. Water intake was also tended to be increased by the injection of LPS in the present study. Why ICV-injected LPS showed biphasic effect on food and water intake was still unknown.

Thus the present study revealed that MDP and LTA did not affect feeding and drinking behavior in chicks after central and peripheral injection. These results demonstrated that LTA and MDP are not related to the suppression of feeding and drinking behavior under infection of Gram-positive bacteria in chicks. It has been reported that the feeding regulatory mechanism of chicks is different from those of young chickens. For example, ICV injection of mammalian leptin, a hormone released from the adipose tissue, suppresses food intake in young chickens (Denbow et al., 2000) while it had no effect on neonatal chicks (Bungo et al., 1999). It is therefore possible that the effect of MDP and LTA might be able to be observed in young chickens. Alternatively, some previous studies revealed that

domestic fowls are relatively resistant to the effects of LPS comparing with mammals (Adler and DaMassa, 1979). Similarly, chicks might possess resistant to the effect of MDP and LTA, and thereby these bacterial components did not affect feeding and drinking behavior of chicks in the present study. Studies using more doses of MDP and LTA would clarify whether the doses used in this study were effective or not in chicks.

Conclusion

IP and ICV injection of LTA and MDP had no effect on feeding and water intake in chicks. Other bacterial components would be related to the inhibition of feeding and drinking behavior under the infection of Gram-positive bacteria.

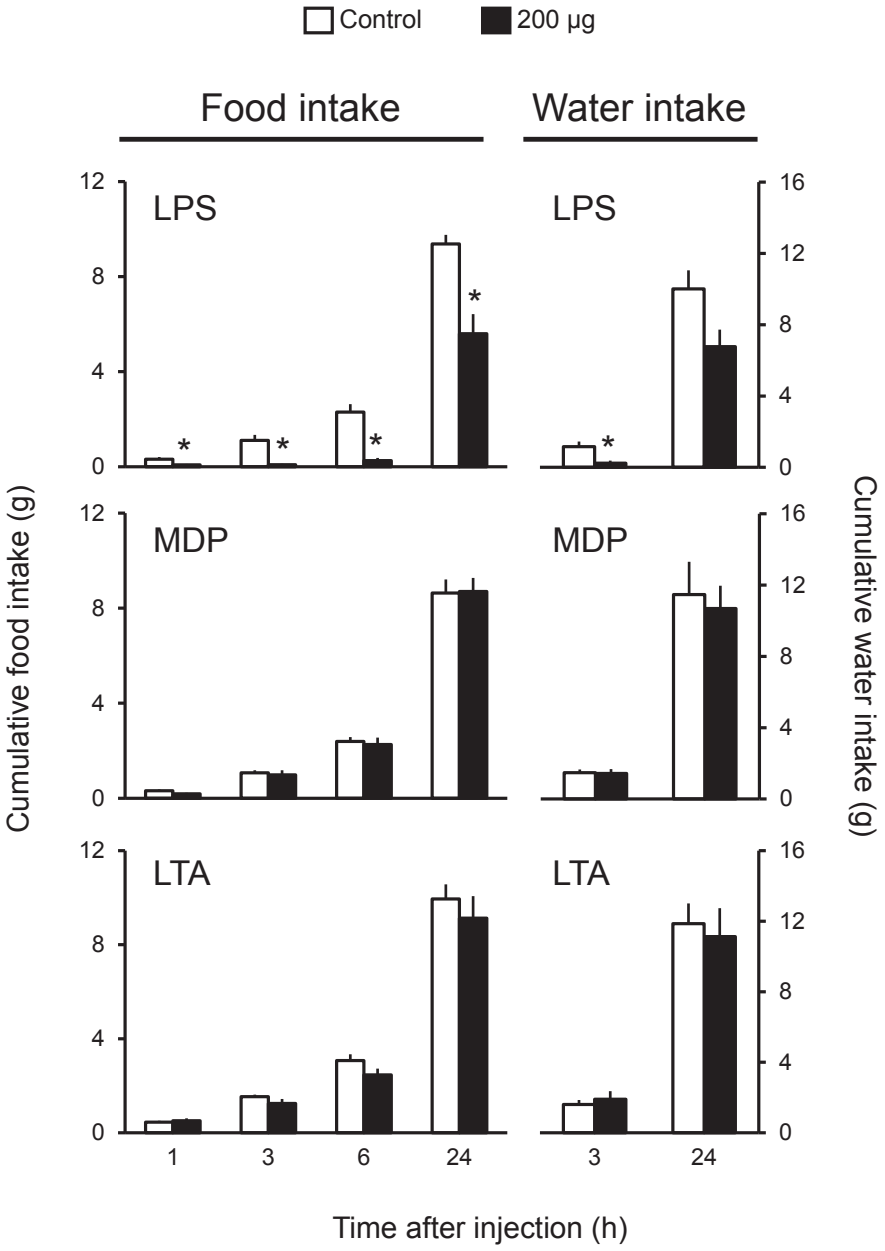

Figure 1. Effect of intraperitoneal (IP) injection of lipopolysaccharide (LPS), muramyl dipeptide (MDP) and lipoteichoic acid (LTA) on food and water intake in chicks. Data are expressed as mean with SEM. The number of chicks in each group was 10 in each study. *Significantly different from the control at each time ($P<0.05$).

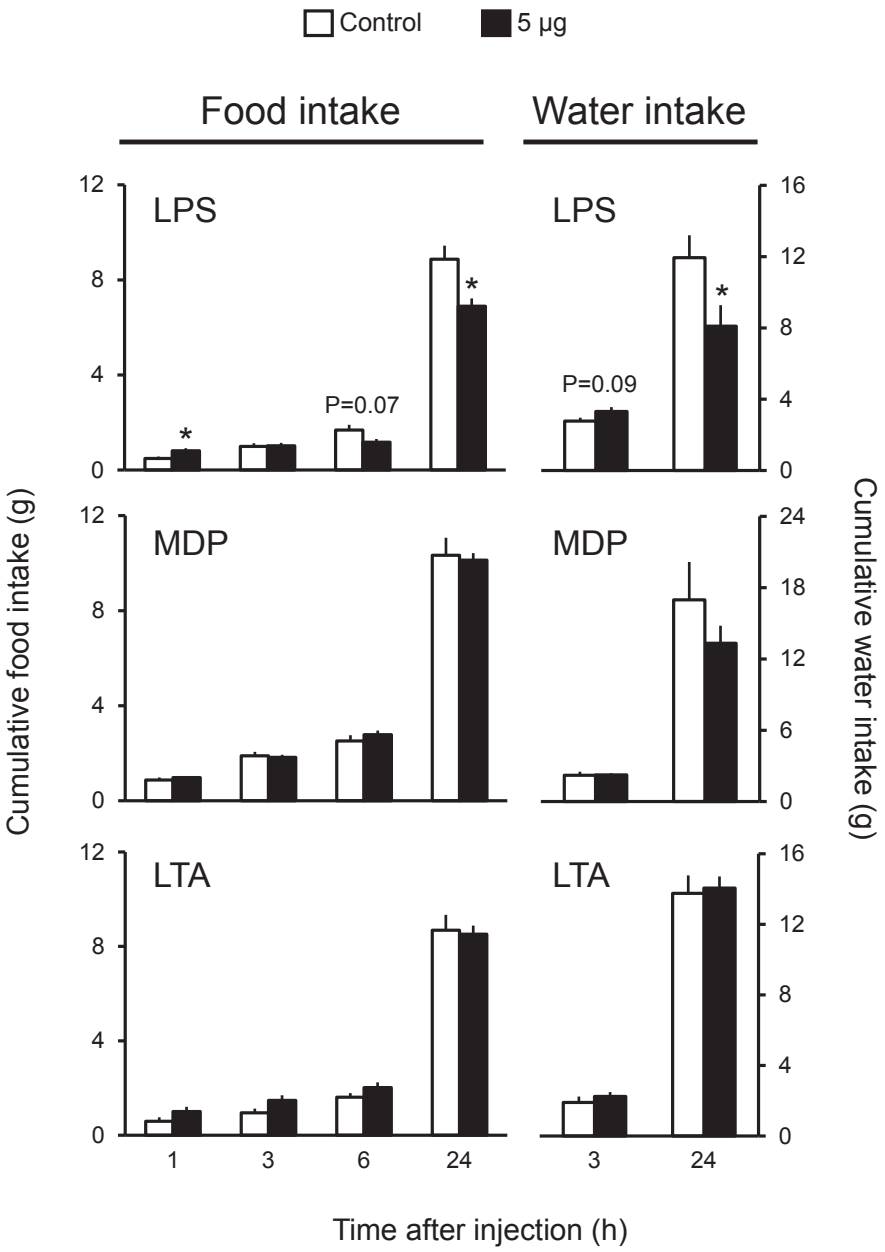

Figure 2. Effect of intracerebroventricular (ICV) injection of lipopolysaccharide (LPS), muramyl dipeptide (MDP) and lipoteichoic acid (LTA) on food and water intake in chicks. Data are expressed as mean with SEM. The number of chicks in the control and 5 μg groups were 9 and 8 for LPS study, 9 and 10 for MDP study, and 9 and 11, respectively. *Significantly different from the control at each time ($P<0.05$).

References

Adler HE, DaMassa AJ. (1979) Toxicity of endotoxin to chicks. Avian diseases, 23: 174–178.

Bungo T, Shimojo M, Masuda Y, Tachibana T, Tanaka S, Sugahara K, Furuse M. (1999) Intracerebroventricular administration of mouse leptin does not reduce food intake in the chicken. Brain Research, 817: 196–198.

Cheng HW, Freire R, Pajor EA. (2004) Endotoxin stress responses in chickens from different genetic lines. 1. Sickness, behavioral, and physical responses. Poultry Science, 83: 707–715.

Davis JL, Masuoka DT, Gerbrandt LK, Cherkin A. (1979) Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol Behav. 22: 693–695.

Denbow DM, Meade S, Robertson A, McMurtry JP, Richards M, Ashwell C. (2000) Leptin-induced decrease in food intake in chickens. Physiology & Behavior, 69: 359–362.

Furuse M, Ando R, Bungo T, Ao R, Shimojo M, Masuda Y. (1999) Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. British Poultry Science, 40: 698–700.

Johnson RW, Curtis SE, Dantzer R, Bahr JM, Kelley KW. (1993a) Sickness behavior in birds caused by peripheral or central injection of endotoxin. Physiology and Behavior, 53: 343–348.

Johnson RW, Curtis SE, Dantzer R, Kelley KW. (1993b) Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiology and Behavior, 53: 127–131.

Kanra GY, Ozen H, Kara A. (2006) Infection and anorexia. Turkish Journal of Pediatrics, 48: 279–287.

Kent S, Bluthé RM, Kelley KW, Dantzer R. (1992) Sickness behavior as a new target for drug development. Trends in Pharmacological Sciences, 13: 24–28.

Langhans W, Harlacher R, Balkowski G, Scharrer E. (1990) Comparison of the effects of bacterial lipopolysaccharide and muramyl dipeptide on food intake. Physiology & Behavior, 47; 805–813.

Plata-Salamán CR. (1999) Brain mechanisms in cytokine-induced anorexia. Psychoneuroendocrinology, 24: 25–41.

Saito ES, Kaiya H, Tachibana T, Tomonaga S, Denbow DM, Kangawa K, Furuse M. (2005) Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. Regulatory Peptides, 125: 201–208.

Tachibana T, Ogino M, Makino R, Khan MSI, Cline MA. (2016) Lipopolysaccharide reduces food passage rate from the crop by a prostaglandin-independent mechanism in chicks. British Poultry Science, in press.

PO-06-39 Effects of nanosize zinc oxide and γ -polyglutamic acid on zinc retention and egg shell quality of aged laying hens

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Abstract

The aim of this study was to investigate the effects of dietary supplementation of nanosize zinc oxide and γ-polyglutamic acid (γ-PGA) on zinc retention, egg production and eggshell quality of aged layers. In trial 1, twenty brown layers (Hendrix) (64wks-old) were assigned to the control, ZnO, organic-Zn (Zn-methionine), nano-Zn (nanosize ZnO, nanoZnO) and γ-PGA + nanosize ZnO (γ-PGA-nanoZnO) groups. The zinc was maintained at 80 mg/kg level in the Zn-added group diet, while the control was at 40 mg/kg level to evaluate the nutrient retention. In trial 2, seventy-five brown layers (Hendrix) (64 wks-old) were randomly allotted to five dietary treatments (as trial 1) to evaluate egg production and eggshell quality. The results of trial 1 indicated that zinc retention in organic Zn and γ-PGA-nanoZnO groups were the highest, follow by nanoZnO, the control and ZnO groups were the lowest ($P<0.05$). The results of trial 2 indicated that the average feed intake in nanoZnO group was the highest follow by γ-PGA-nanoZnO, organic Zn and ZnO groups, the control group was the lowest, there display difference among groups ($P<0.0001$). Compare to ZnO group, eggshell weight in nanoZnO group was increased ($P<0.05$). The eggshell thickness and zinc content in γ-PGA-nanoZnO groups were better than in ZnO group ($P<0.05$). In conclusion, γ-PGA-nanoZnO for dietary supplementation can increase zinc retention, as well as enhance eggshell thickness, zinc content and egg quality of aged layers.

Introduction

Zinc (Zn) bioavailability in monogastric animals is low (Brody, 1994). Although organic zinc can improve absorption, it still remains poor overall (Cao *et al.,* 2002). Zinc is an essential nutrient with a wide range of functions and is closely involved in a variety of enzymatic processes.

The development of nanotechnology has brought new trends to many fields. The surface area of nanosized minerals is much greater (about 1250 times) than that of macrosized minerals (Rajendran, 2013). Furthermore, nanoparticles show new characteristics of transport and uptake, and exhibit high absorption efficiency (Davda and Labhasetwar, 2002, Liao *et al.,* 2010). Some reports have indicated that nanosizing drugs and minerals could increase their absorption (Davda and Labhasetwar, 2002; Win and Feng, 2005). Lien *et al.* (2009) reported that chromium nanoparticles significantly elevated chromium absorption and increased serum chromium levels in rats. Nanosized copper and zinc has also been demonstrated to increase Cu and Zn absorption (Gonzales-Eguia *et al.,* 2009; Tsai *et al.,* 2016). Therefore, reducing macrosized minerals to nanoscale may increase its absorption and utilization.

Poly-γ-glutamic acid (γ-PGA) is apolymerof glutamic acid, naturally secreted from various strains of*Bacillus*during soybean fermentation. It is a safe, water soluble, biodegradable, edible and nontoxic polymer in which the α -amino andγ-carboxylic acid groups of D- or L-glutamic acid are linked by isopeptide bonds (Shih and Van, 2001). γ –PGA has a wide number of potential uses including food, medicine and water treatments. However, as reports focusing on zinc nanoparticles mixed with γ-PGA supplementation in layer diets are rare, this topic seemed worth investigating. We hypothesized that supplementation of the zinc nanoparticles mixed with poly-γ-glutamic acid would have a positive effect on the zinc retention and eggshell quality of aged layers. We therefore investigated the effects of nanosize ZnO mixed with γ-PGA on Zn retention, egg production and eggshell quality of the aged hens.

Materials and methods

Zinc oxide nanoparticle preparation, nanoparticle size determination and zinc content determination

Zinc oxide nanoparticles were prepared using a ball grinding machine (Just Nanotech Co., JBM-B035, Taiwan) following the procedures described by Tsai *et al.* (2016). Particle size was determined using a transmission electronic microscope (JEM 2100, JOEL, Japan). The zinc content was determined by atomic absorption spectrometry (Perkin Elmer, Atomic Analyst 100, USA).

Trial 1: Metabolic trial

Twenty 64-week-old brown layers (Hendrix) were housed in individual metabolism crates (35 cm 20cm each). Treatment groups included: control, ZnO (Shimakyu's Co. Japan), organic-Zn (Zn-methionine, American Elements Inc, USA), nanoZnO and γ-PGA-nanoZnO (γ-PGA, Vedan Company, Taichung, Taiwan). Each group had 4 replicates. Zinc content in the supplemented groups was 80 mg/kg while the control group was only 40 mg/kg. Individual cages and a plastic plate were used for collecting total excreta. Feed (NRC, 1994) was restricted to about 100 g/day (basal diet) and water was supplied freely. The layers were first allowed 7 days to adapt to the metabolic crates, followed by total excreta collection for a 5-day period using Cr_2O_3 as a marker. During the collection period, the diet was supplemented with 1% Cr_2O_3 on the first and final days. Excreta collection began when a green color appeared but halted once the color disappeared in the days following the final Cr_2O_3 supplementation. After a 7 day rest period, the trial was repeated. Excreta were collected and oven-dried at 55°C. After grinding and pooling the samples, they were stored for analysis at 4°C. Nutrients, including dry matter and ash, were determined using AOAC (1980) procedures. Zinc analysis was performed as AOAC (1980) methodology.

Zinc retention =

〔(individual feed intake* feed zinc concentration)-(individual excreta × excreta zinc concentration)〕/(individual feed intake* feed zinc concentration) ×100%

Trial 2: Animal feeding trial

Seventy-five individually caged 64-week-old brown layers (Hendrix) were allocated to five dietary treatment groups: control, ZnO (Shimakyu's Co. Japan), organic-Zn (Zn-methionine, American Elements Inc., USA), nanoZnO and γ-PGA-nanoZnO. Each group had 15 replicates. The zinc was maintained at 80 mg/kg in the supplemented groups' diet, while the control group was at 40 mg/kg. Layers were fed about 120g of feed every day. Water was supplied freely and lighting was controlled (15 h) throughout the 8-week experimental period. Four eggs per hen were collected at the end of the experiment to determine egg quality. Blood (about 10mL) was taken from all the birds via the brachial wing veins at the end of the experiment.

Determination of eggshell weight, thickness, strength and zinc content

Hens and feed were weighed at the beginning and end of the trial, and the egg production and egg weight were recorded daily. This data was used to calculate the average daily feed intake (ADFI), egg production, egg mass and feed conversion ratio (ADFI/egg mass*egg production). After the eggs were cooked, the eggshell was removed and weighed. The eggshell strength was measured using a press meter (FHK, Japan) to gauge the force required to produce cracking under longitudinal compression. The average eggshell thickness was measured with a micrometer at each of its two ends as well as at a point on the midsection.

Statistical analysis

The experimental data was subjected to statistical analysis using SAS software (version 9.1, SAS Institute Inc., Cary, NC. US.). The general linear model procedure (GLM) was used based on the completely randomized design (CRD). Tukey's tests were used to determine the P values among the variables. In addition, the contrast was used to compare the related group difference.

Results

The zinc concentration in nanoZnO was 81.7% and the average particle size was 135 ± 14 nm (Figure 1). The zinc content of the basal diet was 40.3 mg/kg. The ZnO and Zn-methionine particle size was 0.45 ± 0.11 mm and 0.12 ± 0.07 mm, respectively

*Trial 1. **Effect on** nutrient retention*

Zinc retention was highest in the organic-Zn and γ-PGA-nanoZnO groups, followed by the nanoZnO group; the control and ZnO groups had the lowest retention ($P < 0.05$). The γ-PGA-nanoZnO group had better retention than the nanoZnO group ($P < 0.01$). Ash retention in the organic-Zn group was higher than in the control, nanoZnO or ZnO groups ($P < 0.05$); the γ-PGA-nanoZnO group also had higher retention than the nanoZnO and ZnO groups ($P < 0.05$).

Trial 2. Effect on the egg production and egg quality

The average feed intake (ADFI) was highest in the nanoZnO group followed by the γ-PGA-nanoZnO, organic-Zn and ZnO groups; the control group was the lowest ($P < 0.0001$). Egg mass in the ZnO group was higher than in the control, organic-Zn or γ-PGA-nanoZnO groups ($P < 0.05$). The feed conversion ratio (ADFI/egg mass*egg production) was greatest in the ZnO group, followed by γ-PGA-nanoZnO and nanoZnO, control and finally the organic-Zn group ($P < 0.05$).

Effect on eggshell quality

There were no significant differences between the egg weight, eggshell strength among the different groups ($P > 0.05$). The eggshell weight in the nanoZnO group was higher than the ZnO group ($P < 0.05$), while the eggshell thickness in the γ-PGA-nanoZnO group was better than the ZnO group ($P < 0.05$). Supplementation with γ-PGA-nanoZnO and nanoZnO showed increased eggshell zinc content compared to the other groups ($P < 0.05$).

Discussion

*Trial 1: **Effect on** nutrient retention*

Cao *et al.* (2000) indicated that organic zinc supplements in poultry had higher bioavailability values than inorganic zinc. Compared to inorganic zinc, organic zinc (zinc methionine) is absorbed via peptide or amino acid transport systems, resulting in a higher bioavailability. The results of this study also indicated that organic zinc provides the best retention for zinc and ash. Due to nanoparticle reduced dimensions and high surface area, they have higher rates of absorption from the gastrointestinal tract (Hussain *et al*., 2001). The available surface area of nanosized minerals is much greater (about 1250 times) **than that of macrosized minerals** (Rajendran, 2013)**.** Zha *et al.* (2008) used an *in vitro* Caco-2 cell monolayer **model to** screen particles for permeability and identify intestinal transporters; **they found that nanoparticle** chromium significantly increased absorption. Therefore, reducing a material's size to nanoscale may increase its absorption.

Some studies indicated that coating a nanoparticle material with a polymer can increase its absorption and utilization, especially those materials with low absorbability (McClean *et al.,* 1998; Owen and Peppers, 2005). Some low absorption drugs coated with a polymer demonstrated enhanced cell uptake, suggesting that a polymer could be used as a carrier. Win and Feng (2005) used a polyethylene glycol coating on their test drug and found a 4- or 5-fold increase in caco-2 cell uptake. γ-PGA dissolves in water allowing the α -COOH group to degrade to α -COO^- (Liao, 2011); the α -COO^- group has good chelate affinity with Ca^{2+}, Cu^{2+} and Zn^{2+} (Shin and Van, 2001; Ho *et al.,* 2006). NanoZnO is easily dissolved in water, releasing the Zn^{2+} ion which can chelate with γ-PGA via the glutamic acid carboxy group (COO^{2-}). Liao (2011) reported that γ-PGA can chelate with copper and increase the Cu bioavailability in chicks. Using this same mechanism, the bioavailability of the chelated form of γ-PGA-Zn will also improve. The high bioavailability of zinc may reduce its excretion, thus alleviating its environmental impact. In areas of intense animal production, heavy metals (e.g. zinc) that are present at high concentrations in animal excreta may result in soil phytotoxicity.

Trial 2. Effect on egg production and eggshell quality

Most reports indicated that zinc supplementation could improve egg quality. Yang *et al.* (2012) found that zinc supplementation (65mg/kg) increased eggshell thickness, while adding 0.05% γ-PGA to the hens' diet during the second period of laying could enhance egg production and quality (Huo *et al.,* 2012); this is consistent with our results. Zinc supplementation also improved eggshell breaking strength and fracture toughness (resistance to fracture) in aged hens (Mabe *et al.,* 2003). According to Stefanello *et al.* (2014), an increase in the level of zinc supplementation provided a linear increase in the breaking strength and percentage of eggshell since, structurally, zinc provided a higher thickness of the palisade layer and lower mammillary density. In our study, however, zinc supplementation did not significantly improve eggshell strength.

Conclusion

Nanosize zinc oxide mix with γ-PGA for dietary supplementation could increase zinc and ash retention, the eggshell thickness and eggshell zinc content as compared to regular ZnO. Thus, nanosize of ZnO mix with γ-PGA could increase Zn absorption and has positive effects on zinc supplementation of aged layers.

Keywords: Nanosize zinc, γ-PGA, Nutrient retention, Eggshell quality, Laying hens

References

AOAC 1980. Chapter 25: Metals and other elements as residues in foods. In: Official methods of Analysis (13th Ed.). pp. 411-412. Association of Official Analytical Chemistry, Arlington, VA. US.

Brody T 1994. Chapter 9: Inorganic nutrients. In: Nutritional biochemistry. pp. 581-591. Academic press, NY. US.

Cao JPR, Henry SR, Davis RJ 2002. Relative bioavailability of organic zinc sources based on tissue zinc and metallothionein in chicks fed conventional zinc concentrations. Animal Feed Science and Technology 101, 161-170.

Davda J and Labhasetwar V 2002. Characterization of nanoparticle uptake by endothelial cells. International Journal of Pharmaceutics 233, 51-59.

Gonzales-Eguia A, Fu CM, Lu FY and Lien TF 2009. Effects of nanocoper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livestock Science 126, 122-129.

Ho GH, Ho TI, Hsieh KH, Su YC, Lin PY, Yang JYKH and Yang SC 2006. γ-polyglutamic acid produced by Bacillus subtilis (natto): structureal characteristics, chemical properties and biological functionalities. Journal of Chinese Chemistry Society 53, 1363-1384.

PO-06-40

EFFECTS OF VARYING DIETARY RATIOS OF n-6:n-3 FA ALTER GENE EXPRESSION AND FATTY ACID COMPOSITION IN BREAST MEAT OF BROILERS

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Introduction

The increasing consciousness among the consumers regarding the relationship between diet and the incidence of chronic disease has created the drive to develop healthier meat which can offer potential health benefits to consumers whilst enhancing consumer confidence in meat. The low saturated fatty acid (SFA) and high polyunsaturated fatty acid (PUFA) contents in chicken meat have made it a sought after meat by health conscious individuals (Vilarrasa et al., 2015). However, in recent times, the quality perception of chicken meat is grossly affected due to its low level of n-3 fatty acid (FA), high level of n-6 FA and high ratio of n-6: n-3 FA which has been implicated in the incidence of various chronic diseases (Haug et al., 2007; Simopoulos, 2008; Russo, 2009). It is recommended that the ratio of n-6: n-3 FA in human diet should be less than 4 to prevent arteriosclerosis and coronary heart disease (Simopoulos, 2008). Thus, ensuring the nutritional balance of fatty acids in diets is physiologically important. According to Prescott and Calder (Prescott and Calder, 2004), fatty acids have been identified as the major regulators of biological activities. Fatty acids affect cellular metabolism indirectly by changing membrane phospholipid concentrations by instigating the peroxisome proliferator-activated receptors (PPAR). Difference in dietary n-3 and n-6 PUFA and their effects on the modulation of PPAR which affects gene expression in reaction to nutritional and environmental factors (Sampath and Ntambi, 2008). Thus, it was proposed that diets differing in n-6: n-3 FAR will differ in their effects on gene expression in broiler chickens. Unlike red meats, there is paucity of information on how the modification of fatty acid profile, particularly the ratios of n-6: n-3 FA in broiler chicken diets affects fatty acid profile in chicken muscle and gene expression (Jerónimo et al., 2009; Nassu et al., 2011; Ebrahimi, 2012; Ebrahimi et al., 2014). It is known that modifying the fatty acid composition of chicken meat to meet the prevailing health demands of consumers is warranted (Taulescu et al., 2010). Thus, this study is aimed at examining the effects of varying dietary ratios of n-6: n-3 fatty acids alter gene expression and fatty acid composition in breast meat of broilers.

Methods

Birds and Housing

The current finding was piloted following the recommendations made by the animal welfare ethics of the Research Policy of Universiti of Putra Malaysia. A total of 108, one-day old Cobb 500 male broiler chicks were procured from a local farm. Individual weight of birds was recorded, wing-banded and randomly allocated to three different dietary treatments. The feeding trial lasted for a period of 42 days with each dietary group having six replicates (pens) and each replicate having six chicks. The birds were raised in an open house system in the Poultry Unit, Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia. Feeds and water was provided to birds *ad libitum*. Climatic conditions and lighting program followed the commercial recommendations.

Dietary Treatments

Three dietary treatments were formulated in the feed factory at the Poultry Unit, as follows: (T1) basal diet containing 6 % palm oil as a control; (T2) basal diet containing 4% palm oil (PO) + 1% soybean oil (SO) + 1% linseed oil (LO); (T3) basal diet containing 2% PO + 2% SO + 2% LO, with the different ratios of n-6: n-3: 17.68, 3.70, 2.05 and 19.02, 3.28, 2.23 in the started and finisher diets respectively. All the trial feeds were formulated as isocaloric and isonitrogenous based on nutrient requirements of poultry (NRC, 1994).

Samples and Data Collection

At the end of the trial, 12 broiler chickens from each treatment were slaughtered by neck cutting, bled and he *Pectoralis major* muscles were detached within 45 min aging all carcasses, quickly frozen in liquid nitrogen and kept at -80 ℃ until consequent analyses for the determination of fatty acid composition and gene expression.

Analysis of fatty Acid

The extraction of total fatty acids from experimental diets and breast muscle tissues following the protocols of Folch et al. (1957), and described by Abdulla et al. (2015).

Gene Expression

RNA Extraction Purification and Tissue Collection

After slaughtering the birds, samples from breast muscle were removed, iced up in liquid nitrogen and kept at -80 ℃ until the extraction of RNA. About 50 mg of frozen tissue was used in extracting the total RNA using the RNeasy® fibrous tissue mini kit (Cat. No.74704, Qiagen, Hilden, Germany) for breast muscle sample. The RNase-Free DNase set (Qiagen, Hilden, Germany) was used to complete the DNase digestion during RNA purification following the manufacturer's protocol. The purity of the total was determined using a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Complementary DNA Synthesis

A Quantiect® reverse transcription kit (Qiagen, Hilden, Germany) was used for the reverse dictation of the purified total RNA (1 μg) in line with the manufacturer's procedure.

Real-Time Polymerase Chain Reaction

A real-time PCR was achieved using a Bio-Rad CFX96 Touch (Bio-Rad Laboratories, Hercules, CA, USA) via an optical grade plate using Quantinova™ SYBR® green PCR kit (Cat. no. 208052, Qiagen, Hilden, Germany). Real-time quantitative PCR was performed with Quantitect primer assays for PPARα (Cat. QT00596085, Qiagen), PPARγ (Cat. QT00595301, Qiagen) and SCD (Cat. QT00592214, Qiagen). The β-actin (Cat. QT00600614, Qiagen) was used as the reference gene to normalize the tested genes. Each reaction (20 μl) contains about 10 μl SYBR green PCR mix, 1 μl cDNA, 7 μl RNase free water and 2 μl primer and. The thermo cycling program were used to amplify the target genes as described by Ebrahimi et al. (2013).

Statistical Analysis

A completely randomized design was used for the study. All data generated for all parameters were subjected to analysis using a general linear model (GLM) of SAS (SAS, 2007). Duncan's Multiple Range was used to test the level of significance between treatment means and compared using Test and significant levels were set at $P< 0.05$. Orthogonal contrasts (linear and quadratic effects) were tested with coefficients calculated based on the ratios of n-6: n-3 fatty acid.

Results and Discussion

Fatty Acid Profiles of Breast Muscle Tissues

Lowering levels of n-6: n-3 FAR increased (linear, $P < 0.01$) the proportions of C18:0, C18:2n-6, C18:3n-3, C22:5n-3 and C22:6n-3, but decreases (linear, $P < 0.01$) the concentrations of C14:0, C16:0, C16:1n-7, C18:1n-9 and C20:5n-3. The concentrations of C20:4n-6 did not differ among the treatments. The total n-3 PUFA, PUFA n-6, USFA, USFA: SFA and PUFA: SFA ratio increased (linear, $P < 0.01$) while the total SFA, MUFA and n-6: n-3 FA ratio decreased (Linear, $P < 0.01$) with lowering n-6: n-3 FAR.

The composition n-6: n-3 FAR have effects on diets offered as nourishment to birds (Jankowski et al., 2012). This findings examines, the influence of modifying dietary n-6: n-3 FAR in palm oil-based diet of broiler on the composition of fatty acids in breast meat samples were analysed. Inclusion levels of different ratios of n-6: n-3 in diets caused changes in their FA profile. The shrinkage in the ratios of dietary n-6: n-3 FAR caused by subsituting palm oil with linseed oil in feeds, lower the n-6: n-3 FAR of breast samples. Lowering the ratio of n-6 to n-3 FA in meat and meat products have been recommended to prevent or modulate certain diseases in humans (Simopoulos, 2011). The n-6: n-3 FAR in food should range between 1 and 4 (Simopoulos, 2004). The T2 and T3, treatment

groups resulted in n-6: n-3 FAR below 4:1 in comparison with T1 (control group), which is the highest value recommended for human diets (Simopoulos, 2004). In this study, the upsurge in the concentrations of C22:6n-3 and C22:5n-3 in breast meat samples with the increase in α-linolenic acid (C18:3n-3) could be as a result of synthesis of long chain n-3 FA from C18;3n-3. Vertebrate species possess the capacity to synthesize C22:6n-3 and C22:5n-3 form C18:3n-3. This observation corroborates the report of Jing et al. (2013) who observed that the concentrations of liver C22:6n-3, C22:5n-3 and C20:5n-3 was generally amplified with a decrease in the ratio of dietary n-6: n-3 FAR.

The mRNA expression of SCD, PPARγ and PPARα in Breast Muscle of Broiler Chickens

The expression of PPARγ and PPARα was greater ($P < 0.05$) in birds fed T3 diets in comparison with those fed T1 and T2. Birds fed T2 diets had lower expression of SCD gene in comparison to this nourished with treatment 1 diets. There was no significant difference in SCD gene expression between birds nourished with T3 than those fed T1 and T2 diets.

Generally, the PPARγ and PPARα expression in the breast muscle tissue amplify with decrease in n-6: n-3 FAR, indicating that increasing the levels of dietary C18:3n-3 up regulated the PPARγ and PPARα gene expression. Contrarily, the level of SCD expression decrease with decreasing ratios of n-6: n-3 FA and it seems that the SCD gene was down regulated by increasing dietary α-linolenic acid. Although numerous findings have shown the effects of n-3 PUFAs on SCD and PPAR gene expression in mammals, knowledgeable to us, the current findings is the first which tries investigated the effects of different dietary ratio of n-6: n-3 FA on SCD, PPARγ and PPARα mRNA expression in the broiler breast meat tissue.

The impact of different n-6: n-3 FAR on the expression of PPARγ genes, SCD and PPARα, which are the key lipogenic transcription factors in avian tissues, has successfully been shown in this experiment. The substantial differences in the SCD, PPARγ and PPARα expression in the breast muscle among the diets indicate that the major pathways via which FAs act to lessen the expression of lipogenic genes is via the altered expression of PPARγ, SCD and PPARα. The result of the present study obviously agrees with the assertion that an association amongst the intakes of gene expression and n-3 fatty acids of PPAR those exist. Reducing dietary n-6: n-3 FAR might excite PPAR target genes expression such as acyl-CoA synthase, transportation of fatty acids and proteins and lipoprotein lipase (Michaud and Renier, 2001; Kaplins' kyĭ et al., 2008). PPARα genes are involved in the oxidation of fatty acid by up controlling the expression of acyl CoA oxidase and hydrolysis, fatty acid desaturation and elongation (Pawar and Jump, 2003; Zhang et al., 2010). The current finding is in line with the numerous findings in rodents where fish oil is administered as a source of n-3 PUFA causing a strong up-regulation of PPARα and its target genes (Nakatani et al., 2005). Akin results were observed in turkeys (Ding et al., 2003), mice (Issemann and Green, 1990), and humans (Schmidt et al., 1992). Nourishments such as carbohydrates and fatty acids, and hormones modulate the expression of SCD (Ntambi and Miyazaki, 2004; Waters et al., 2009), and cholesterol (Kim et al., 2002). The current finding shows that lowering the dietary n-6: n-3 FAR, down regulates the SCD gene expression. Similarly, Ebrahimi et al. (2014) reported a decrease in SCD gene expression in the muscle of goat fed diet supplemented with high C18:3n-3 group compared with the low C18:3n-3 group. Bellinger et al. (2004) a negative association between the expression SCD gene and n-3 PUFA in the young adult rat was observed which is in tandem with the current findings where the down regulation of SCD takes place in breast muscle tissue with decreasing ratios of n-6: n-3 FA in broiler diets.

Conclusion

A result from the current finding shows that lowering dietary n-6: n-3 FAR in palm oil -based diets resulted in a sharp decline in n-6: n-3 FAR in breast muscle tissue. Additionally, lowering the concentrations of dietary n-6: n-3 FAR attained a nutritionally improved meat with higher contents of desirable C18:3n-3, C22:5n-3 and C22:6n-3. Adjustments may likely better the choice of broiler meat as a healthy food. Decreasing n-6: n-3 FAR in broiler diets up regulated the expression of PPARα and PPARγ but down regulated the expression of SCD ($P<0.05$) in the breast sample.

References

Abdulla, N., Loh, T., Akit, H., Sazili, A., Foo, H., Mohamad, R., Rahim, R.A., Ebrahimi, M., Sabow, A., 2015. Fatty acid profile, cholesterol and oxidative status in broiler chicken breast muscle fed different dietary oil sources and calcium levels. South African Journal of Animal Science 45, 153-163.

Bellinger, L., Lilley, C., Langley-Evans, S.C., 2004. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. British Journal of Nutrition 92, 513-520.

Ding, S., Li, Y., Nestor, K., Velleman, S., Mersmann, H., 2003. Expression of turkey transcription factors and acyl-coenzyme oxidase in different tissues and genetic populations. Poultry Science 82, 17-24.

Ebrahimi, M., 2012. Production of Omega- 3 Polyunsaturated Fatty Acid Enriched Chevon Using Treated Oil Palm Fronds. Ph. D. Thesis research. Universiti Putra Malaysia. Accessed September 2012.

Ebrahimi, M., Rajion, M.A., Goh, Y.M., 2014. Effects of oils rich in linoleic and α-linolenic acids on fatty acid profile and gene expression in goat meat. Nutrients 6, 3913-3928.

Ebrahimi, M., Rajion, M.A., Goh, Y.M., Sazili, A.Q., Schonewille, J., 2013. Effect of linseed oil dietary supplementation on fatty acid composition and gene expression in adipose tissue of growing goats. BioMed Research International 2013.

Folch, J., Lees, M., Sloane-Stanley, G., 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497-509.

Haug, A., Eich-Greatorex, S., Bernhoft, A., Wold, J.P., Hetland, H., Christophersen, O.A., Sogn, T., 2007. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. Lipids in Health Disease 6.

Issemann, I., Green, S., 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347, 645-650.

Jankowski, J., Zdunczyk, Z., Mikulski, D., Juskiewicz, J., Naczmanski, J., Pomianowski, J.F., Zdunczyk, P., 2012. Fatty acid profile, oxidative stability, and sensory properties of breast meat from turkeys fed diets with a different n-6/n-3 PUFA ratio. European Journal of Lipid Science and Technology 114, 1025-1035.

Jerónimo, E., Alves, S.P., Prates, J.A., Santos-Silva, J., Bessa, R.J., 2009. Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. Meat Science 83, 499-505.

Jing, M., Gakhar, N., Gibson, R., House, J., 2013. Dietary and ontogenic regulation of fatty acid desaturase and elongase expression in broiler chickens. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA) 89, 107-113.

Kaplins' kyĭ, S., Shysh, A., Nahibin, V., Dosenko, V., Klimashevs' kyĭ, V., Moĭbenko, O., 2008. [Omega-3 polyunsaturated fatty acids stimulate the expression of PPAR target genes]. Fiziolohichnyi zhurnal (Kiev, Ukraine: 1994) 55, 37-43.

Kim, H.-J., Miyazaki, M., Ntambi, J.M., 2002. Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism. Journal of Lipid Research 43, 1750-1757.

Michaud, S.É., Renier, G., 2001. Direct regulatory effect of fatty acids on macrophage lipoprotein lipase potential role of PPARs. Diabetes 50, 660-666.

Nakatani, Y., Kaneto, H., Kawamori, D., Yoshiuchi, K., Hatazaki, M., Matsuoka, T.-a., Ozawa, K., Ogawa, S., Hori, M., Yamasaki, Y., 2005. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. Journal of Biological Chemistry 280, 847-851.

Nassu, R., Dugan, M., He, M., McAllister, T., Aalhus, J., Aldai, N., Kramer, J., 2011. The effects of feeding flaxseed to beef cows given forage based diets on fatty acids of longissimus thoracis muscle and backfat. Meat Science 89, 469-477.

NRC, 1994. Nutrient requirements of poultry. National Research Council. National Academy Press Washington^ eUSA USA.

Ntambi, J.M., Miyazaki, M., 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Progress in Lipid Research 43, 91-104.

Pawar, A., Jump, D.B., 2003. Unsaturated fatty acid regulation of peroxisome proliferator-activated receptor α activity in rat primary hepatoctes. Journal of Biological Chemistry 278, 35931-35939.

Prescott, S.L., Calder, P.C., 2004. N-3 polyunsaturated fatty acids and allergic disease. Current Opinion in Clinical Nutrition & Metabolic Care 7, 123-129.

Russo, G.L., 2009. Dietary n− 6 and n− 3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochemical Pharmacology 77, 937-946.

Sampath, H., Ntambi, J., 2008. Polyunsaturated fatty acids and regulation of gene expression. Food Science And Technology-New York-Marcel Dekker- 170, 727.

SAS, 2007 "User's Guide. 9.2 ed,". In SAS institute, Inc., Cary, NC, USA.

Schmidt, A., Endo, N., Rutledge, S.J., Vogel, R., Shinar, D., Rodan, G.A., 1992. Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Molecular Endocrinology 6, 1634-1641.

Simopoulos, A.P., 2004. Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food Reviews International 20, 77-90.

Simopoulos, A.P., 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental Biology and Medicine 233, 674-688.

Simopoulos, A.P., 2011. Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. Healthy Agriculture, Healthy Nutrition, Healthy People 102, 10-21.

Taulescu, C., Mihaiu, M., Bele, C., Matea, C., Dan, S.D., Mihaiu, R., xandra Lapusan, A., Ciupa, A., 2010. Manipulating the Fatty Acid Composition of Poultry Meat for Improving Consumer's Health. Bulletin UASVM 67, 2.

Vilarrasa, E., Guardiola, F., Codony, R., Esteve-Garcia, E., Barroeta, A., 2015. Use of combinations of re-esterified oils, differing in their degree of saturation, in broiler chicken diets. Poultry Science 94, 1539-1548.

Waters, S., Kelly, J., O'Boyle, P., Moloney, A., Kenny, D., 2009. Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ-desaturase in muscle of beef cattle. Journal of Animal Science 87, 244-252.

Zhang, J., Chen, H., Sun, Z., Liu, X., Qiang-Ba, Y., Gu, Y., 2010. Genetic Variation of the Peroxisome Proliferator-Activated Receptor α Gene (PPARA) in Chickens Bred for Different Purposes. Biochemical Genetics 48, 465-471.

PO-06-42

Effect of Dietary Turmeric Rhizome Powder and White Corn On Egg Production Performance of Laying Hens

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INTRODUCTION

Turmeric (*Curcuma longa* L.) is a perennial herb belongs to the family of *Zingiberaceae*. It is distributed throughout tropical and subtropical regions of the world (Beevers and Huang, 2011). Turmeric is widely used as a medicinal plant (Srimal, 1997), and it is belongs to a group of aromatic spices, that has been originally used as a food additive in curries to improve the storage condition, palatability and preservation of foods (Jayaprakasha et al., 2005).

The rhizome of turmeric, in Indonesia is known as kunyit, was used as traditional remedy and usually mixed with other herbs (called "jamu") for various biological activities. Curcumin is the main important bioactive ingredient responsible for the biological activity (Chattopadhyay et al., 2004). Curcumin, a natural phenolic yellow pigment, obtained from the rhizomes of *Curcuma longa* L. It is a major component of turmeric and is commonly used as a spice and food-coloring agent (Govindarajan, 1980; Chattopadhyay et al., 2004). Curcumin has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-bacterial, anti-fungal, anti-protozoal, anti-viral, and immunomodulatory (Chandra and Gupta, 1972; Soni et al., 1997; Srimal, 1997; Antony et al., 1999; Igbal et al., 2003; Chattopadhyay et al., 2004; Holt et al., 2005; Gowda et al., 2008; Yarru et al., 2009; Beevers and Huang, 2011; Li, 2011; Krup, 2013).

The potency of turmeric is approved by researchers (Hallagan et al., 1995; Srinivasan, 2005). Studies showed that supplementing of turmeric in broiler diets enhance their performance (Al-Sultan,.2003; Durrani et al., 2006). However, Mehala and Moorthy (2008) demonstrated that 0.1 and 0.2 % turmeric powder used as feed additive had no significant effect on the performance and carcass yield of broiler chickens. There are limited studies on the effects of turmeric powder supplementation in birds, especially laying hens (Radwan et al., 2008). It is therefore, this experiment was designed to investigate the efficacy of different levels of turmeric powder substituted to white corn in diet on egg production performance of laying hens.

MATERIALS AND METHODS

A total of 100 laying hens 15 months of age used in this study. They were caged individually, and randomly assigned into four dietary treatments (R0 = based diet with 58% yellow corn; R1 = diet with 58% white corn and without turmeric rhizome powder (TRP); R2 = based diet with 57.5% white corn and 0.5% TRP; and R3 = based diet with 57% white corn and 1% TRP) with five replications of 5 birds each using a completely randomized design. Chemical composition of yellow corn, white corn and turmeric is shown in Table 1 and chemical composition of the experimental diet is shown in Table 2.

The chicks were allowed to have free access to diet and water. The experimental diets were freshly added everyday, feed intake was recorded daily and egg weight and yolk color were determined. The number of eggs laid by each replicate was recorded daily and the hen-day egg production (HDP) calculated as: HDP (%) = (Total eggs produced : Number of birds alive) x 100. Eggs produced by each replicate were weighed and the mean egg weight (g) recorded. Feed conversion ratio (FCR) was calculated for each replicate as the ratio of the feed consumed (g) to the total egg weight (g) (Kwari et al., 2005).

Data were analyzed by one-way analysis of variance and the treatment means were compared using Duncan's multiple range test, using the statistical analysis system, IBM SPSS 22.

RESULTS AND DISCUSSION

The results of egg production performance of laying hens fed experiment diets were shown in Table 3. The results indicated that the supplementation of TRP combine with white corn decreased feed intake, dry matter, protein, crude fiber and energy intake of the hen, while there were no significant changes in hen-day production, egg mass, Ca and fat intake. However, feed efficiency was almost the same between treatments. Moreover, the yolk color were significantly lowered in fan value compared to control (diet with yellow corn). Diet with yellow corn

was reflected on the highest significant records of yolk color fan value.

Our observation was not consistent with the finding of several researcher. Samarasinghe et al. (2003) reported that feeding of turmeric did not affect to feed intake, egg production, egg weight and egg mass. Moreover, Radwan et al. (2008) who used TRP in diets of laying hen reported that the feed intake numerically increased. This discrepancy could be due to the higher levels of turmeric in the previous study and the probably effects of turmeric aroma on birds' appetite. That TRP had beneficial effects on older laying hens, because it improved the FCR. Also, had potential to improve the yolk color value of the eggs produced by older laying hens and increased egg mass, egg production.

The yolk color of laying hens fed TRP combine with white corn in this study showed lower yolk color value than that fed yellow corn. The coloring agent of the TRP didn't be able to increase the yolk color value if they combine with white corn. This study was in contrast with Park et al. (2012) that reported that TRP improves egg production and yolk color. Riasi et al. (2012) also stated that supplementation of TRP had higher yolk color fan value compared with the control group. However, Jacqueline et al. (1998) reported that yolk color depends on the diet of the hen. If layers get plenty of yellow-orange plant pigments known as xanthophylls, they will be deposited in the yolk. Natural yellow-orange substances in turmeric might be added to light-colored feeds to enhance yolk color.

Egg mass that was reported by Radwan et al. (2008) was in contrast with our study, that TRP significantly increased egg mass production over the 4 weeks assay. Moreover, egg production performance of old laying hen fed TRP could be markedly improved (Rahardja et al., 2015). It is an indication that the quality of egg produced by laying hen fed TRP was maintained regardless of the percentages and periods of supplementation.

CONCLUSION

It was concluded that supplementation of TRP up to 1% combined with white corn was not decreased FCR of the hens but did not be suggested to the laying hens diet because of its effect to yolk color.

Table 1. Chemical composition of yellow corn, white corn and turmeric

Nutrient	Ingredient		
	Yellow corn	White corn	Turmeric
Crude protein (%)	9.67	8.68	9.89
Crude fiber (%)	2.11	1.30	2.57
Fat (%)	3.97	4.26	7.33
Ca (%)	0.02	0.03	0.06
P (%)	0.28	0.02	0.08
GE (Kcal/kg)	3774	3891	3921

Table 2. Diet and chemical compositions of the experimental diet

	R0	R1	R2	R3
Ingredients, %				
Yellow corn	58	-	-	-
White corn	-	58	57.5	57
Rice bran	10	10	10	10
Coconut cake	9	9	9	9
Soybean meal	8	8	8	8
Meat meal	12.5	12.5	12.5	12.5
Bone meal	2	2	2	2
Turmeric	-	0	0.5	1
Grit	0.5	0.5	0.5	0.5
Calculated nutrient content:				
Crude protein (%)	16.22	15.65	15.65	15.66
Crude fiber (%)	4.25	3.78	3.79	3.79
Fat (%)	7.8	7.5	8.0	8.0
Ca (%)	1.11	1.11	1.12	1.22
GE (Kcal/kg)	3917	3985	3985	3985

Table 3. Dietary effects of turmeric and white corn on the performance of laying hens

Parameter	YC, 0% T	WC, 0% T	WC, 0.5%T	WC, 1% T	SEM[1]	pValue
Feed Intake(g/d/bird)	96.16^{b}	95.39^{b}	85.86^{a}	86.69^{a}	1.50	.008
Dry Matter Intake (g/d/bird)	84.20^{b}	85.73^{b}	77.22^{a}	77.88^{a}	1.28	.020
Protein Intake (g/d/bird)	13.66^{b}	13.41^{b}	12.08^{a}	12.19^{a}	0.22	.005
Crude Fiber Intake (g/d/bird)	3.58^{c}	3.24^{b}	2.92^{a}	2.95^{a}	0.71	.000
Fat Intake(g/d/bird)	6.56	6.43	5.79	6.23	0.82	.172
Ca Intake (g/d/bird)	0.70	0.95	0.84	0.88	0.01	.173
P Intake (g/d/bird)	0.32^{b}	0.16^{a}	0.16^{a}	0.16^{a}	0.02	.000
Energy Intake (Kcal/kg)	301^{b}	304^{b}	274^{a}	276^{a}	4.68	.016
Hen-day Production (%)	59.04	58.57	58.33	58.81	0.31	.870
Egg Mass	36.60	36.55	36.42	37.09	0.23	.782
Feed Efficiency	0.38^{a}	0.38^{ab}	0.42^{bc}	0.42^{c}	0.04	.013
Egg Yolk Color	11.67^{b}	1.0^{a}	1.2^{a}	1.0^{a}	1.05	.000

[1]SEM = standard error of mean

YC = yellow corn, WC = white corn; T = turmeric

REFERENCES

Al-Sultan, S.I. 2003. The effect of *Curcuma longa* (turmeric) on overall performance of broiler chickens. Int. J. Poult. Sci. 2: 351-353.

Antony, S., R. Kuttan, and G. Kuttan. 1999. Immunomodulatory activity of curcumin. Immunol. Invest. 28: 291-303.

Beevers, C.S. and S. Huang. 2011. Pharmacological and clinical properties of curcumin, Review. Botanics: Targets and Therapy, 1: 5-18

Chandra, D., and S.S. Gupta. 1972. Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa* (Haldi). Indian J. Med. Res. 60: 138-142.

Chattopadhyay, I. K., U. Biswa, Bandyopadhyay, and R. K. Banerjeeil. 2004. Turmeric and Curcumin: Biological actions and medicinal applications. Current Sci. 87 (1): 44-53

Durrani, F.R, M. Ismail, A. Sultan, S.M Suhail, N. Chand, and Z. Durrani. 2006. Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler chicks. J.Agric. Biol. Sci. 1:9-11.

Govindarajan, V.S. 1980. Turmeric-chemistry, technology and quality. Crit. Rev. Food Sci. Nutr. 12:199-301.

Gowda, N.K.S., D.R. Ledoux, G.E. Rottinghaus, A.J. Bermudez, and Y.C. Chen. 2008. Efficacy of turmeric (*curcuma longa*), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poult. Sci. 87: 1125-1130.

Hallagan, J.B., D.C. Allen, and J. Borzelleca. 1995. The safety and regulatory status of food, drugs and cosmetics, colors additives exempt from certification. Food Chem. Toxicol. 33: 515-528.

Holt, P.R, R. Katz, and R. Kirschoff. 2005. Curcumin therapy in inflammatory bowel disease: a pilot study. Dig. Dis. Sci. 50: 2191-2193.

Iqbal, M., S.D. Sharma, Y. Okazaki, M. Fujisawa, and S. Okada. 2003. Dietary supplementation of curcumin enhance antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. Pharmacol.Toxicol. 92: 33-38.

Jayaprakasha, G.K., I.J.M. Rao, and K.K. Sakariah. 2005. Chemistry and biological activities of *C. longa*. Trends Food Sci. Tech. 16: 533-548.

Krup, V., L.H. Prakash, and A. Harini. 2013. Pharmacological Activities of Turmeric (*Curcuma longa* Linn): A Review. J. Homeop. Ayurv. Med. 2:133.

Kwari, I.D., S. S. Diarra, A. O. Raji, and S. B. Adamu. 2011. Egg production and egg quality laying hens fed raw or processed sorrel (*Hibiscus sabdariffa*) seed meal. Agric. Biol. J. N. Am.. 2(4): 616-621.

Li, S., Y. Wei, D. Guangrui, W. Ping, Y. Peiying, and B. A. Aggarwal. 2011. Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa* L.). Pharmaceutical Crops. 2: 28-54

Mehala, C., and M.Moorthy. 2008. Production performance of broilers fed with Aloe vera and Curcuma longa (Turmeric). Int. J. Poult. Sci. 7: 852-856.

Park, Sang-Sul, Jin-Man Kim, Eun-Jib Kim, Hee-Sung Kim, Byoung-Ki An, and Chang-Won Kang†. 2012. Effects of Dietary Turmeric Powder on Laying Performance and Egg Qualities in Laying Hens. Korean J. Poult. Sci. 39 (1): 27-32.

Radwan, N.L., R.A. Hassan, E.M. Qota, and H.M. Fayek. 2008. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. Int. J. Poult. Sci. 7: 134-150.

Rahardja, D.P, M. Rahman Hakim, and V. Sri Lestari. 2015. Egg Production Performance of Old Laying Hen Fed Dietary Turmeric Powder. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering. 9 (7): 712-716.

Riasi, A., H. Kermanshahi, and A. H. Mahdavi. 2012. Production performance, egg quality and some serum metabolites of older commercial laying hens fed different levels of turmeric rhizome (*Curcuma longa*) powder. J. of Med. Plants Res. 6 (11): 2141-2145.

Samarasinghe, K., C. Wenk, K.F.S.T. Silva, J.M/D.M. Gunasekera. 2003. Turmeric (*Curcuma longa*) root powder and mannanoligosaccharides as alternatives to antibiotics in broiler chicken diets. Asian-Aust. J. Anim. Sci. 10: 1495-1500.

Soni, K.B., M. Lahiri, P. Chackradeo, S.V. Bhide, and R. Kuttan. 1997. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer letters. 115: 129-33.

Srimal, R.C. 1997. Turmeric: A brief review of medicinal properties. Fitoterapia. 68: 483-493.

Srinivasan, K. 2005. Spices as influencers of body metabolism: an overview of three decades of research. Food Res. Int. 38: 77-86.

Yarru, L.P., R.S. Settivari, N.K.S. Gowda, E. Antoniou, D.R. Ledoux, G.E. Rottinghaus. 2009. Effects of turmeric (*Curcuma longa*) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poult. Sci. 88: 2620-2627.

Zainali, A, A. Riasi, H. Kermanshahi, and H. Ziaei. 2008. Effect of selenium and (or) turmeric powder on growth performance of broiler chickens reared under heat stress condition. 1st Mediterranean Summit of WPSA, Advance and Challenges in Poult. Sci. Greece. pp. 790-792.

PO-06-46

Effect of Dietary Supplementation of Blood Meal on Productive Performance and Egg Qulity in Laying Hens

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INTRODUCTION

Eggshell color for brown eggs is a trait of great economic importance to the egg industry. Brown color or pigment of eggshell comes mainly from protoporphyrin IX in eggshell, which is almost completely lacking from white eggs. The protoporphyrin IX in eggshells is derived from heme metabolism in birds. The complex nature of eggshell color is still clearly undefined; however, it is known to be associated with a genetic basis for shell color, possibly sex-linked genes (Samiullah et al., 2015). However, the eggshell color of laying hens is also affected by various factors including housing system, nutrition, health status, stress, and age of laying hens (Samiullah et al., 2015). For nutrition, several nutritional components, especially for trace minerals such as Fe, Cu, Mn, and Zn have been reported to affect eggshell color in laying hens; however, the effects of nutritional regimens have been inconsistent (Samiullah et al., 2015). Thus, more nutritional researches are required to improve eggshell color in laying hens.

Blood meal (BM) is an important by-product of the meat industry and is a common feed ingredient for animals. Blood meal contains relatively high amounts of protoporphyrin IX and heme (Zheng et al, 2014). Accordingly, feeding diets containing BM to laying hens may exert positive effects on the eggshell color because protoporphyrin IX is the main colorant in eggshells. However, this hypothesis has not been tested before. Therefore, the objective of this experiment was to investigate the effects of dietary supplementation of blood meal on productive performance and egg quality, especially for eggshell color, in laying hens.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Birds, diets and experimental design

A total of 120 43-week-old Hy-Line Brown laying hens were randomly allotted to one of three dietary treatments with 5 replicates per treatment. Each replicate consisted of 4 consecutive cages with 2 hens per cage. A commercial-type control diet was formulated to be adequate in energy and all nutrients. Two additional diets were prepared by adding 0.5% or 1.0% BM, respectively. All nutrients and energy were included to meet or exceed NRC (1994) requirement estimates for laying hens. The experimental diets were given to the hens on an ad libitum basis for 6 weeks. A 16-h lighting schedule was used during the entire experiment.

Sample collection and chemical analyses

Laying performance including hen-day egg production, egg weight, egg mass, and broken and shell-less egg production rate was recorded daily. However, feed intake (FI) and feed conversion ratio (FCR) were recorded weekly. The data for laying performance were summarized for 6 weeks of feeding trial. Egg quality was assessed with all eggs that were collected at the conclusion of the experiment. Eggshell strength was determined using a Texture analyzer (model TAHDi 500, Stable Micro System, Godalming, UK) and was displayed as unit of compression force per unit eggshell surface area. Eggshell thickness was measured at three different regions (top, middle and bottom) using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan). Eggshell color was determined using the eggshell color fan (Samyangsa, Kangwon-do, Repulic of Korea). The Hunter values for lightness (L*), redness (a*), and yellowness (b*) were determined using the Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). Egg yolk color was estimated by using the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland). Haugh units (HU) were measured using a micrometer (model S-8400, B.C. Ames Co., Waltham, MA), and the HU values were calculated from the egg weight (W) and albumen height (H) based on the following equation: HU = 100 log (H - 1.7W0.37 + 7.6) as described by Eisen et al. (1962).

Statistical analysis

All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Each replicate was considered an experimental unit in the analysis of laying performance and egg quality. Outlier data were examined according to the method of Steel et al. (1997), using the UNIVARIATE

procedure of SAS. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $P < 0.05$.

Results

Laying performance

Supplementation of blood meal in diets had no effects on productive performance including egg production, feed intake, and feed conversion ratio (Table 1).

Egg quality

Supplementation of blood meal in diets had no effects on egg quality including eggshell strength, eggshell thickness, and haugh unit (Table 2). However, increasing inclusion levels of blood meal in diets linearly decreased egg yolk color, and quadratically decreased eggshell hunter b* value. No effects of dietary supplementation of BM were observed for eggshell hunter L* and a* value, and for eggshell color that was based on the 15-scaled color fan (Table 3).

Conclusion

In conclusion, dietary supplementation of BM has no positive effects on productive performance and egg quality, but except that it shows negative effects on egg yolk color and yellowness of eggshell color.

Table 1. Effects of dietary supplementation of blood meal (BM) on productive performance in laying hens

	Dietary treatments[1]				*P*-value[2]		
Item	NC	BM0.5	BM1.0	SEM	T	L	Q
Laying performance							
Hen-day egg production, egg, %	96.6	95.3	95.4	1.04	0.628	0.435	0.586
Broken and shell-less egg, %	0.01	0.10	0.02	0.037	0.282	0.860	0.121
Egg weight, g	69.9	68.9	67.9	0.84	0.254	0.105	0.985
Egg mass, g	67.6	65.7	64.8	1.32	0.340	0.157	0.770
Feed intake, g	132	129	127	2.0	0.288	0.130	0.728
Feed conversion ratio, g/g	1.95	1.96	1.97	0.035	0.962	0.787	0.975

[1]NC = basal diets; BM0.5 = NC + 0.5% blood meal; BM1.0 = NC + 1.0% blood meal;

[2]T = Overall effects of treatments; L = Linear effect; Q = Quadratic effect.

[a,b]Means with different superscripts differ significantly ($p<0.05$).

Table 2. Effects of dietary supplementation of blood meal (BM) on egg quality in laying hens

Item	Dietary treatments[1]			SEM	*P*-value[2]		
	NC	BM0.5	BM1.0		T	L	Q
Egg quality							
Eggshell color	11.50	10.91	10.69	0.38	0.292	0.137	0.665
Eggyolk color	8.46[a]	8.10[ab]	7.94[b]	0.17	0.031	0.011	0.524
Egg strength	3.28	3.46	3.14	0.14	0.32	0.493	0.183
Egg thickness	431	441	428	4.7	0.16	0.704	0.06
Egg weight	67.71	66.73	65.25	1.00	0.251	0.106	0.845
Haugh unit	96.55	95.88	97.13	0.88	0.612	0.644	0.389

[1]NC = basal diets; BM0.5 = NC + 0.5% blood meal; BM1.0 = NC + 1.0% blood meal;

[2]T = Overall effects of treatments; L = Linear effect; Q = Quadratic effect.

[a,b]Means with different superscripts differ significantly ($p<0.05$).

Table 3. Effects of dietary supplementation of blood meal (BM) on eggshell color in laying hens

Item		Dietary treatments[1]			SEM	*P*-value[2]		
		NC	BM0.5	BM1.0		T	L	Q
6 week								
Color fan		11.55	10.91	10.69	0.381	0.292		
Hunter color value	*L**	54.28	54.67	55.47	0.408	0.152	0.062	0.684
	*a**	20.70	20.51	20.07	0.398	0.537	0.287	0.796
	*b**	28.22[a]	26.94[b]	27.88[a]	0.286	0.021	0.414	0.008

[1]NC = basal diets; BM0.5 = NC + 0.5% blood meal; BM1.0 = NC + 1.0% blood meal;

[2]T = Overall effects of treatments; L = Linear effect; Q = Quadratic effect.

[a,b]Means with different superscripts differ significantly ($p<0.05$).

Reference

1. Eisen, E. J., B. B., Bohren, and H. E. Mckean. 1962. The Haugh unit as a measure of egg albumen quality. Poult. Sci. 41:1461-1468.

2. Samiullah, S., and J. R. Roberts. 2014. The eggshell cuticle of the laying hen. World's. Poult. Sci. J. 70:693.

3. Steel, R. G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw Hill Book Co., New York, NY.

4. Zheng, C., Z. Li., N. Yang., and Z. Ning. 2014. Quantitative expression of candidate genes affecting eggshell color. Anim. Sci. J. 85:506-510.

PO-06-47 Effect of herbal cocktail (Capsicum spp., Curcuma longa and Allium sativum) powder dietary supplementation on performance and immunity of broilers

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Introduction

Presently, the poultry industry raised under intensive production systems in densely populated colonies or flocks to achieve high levels of economic efficiency (Kabir et al., 2004). The chicken may get stress from possible many factors such as vaccination, ventilation etc. Then these factors induce to lower performance and easy to get disease outbreak. Phytogenic feed additives may positively affect poultry health and productivity. Several studies have reported effects with inconsistent results of phytogenic feed additives in feeding experiments conducted with broiler chickens (Amad et al., 2011). Positive effects were observed on the daily weight gain and feed conversion ratio (FCR) of chickens when fed a diet supplemented with a mixture (300 mg/kg of feed) containing capsaicin, cinnamaldehyde and carvacrol (as cited in Amad et al., 2011). The addition of 100, 1,000 and 10,000 mg of garlic/kg of feed increased average daily weight gain during the first 21 d on feed (Horton et al., 1991).Jafari et al. (2008) reported that adding garlic powder to the diet for broilers don't have any beneficial effect on humoral immune response to live NDV vaccine. The feed conversion of the chickens in the group offered 0.5% tumeric (*Curcuma longa*) in their diets were the best as compared with controls and the other treated groups. The levels of turmeric 0.5% and 1.0% increased both erythrocytic and total leukocytic count (AL-Sultan, 2003).

The objective of this study was to determine the effect of diet supplementation with a herbal cocktail on growth performance and immunity of broilers. The herbs used as herbal cocktail powder in this study were chili (*Capsicum* spp.), turmeric (*Curcuma longa*) and garlic (*Allium sativum*).

Materials and methods

Animals and diets

Ninety six (Ross 308), one day old broilers, mixed sex, were randomly allotted into 3 groups with 4 replications for each (1 x 1 m^2, 8 birds per replication). The dietary treatments consisted of 1) the basal (control) diet (C), 2) the basal diet supplemented with recommended dose of herbal cocktail powders (chili 3.04, turmeric 7.00 and garlic 4.00 g/kg diet); Treatment 1 (T1) and 3) the basal diet supplemented with half recommended dose of herbal cocktail powders (chili 1.52, turmeric 3.50 and garlic 2.00 g/kg diet); Treatment 2 (T2). The dietary period was divided in two phases: starter (0 to 3 week of age) and grower (3 to 5 week of age). All studied chickens were offered experimental diet and drinking water *ad libitum*. . Growth performance data were collected and then determined for periods of 0 to 3, 3 to 5, and 0 to 5 week of age. The average daily gain, average daily feed intake and feed conversion ratio were calculated. Blood samples were collected at 7, 14, 21, 28 and 35 days of age for the analyzed of hemagglutination inhibiion titer (HI-titer) against Newcastle's disease virus (NDV) and Heterophil : Lymphocyte (H:L ratio).

Statistical analysis

Data from all experiments were analyzed by using the ANOVA procedure with Duncan multiple comparison tests to detect a significant level at P£0.05.

Result and Discussion

The effect of herbal cocktail powder dietary supplementation at different level on performance was shown in Table 1. Average daily gain (ADG) at the starter period (0-3wk) of the chickens in the T2 was higher than those in the C ($P<0.05$) while feed conversion ratio (FCR) and average daily feed intake (ADFI) of the chicken were not different ($P>0.05$) among studied groups. Doungmali (2014) found that chili and turmeric can increase feed intake because the active ingredient in crude extract of chili is capsaicin, which stimulate the secretion of enzymes in the digestive tract and help optimize digestion even more Sangaunphun report that chili and turmeric had no effect on FCR of chickens. Khacharoen found that garlic can improve body weight and FCR of chickens and pigs.

The effect of herbal cocktail powder dietary supplementation on immunity was shown in Table 2 and Table 3. The hemagglutination inhibition (HI) titer against NDV, at 3 week old chickens in the T2 group had higher ($P<0.05$)

than those in the C group. Niyomdecha and Khongsen (2015) reported that the active ingredient in crude extract of turmeric is curcumin, which stimulate B lymphocyte, T lymphocyte, Macrophage, Heterophil and NK cell, and affect to increase antibody. In broiler chickens, capsaicin in chili can inhibit lipid peroxidation Kaewdirak, 2006) but there was not agree with Jindamongkon et al. (2005), who reported that turmeric, fever vine and heart-leaved moonseed had no effect on immunity. Heterophil : Lymphocyte (H:L ratio) is an indicator of stress in chickens. In this study, H:L ratio at 2, 4 and 5 weeks old chicken in the T1 and T2 group had lower ($P<0.05$) than the C group, which was in agreement with earlier reports. Ganesh and Bharat (2007) reported that curcumin in turmeric affecting H:L ratio by lowering the ratio. In general, when chicken was in stress condition, corticosterone in adrenal cortex was released into the blood stream and then glucocorticoid would decrease the lymphocyte but increase the heterophil by increasing the secretion of the heterophil from hemopoetic system into the bloodstream (Harmon, 1998). This resulted in higher the ratio of H:L. From overall results of this study, the chickens offered half dose of dietary herbal cocktail powder supplementation had better growth rate and immunity, possibly the results of synergistic effect of active ingredient in supplemented herbal cocktail at optimal level. However, further study is required to elucidate this influence.

Conclusion

Dietary herbal cocktail powder supplementation at half dose of recommendation (chili 1.52, turmeric 3.50 and garlic 2.00 g/kg diet) improved average daily gain and immunity of broilers.

Table.1 Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of studied broiler chickens in periods of 0 to 3, 3 to 5, and 0 to 5 week of age.

Parameter	Group		
	Control	T1	T2
	0-3 week		
ADG	$42.25^{ab} \pm 1.25$	$39.61^{a} \pm 2.92$	$43.03^{b} \pm 0.95$
ADFI	55.11 ± 8.21	54.48 ± 2.28	56.94 ± 0.92
FCR	1.31 ± 0.23	1.38 ± 0.08	1.33 ± 0.02
	3-5 week		
ADG	65.97 ± 5.79	63.35 ± 6.13	64.54 ± 7.14
ADFI	145.57 ± 22.61	141.46 ± 20.79	161.98 ± 8.68
FCR	2.21 ± 0.31	2.26 ± 0.49	2.55 ± 0.44
	0-5 week		
ADG	51.74 ± 3.06	49.1 ± 3.98	51.63 ± 3.25
ADFI	91.29 ± 7.85	89.27 ± 9.61	98.96 ± 3.50
FCR	1.77 ± 0.20	1.83 ± 0.27	1.93 ± 0.19

Table.2 Hemagglutination inhibiion titer (HI-titer) against Newcastle's disease virus (NDV) of studied broiler chickens at 7, 14, 21, 28 and 35 days of age.

HI	Group		
	Control	T1	T2
7 day	4.75 ± 1.49	5.00 ± 1.20	4.88 ± 1.55
14 day	$4.88^{a} \pm 1.46$	$4.88^{a} \pm 0.83$	$6.63^{b} \pm 1.85$
21 day	$4.00^{a} \pm 1.69$	$5.25^{ab} \pm 1.04$	$6.25^{b} \pm 2.05$
28 day	7.88 ± 1.64	7.00 ± 2.88	8.13 ± 1.81
35 day	7.75 ± 2.25	7.20 ± 0.84	7.75 ± 1.49

Table.3 Heterophil : Lymphocyte (H:L ratio) of studied broiler chickens at 7, 14, 21, 28 and 35 days of age.

H:L Ratio	Group		
	Control	T1	T2
7 day	0.082 ± 0.038	0.054 ± 0.043	0.071 ± 0.045
14 day	0.245^{b} ± 0.175	0.123^{a} ± 0.029	0.134^{ab} ± 0.070
21 day	0.032 ± 0.031	0.029 ± 0.022	0.018 ± 0.038
28 day	0.227^{c} ± 0.075	0.096^{b} ± 0.044	0.022^{a} ± 0.023
35 day	0.138^{b} ± 0.058	0.076^{a} ± 0.052	0.099^{ab} ± 0.058

References

AL-Sultan S. I., 2003. The Effect of Curcuma longa (Tumeric) on Overall Performance of Broiler Chickens. International Journal of Poultry Science. 2 (5): 351-353.

Amad, A.A., Männer, K., Wendler, K.R., Neumann, K. and Zentek, J. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poultry Science. 90: 2811–2816.

Doungmali Pannee. 2014. The Effects of Curcuma Longa. And Capsicum Spp. On Productive Performance In Broiler. SKRU Academic journal. 7(1): 20-26.

Ganesh Chandra Jagetia and Bharat Aggarwal. 2007. "Spicing Up" of the Immune System by Curcumin. Journal of Clinical Immunology. 27(1):19-35

Harmon BG. 1998. Avian heterophils in inflammation and disease resistance. Poult Sci. 77(7): 972-977.

Horron,G. M. J.,Fennell,M. J.and Prasad,B. M. 1991. Effectsof dietarygarlic(Allium sativum) on performance, carcass composition and blood chemistry changes in broiler chickens. Canadian Journal of Animal science. 7l(3): 939-942.

Jafari RA , Jalali MR , Ghorbanpoor M , Saraei SM, 2008, Effect of dietary garlic on immune response of broiler chicks to live Newcastle Disease vaccine. Pakistan Journal of Biological Sciences. 11(14):1848-1851.

Jindamongkon Kitima, Isariyodom Supaporn, Tirawattanawanich Chanin, Kongkathip Ngampong, Mongkolsook Yupa, Santisopasri Wilai and Kongkathip Boonsong. 2005. The Effects of Extracts from *Curcuma longa*, *Paederia tomentosa* and *Tinospora crispa* on Stress and Immunity in Broiler Chickens. Available source : http://www.lib.ku.ac.th/kuconf/kc4302025.pdf. February 15, 2016.

Kabir S.M.L., Rahman M.M., Rahman M.B., Rahman M.M. and Ahmed S.U. 2004. The Dynamics of Probiotics on Growth Performance and Immune Response in Broilers, International Journal of Poultry Science. 3 (5): 361-364.

Kaewdirak Aungsumalee. 2006. Supplementation of Crude Extract from Capsicum spp. in Broilers Diet. Master's thesis. Department of animal science. Kasetsart University.

Khacharoen Yaowaman, Khacharoen Sarot, Ratanasethakul Cherdchai, Pakdee Pawadee and Srepraya Pituk. 2011. Effect of substituting garlic herbal for antibiotic growth promotors and dietary supplementation in commercial broilers and pig farm. Research abstracts Khon Kaen University

Niyomdecha Atchara and Khongsen Mongkon. 2015. Effects of turmeric(Curcuma longa Linn.) powder supplementation in diet on carcassquality and immune response of broiler chickens. Proceedings of 53rd Kasetsart University Annual. Bangkok

Sangaunphun Suchart, Songserm Ornprapun, Triwutanon Oratai and Plaiboon Atthawoot. 2009. Effect of Curcuma longa Linn. Marc Supplementation on Broiler Production Performance. The 6th KU-KPS Conference. pp. 249-256.

Yanin Tungsit, Poltee Nirun, Chukiatsiri Kridda and Panatuk Julakorn. 2016 Effects of dietary ground capsicum (Capsicum frutescens Linn.) supplementation on productive performances and carcass characteristic in Pradu Hangdum chicken. KHON KAEN AGR. J. 44 SUPPL. 1 : (2016). 459-464.

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Efficacy of Thai bentonite to ameliorate the adverse effects of mycotoxins contaminated diet in Cherry Valley ducks

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Introduction

Aflatoxins (AF) are the most common toxin produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B_1 (AFB_1) is the most hepato-toxic among AF (Godfrey et al., 2013). The symptoms of aflatoxicoses are loss of appetite, reduction of growth performance, feed efficiency, immunosuppression and mortality. (Bennett et al., 2007). Fumonisins (FMN) are a group of toxins produced by *Fusarium* fungi that can cause toxicitiesin liver andkidney of all animals. The toxins involve alteration of sphingolipid metabolism, cardiovascular dysfunction, oxidative stress, lipid peroxidation, DNA fragmentation, apoptosis and immunity (European Commission, 2003; Stockmann and Savolainen, 2008).

One suitable approach to detoxify the toxin is to combine toxin binder or adsorbent into animal feed to inhibit the bioavailability of the toxin (Basalan et al., 2006). Several reports demonstrated that toxin binders have shown considerable promise in preventing mycotoxicoses (Kossolova et al., 2009). Thai bentonite (TB) clay from Lopburi Province is capable to bind AFB_1 *in vitro* (Tengjaroenkul et al., 2013). However, *in vivo* report on efficacy of TB to reduce the toxic effects of mycotoxins is very limited. Thus, the purpose of the present study was to determine the efficacy of TB to ameliorate the adverse effects of the mixed AF and FMN contaminated in diet, and to compare its efficacy with HSCAS in the Cherry Valley ducks.

Materials and Methods

Animal, Diet and Experimental design

A total of 210, 7-d-old Cherry Valley ducks were randomly allocated into 4 treatments for a 6 weeks feeding trial. Experimental diets were formulated to meet nutritional requirements of ducks as recommended by NRC (1994)(Table 1). In this study, the contaminated diet contained total AF and total FMN as 35.78-39.62 and 286.46-326.40 ppb, respectively. The diets were prepared as follow: 1) basal diet (control group), 2) MD with contaminated corn (total AF = 70.42 ppb and total FMN = 580 ppb), 3) MD + 0.1% commercial HSCAS (ALCA Co., LTD., Thailand), and 4) MD + 0.2% TB. Each treatment had three replication pens with 14 birds per pen. Feed and water were provided ad libitum.

Productive performance

The ducks were daily recorded for clinical signs and mortality. At the end of the experiment, the ducks were weighed, and feed intake of each duck was brought to determine body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), feed consumption ratio (FCR) and coefficient of variation of body weight (CVBW).

Hematology and serum enzymes

At the end of the experiment, blood samples from wing veins of 9 birds from each treatment were collected to determine serum enzyme values. For the enzyme study, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analyzed.

Statistical analyses

Statistical analyses were performed using Proc. GLM (SAS). The differences of means were compared using Duncan's multiple range test (DMRT). Statements of statistical significance were based on $p<0.05$.

Results and Discussion

Productive performance

Our results indicated that both natural mycotoxins affected on productive performance by decrease BWG, ADG and ADFI, and increase FCR and CVBW ($p<0.05$) when compared with the control diet. HSCAS and TB mixed in MD reduced ADG, FCR, ADFI, CVBW and SVR ($p<0.05$) when compared with the MD. These observations are similar to several previous studies. Wan et al. (2013) reported that the natural AFB_1 less than 100 ppb linearly decreased the ADG ($p<0.05$) and linearly increased the mortality ($p<0.05$). Han et al. (2008) demonstrated that the ducks'

diet containing AFB_1 decreased BWG and FI ($p<0.05$), and increased FCR ($p<0.05$). In addition, Yang et al. (2014) demonstrated that ducks fed mycotoxin significantly reduced nutrient digestibility ($p<0.05$).

Serum enzymes

Serum enzyme data are presented in Table 3. ALT, AST and ALP activities can be used as specific measurement of liver function or injuries in poultry (Quist et al., 2000). In this study, MD alone demonstrated an increase of AST, ALT and ALP levels compared with the control diet. HSCAS and TB supplementation in MD significantly reduced ALT, AST and ALP levels ($p<0.05$) when compared with the MD. Similarly, previous study reported that ducks fed AFB_1 showed the increase of AST and ALT activities as well as AST:ALT ratio ($p<0.05$) (Chen et al., 2014). The MD in this study demonstrated the adverse effects by increase AST, ALT and ALP activities. These occur when liver tissue is injured, and the enzymes containing in the liver cells are released into the blood stream. Therefore, the levels of these enzymes were increased in the serum.

Toxin binders

In present study, both 0.1% HSCAS and 0.2%TB could ameliorate the adverse effects of mycotoxins on productive performance as well as serum enzymes when compared with the MD ($p<0.05$). HSCAS demonstrated a better efficacy to prevent mycotoxicoses than the TB. These results are consistent with previous studies reported by Miazzo et al. (2005) supplemented 0.3% sodium bentonite (SB) to AFB_1 and $FMNB_1$ in broiler diet, and reported that the SB could reduce the deleterious effects, particularly weight gain, serum biochemistry and liver lesions associated with the mycotoxins. Magnoli et al. (2008) added 0.3% natural bentonite to AF in broiler, and the bentonite reduced severity of hepatic histopathologyassociated with aflatoxicosis. Manafi et al. (2009) added 1% SB to AF broiler diet, and the SB reduced the toxicity of AF on growth performance and serum biochemistry. Kermanshahi et al. (2009) suggested that 0.5-1.0% SB might be used for reducing the AFB_1 effects in terms of growth performance, carcass quality and serum biochemistry in broiler.

Conclusions

The AF and FMN contaminated diet decreased productive performance and serum enzyme levels of Cherry Valley ducks. Supplementation mycotoxins contaminated diet with 0.1% HSCAS and 0.2% TB can reduce the adverse effects; however, 0.1% HSCAS almost completely ameliorated the adverse effects when compare with the control.

Table 1. Ingredient and nutrient composition (%) of experimental diets[1] of Cherry Valley duck

Item	Starter phase (1-3wk)	Grower phase (3-6wk)
Ingredients, %		
Corn	49.39	56.26
Soybean meal	28.00	24.50
Rice bran oil	4.00	4.50
Rice bran	6.80	7.50
Fish meal	9.00	3.50
Limestone	0.10	1.46
Dicalcium phosphate	1.50	1.10
Choline chloride	0.10	0.10
Salt	0.18	0.30
DL-methionine	0.23	0.15
L-lysine	0.20	0.13
Vitamin-mineral premix[2]	0.50	0.50
Total	100.00	100.00
Chemical composition[3]		
CP, %	22.50	18.41
Total phosphorus, %	0.65	0.58
Calcium, %	1.24	1.22
ME, kcal/kg[4]	3,169	3,250

[1]The diet of treatment 1 (T1, basal diet) was formulated without contaminated corn; the diets of treatment 2, 3 and 4 (T2 to T4) were formulated by replacing normal corn with mycotoxins contaminated corn at 100%.

[2]Vitamin-mineral premixes supplied the following per kilogram of diet: vitamin A 9,500 IU; vitamin D, 3,600 IU; vitamin E, 40 IU; vitamin K_3, 5.5 mg; thiamine, 3.0 mg; riboflavin, 12.6 mg; pyridoxine, 5.0 mg; vitamin B_{12}, 0.025 mg; pantothenic acid, 12 mg; niacin, 75 mg; choline, 1,000 mg; folic acid, 2.1 mg; biotin, 0.25 mg; Mn, 90 mg; Zn, 86 mg; Fe, 80 mg; Cu, 8.6 mg; Co, 1.5 mg; I, 0.35 mg; Se, 0.31 mg; thoxyquin, 125 mg

[3]The chemical composition of the diets was determined according to AOAC (1995)

[4]ME = Metabolizable energy calculated (NRC, 1994)

Table 2. Efficacy of adsorbents to ameliorate the toxin effects of AF and FMN on productive performance and survival of Cherry Valley ducks

Item	T1	T2	T3	T4	SEM
BWG, (g)	2,492.2[a]	2,304.7[e]	2,453.9[b]	2,419.8[c]	10.67
ADG, (g)	59.3[a]	54.9[e]	58.4[b]	57.6[c]	0.76
ADFI, (g)	167.6[a]	165.3[c]	167.0[b]	166.9[b]	0.31
FCR	2.35[d]	2.51[a]	2.38[c]	2.41[b]	0.02
CVBW, (%)	6.83[b]	11.39[a]	7.13[b]	7.06[b]	0.38
SVR, (%)	100[a]	94.45[b]	100[a]	100[a]	1.24

[a-d] Values within a row with the difference superscripts significantly different ($p<0.05$)
BWG = body weight gain; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed consumption ratio; CVBW = coefficient of variation of body weight; SVR = survival rate; SEM (standard error of mean) = dividing the standard deviation by the square root of sample size
T1 = basal diet (control diet), without contaminated corn, T2 = mycotoxins contaminated diet (MD), with contaminated corn (total AF (B1, B2), 70.42 ppb and total FMN (B1, B2), 580 ppb), T3 = MD + 0.1% HSCAS (hydrated sodium calcium aluminosilicate), and T4 = MD + 0.2% Thai bentonite clay (TB) (collected from Lopburi Province)

Table 3. Efficacy of adsorbents to ameliorate the toxin effects of AF and FMN on hematology and serum enzymes of Cherry Valley ducks

Item	T1	T2	T3	T4	SEM
Blood hematology					
PCV, (%)	38.33[a]	32.33[d]	36.11[b]	35.22[bc]	0.59
Hb, (g/dl)	12.77[a]	9.60[d]	12.22[b]	11.72[c]	0.22
Serum enzyme					
ALT, (U/L)	29.33[c]	49.33[a]	38.33[b]	38.00[b]	2.01
AST, (U/L)	30.50[c]	62.67[a]	39.33[c]	41.33[b]	2.50
ALP, (U/L)	1,196.33[d]	2,067.33[a]	1,369.33[c]	1,424.67[c]	30.34

[a-d]Values within a row with the difference superscripts significantly different ($p<0.05$)
PCV = pack cell volume; Hb = hemoglobin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; SEM (standard error of mean) = dividing the standard deviation by the square root of sample size

T1 = basal diet (control diet), without contaminated corn,T2 = mycotoxins contaminated diet (MD), with contaminated corn (total AF (B1, B2), 70.42 ppb and total FMN (B1, B2), 580 ppb), T3 = MD + 0.1% HSCAS (hydrated sodium calcium aluminosilicate), and T4 = MD + 0.2% Thai bentonite clay (TB) (collected from Lopburi Province)

References

Basalan M, Gungor T, Aydogan I, Hismiogullari S E, Erat S and Erdem E 2006 Effects of feeding mycotoxin binder (HSCAS) at later ages on gastrointestinal environment and metabolism in broilers. Archiva Zootechinca, 9(1): 5-9

Bennett J W, Kale S and Yu J 2007 Aflatoxins: Background, Toxicology, and Molecular Biology. In: Simjee S. Infectious Disease: Foodborne Diseases. Humana Press Inc, Totowa NJ, pp 355-373

Chen X, Horn N, Cotter P F and Applegate T J 2014 Growth, serum biochemistry, complement activity, and liver gene expression responses of Pekin ducklings to graded levels of cultured aflatoxin B1. Poultry Science, 93:2028–2036

European Commission 2003 Fumonisins. Updated opinion of the Scientific Committee on Food on Fumonisin B1, B2 and B3. SCF/CS/CNTM/MYC/28 Final

Godfrey S B, David K, Lubega A, Ogwal-Okeng J, Anokbonggo W W and Kyegombe D B 2013 Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In: Aflatoxins-Recent Advances and Future Prospects. MR Abyaneh (ed). Croatia: Intech, 239-265

Han X Y, Huang Q C, Li W F, Jiang J F and Xu Z R 2008 Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livestock Science, 119:216–220

Kermanshahi H, Hazegh A R and Afzali N 2009 Effect of Sodium Bentonite in Broiler Chickens Fed Diets Contaminated with Aflatoxin B1. Journal of Animal and Veterinary Advances, 8: 1631-1636

Kossolova A, Stroka J, Breidbach A, Kroeger K, Ambrosio M, Bouten K and Ulberth F 2009 Evaluation of the effect of mycotoxin binders in animal feed on the analytical performance of standardized methods for the determination of mycotoxins in feed. JRC Scientific and Technical Reports, The European Commission, 5-12

Magnoli C E 2008 Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens, Poultry Science 90(2):352-357

Manafi M, Umakantha B, Swamy H D N and Mohan K 2009 Evaluation of high-grade SB on performance and immune status of broilers, fed ochratoxin and AF. World Mycotoxin Journal, 2:435-440

Miazzo R, Peralta M F, Magnoli C,. Salvano M, Ferrero S, Chiacchiera S M, Carvalho E C Q, Rosa C A R and Dalcero A 2005 Efficacy of Sodium Bentonite as a Detoxifier of Broiler Feed Contaminated with aflatoxin and fumonisin, Poultry Science 84:1–8

Quist C F, Bounous D I, Kilburn J V, Nettles V F and Wyatt R D 2000 The effect of dietary aflatoxin on wild turkey poults. Journal of Wildlife Disease, 36:436–444

Stockmann J and Savolainen K 2008 A review of the toxic effects and mechanisms of action of fumonisin B1. Human & Experimental Toxicology**,** 27(11):799–809

Tengjaroenkul B, Pimpukdee K and Saipan S W P 2013 An In Vitro Comparative Study of Aflatoxin B1 Adsorption by Thai Clay and Commercial Toxin Binders. Thai Journal of Veterinary Medicine, 43:491–495

Wan X L, Yang Z B, Yang W R, Jiang S Z, Zhang G G, Johnston S L and Chi F 2013 Toxicity of increasing aflatoxin B1 concentrations from contaminated corn with or without clay adsorbent supplementation in ducklings. Poultry Science, 92:1244–1253

Yang Z B, Wan X L, Yang W R, Jiang S Z, Zhang G G, Johnston S L and Chi F 2014 Effects of naturally mycotoxin-contaminated corn on nutrient and energy utilization of ducks fed diets with or without Calibrin-A. Poultry Science, 93:2199–2209

PO-06-53

Effects of Energy Source in Meat Type Ducks Diets during Starter to Grower Period

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Introduction

The commercial production of meat type ducks has expanded rapidly nowadays. Feedis the largest singlecostitem for poultry production. Reduced feed costslead to improvedproductionefficiency and profitability of poultry enterprises. Manipulation of feed ingredients is important strategies for reducing feed costs. Corn is themainingredient used in poultry diets and contributes themajor feedingredientcostin animal production.The demand for corn in commercial poultry production has risen. However, aflatoxin contamination of corn is an important problem in poultry production. AFs are the secondary metabolite produced by fungi, is extremely toxic, mutagenic and carcinogenic. Meat duck is one of the animals most sensitive to AFs. High levels of AFs can cause acute death in meat-type ducks, and prolonged exposure to low levels of AFs can induce chronic toxicity, resulting in their slow growth and reduced production performance (Shi *et al.*, 2010; Lv *et al.*, 2013; Xie *et al*., 2015). Replacingcorn with alternativeenergy feed sources has been interested recently in commercial production of meat type ducks.

Carbohydrate especially, maize is a majorenergy sourcein the diet of meat type ducks. Replacingmaize with alternativefeed sourcessuch as broken rice, which has high price, is quite acceptable in poultry diets. The present study aims to study the effects of energy feedstuffs which are cassava and corn in meat type ducks diet during starter to grower Period (1-16 days) on production performance, carcass quality, lipid metabolism, and intestinal morphology.

Materials and methods

Experimental design, animals and diets

This study was conducted at The Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. A total of 160 one-day-old Cherry Valley meat ducklings (male duck) were randomly assigned to 2 dietary treatment groups. There were 8 replicates for each treatment group and 10 male ducks for each replicate. This experiment employed the completely randomized design. Proximate analyses of the two diets were conducted using the AOAC (2000) protocols. The control and experimental ducks were fed with basal diets which were formulated to meet or exceed the nutrient requirements for meat ducks inNRC (1994)(Table 1). However, the diets were difference in the energy feedstuffs. Cassava and corn were used in diets which were modified by age-dependent of ducks. There were 2 periods which are starter (d 1 to 9) and grower (d 10 to 16). Total ducklings were divided into 2 groups, as corn based diets groups (corn 1-42 days) and cassava based diets groups (cassava 1-16 days after that corn until 42 days).

The ducklings were raised in closed farming system using the Evaporative Cooling System to control air ventilation and temperature. They had free access to food and water. 23-hr light was provided throughout the experimental period.

Sampling and measurement

Production performances:

At d 9 and 16, after feed withdrawal for 12 h, the ducks and feed in each cage were weighed. Body weight gain (BWG), average daily gain (ADG), cumulative feed intake (FI), and feed conversion ratio (FCR) were calculated.

Lipid metabolism:

At d 42 of age, 2 ducks from each replicate were selected and bled through the jugular vein. The blood samples were immediately placed on ice, transported to the laboratory within 3 h of collection, and centrifuged at 3,000 $\times g$ for 15 min in a refrigerated centrifuge at about 4 °C. Serum was collected and stored at –20 °C until certain biochemical parameters were assayed. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) concentrations were analyzed. They are important indices to evaluate lipid metabolism.

Carcass quality and intestinal morphology:
At 42 d of age, 6 ducks from each replicate were randomly selected for evaluation of carcass traits and intestinal morphology. Feed was withdrawn 4 h before processing, and then the birds were weighed, slaughtered, defeathered, and weighed to obtain carcass weights, breast meat, thigh, drumstick, wing, abdominal fat, liver and gizzard weight. For intestinal morphology, the villus height and crypt dept of each intestinal tract segments were measured. The ratio of villus height and crypt dept was calculated (Nunez *et al*., 1996).
Statistical analysis
Using the Repeated Measurements in Completely Randomized Design, all the experimental data were analyzed using the Analysis of Variance (ANOVA). Means where significant differences occurred were separated using the Duncan's new multiple range test (Duncan, 1955). Statements of significance were based on P
$Yij = \mu + Ai + \varepsilon ij$
Where; Yij is the observed response, Ai is the effect of diet and ε ij is experimental error; ε ij ~NID (0, Δ2).

Result and Discussion

The comparative effects of corn versus cassava as dietary energy sources on production performance of male Cherry Valley ducks are shown in Table 2. The result showed that at starter period (d9) significantly increased body weight and ADG, thus decrease in FCR ($P<0.01$). Whereas at grower period (d16), significantly increased body weight, feed intake, and ADG ($P<0.01$), thus no changed in FCR. The results of this study demonstrate that cassava is comparable to replace corn in providing the energy requirements of growing meat-type ducks. Moreover, the replacingcorn with cassava in diets of starter meat type ducks could solve the aflatoxin toxicity problem. Since the ducklings are more sensitive to both the toxic and the carcinogenic properties of aflatoxin (Carnaghan, 1965).
Table 3 shows the effects of the corn-based diet and the cassava-based diets on the serum lipid contents. After 42d of feeding, the cassava-fed (1-16 day) ducks had serum triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol levels that were not significantly different from those of the control group. Ducks fed the cassava-based diets from 1-16 d of age showed not significantly difference in carcass quality visceral organ (Table 4) and intestinal morphology (villus height, crypt dept and the ratio of villus height and crypt dept).

Conclusion

The results of this study suggest that cassava has nutritional qualities that are similar to those of corn. Its effects on growth rate were comparable to that of corn up to the end of the starter stage. Cassava also proved to be comparable to corn in its effects on the ducks' blood and liver lipid levels and on carcass quality. While feeding cassava could result to more feeds being consumed and thus, poorer FCR, this could be balanced by the fact that cost-wise, cassava is usually cheaper than corn. The high fiber content of cassava and its dusty texture, which can limit the level of its inclusion in the diet, can be minimized with the help of pelletizing machines.

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Table1. Ingredients and chemical composition of the experimental diets.

Ingredients (%)	Starter period (1-9 d)		Grower period (10-16 d)	
	Corn	Cassava	Corn	Cassava
Corn	54.68	-	60.20	-
Cassava	-	47.03	-	51.78
Palm Oil	1.18	1.52	1.05	1.43
Soybean Meal (48 %CP)	39.29	46.73	33.76	41.95
L-Lysine	0.19	0.06	0.17	0.02
DL-Methionine*	0.20	0.29	0.19	0.29
L-Threonine	0.05	0.06	0.08	0.09
MDCP**	2.16	2.25	2.19	2.30
Limestone	1.40	1.17	1.43	1.17
Salt	0.18	0.18	0.18	0.18
Premix***	0.50	0.50	0.50	0.50
Choline Chloride, 75%	0.11	0.16	0.19	0.25
Antioxidant	0.02	0.02	0.02	0.02
Mycotoxin binder	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Calculated nutrients composition				
Energy (ME, Kcal/kg)	2,850.00	2,850.00	2,900.00	2,900.00
Protein (%)	22.00	22.00	20.00	20.00
Fat (%)	3.75	2.32	3.79	2.22
Fiber (%)	4.12	5.01	3.87	4.85
Calcium (%)	1.00	1.00	1.00	1.00
Total Phosphorus (%)	0.85	0.82	0.83	0.81
Avail. Phosphorus (%)	0.50	0.50	0.50	0.50
Salt (%)	0.20	0.20	0.20	0.20
Lysine (%)	1.35	1.35	1.20	1.20
Methionine + Cystine (%)	0.90	0.90	0.84	0.84
Methionine (%)	0.54	0.58	0.50	0.54
Threonine (%)	0.90	0.90	0.85	0.85
Tryptophan (%)	0.29	0.30	0.26	0.28
Choline (%)	0.15	0.15	0.15	0.15

*Synthetic DL-Methionine was supplied by Sumitomo Chemical, Japan. **Monodicalciumphosphate (P 21%).

***Premix, provided/kg of diet: vitamin A 11,000 IU, vitamin D3 5,000 IU, vitamin E 75 IU, vitamin K1 3 mg, vitamin B1 3 mg, vitamin B2 8 mg, niacin 60 mg, pantothenic acid 15 mg, pyridoxine 4 mg, folic acid 2 mg, biotin 0.15 mg, choline 1,600 mg, vitamin B12 0.016 mg, Mn 120 mg, Zn 100 mg, Cu 16 mg, Selenium 0.30 mg, I 1.25mg, Fe 40 mg.

Table 2. Comparative effects of corn *vs*. cassava on production performance of meat-type ducks

Period (d)	Items	Corn Based Diet	Cassava Based Diet	P-value	SEM
1	Initial weight (g/duck)	53.56	53.61	0.52	0.04
1-9	Body weight (g/duck)	293.85	320.73**	<0.01	1.23
	Feed Intake(g/duck /d)	44.13	45.28	0.55	0.27
	ADG***	26.71	29.67 **	<0.01	0.21
	FCR	1.65	1.53**	<0.01	0.01
1-16	Body weight (g/duck)	733.82	812.50**	<0.01	3.66
	Feed Intake(g/duck /d)	65.60	72.15**	<0.01	0.56
	ADG	42.47	47.40**	<0.01	0.34
	FCR	1.54	1.52	0.92	0.01
1-28	Body weight (g/duck)	1789.14	1882.51**	<0.01	8.37
	Feed Intake(g/duck /d)	103.58	108.91**	<0.01	0.97
	ADG	61.99	65.24**	<0.01	0.48
	FCR	1.67	1.67	0.23	0.01
1-35	Body weight (g/duck)	2503.97	2604.11**	<0.01	10.52
	Feed Intake(g/duck /d)	118.08	125.34**	<0.01	1.07
	ADG	69.88	72.80**	<0.01	0.43
	FCR	1.69	1.72	0.06	0.01
1-42	Body weight (g/duck)	3015.16	3068.46	0.03	11.77
	Feed Intake(g/duck /d)	132.04	138.35**	<0.01	1.27
	ADG	70.43	71.84	0.15	0.39
	FCR	1.90	1.93	0.06	0.01

** indicates significantly different between the corn based diet and cassava based diet (P<0.01).
***Average daily gain.

Table 3. Effects of dietary energy source on chemical composition analysis

Items (mg/dl)	Corn Based Diet	Cassava Based Diet	P-value	SEM
Serum lipid profiles				
Triglyceride	84.47	75.89	0.49	3.07
Cholesterol	160.96	169.36	0.76	2.31
HDL - Cholesterol	95.05	109.44	0.43	3.32
LDL - Cholesterol	53.88	67.56	0.39	2.15
VLDL - Cholesterol	16.89	15.18	0.34	0.64

Table 4. Effects of difference energy source on carcass quality and visceral organ

Items (%)	Corn Based Diet	Cassava Based Diet	P-value	SEM
Dressed weight	87.66	88.09	0.42	0.11
Skin	16.25	15.94	0.10	0.17
Outer breast	11.69	12.29	0.06	0.13
Inner breast	1.74	1.83	0.06	0.02
Thigh	9.02	8.9	0.17	0.04
Drumstick	7.72	7.68	0.72	0.04
Wing	11.07	11.1	0.74	0.03
Abdominal fat	0.84	0.77	0.06	0.02
Liver	2.56	2.55	0.12	0.04
Gizzard	3.33	3.37	0.56	0.03

References

AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th Ed. Association of official agriculture chemists, Washington, D.C.

Carnaghan, R.B.A. 1965. Hepatic tumors in ducks fed a low level of toxic groundnut meal. **Nature**. 208: 308.

Duncan, D.B. 1955. Multiple range and multiple F test. **Biometrics.** 11: 1-42.

Lv, W.X., J.H. He, H.G. Song, M.M. Liu, X.L. Jin, Li JB. 2013. Aflatoxin B1 affects growth, liver tissue structure and immune related gene expression of meat duckling. **Chinese Journal of Animal Nutrition**. 4: 812-818.

National Research Council. 1994. Nutrition Requirement of Poultry–NRC-. 9th ed. National Academy of Science, Washington D. C. p. 155.

Nunez, M.C., J.D. Bueno, M.V. Ayudarte, A. Almendros, A. Rios, M.D. Suarez, A. Gil. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursery piglets. **J. nutr.** 126: 933-944.

Shi D.Y., P.F. Li, M.S. Guo, N. Liu, J.Y. Guo, Z.X. Tang. 2010. Effects of different doses of aflatoxin B1 on growth of ducklings. **Chinese Journal of Veterinary Medicine**; 4: 22-23.

Xie Q., M.J. Sun, W.H. Chang, Z.Y. Liu, J.S. Ma, G.H. Liu. 2015. Effects of Aflatoxins and Absorbents on Growth Performance and Immune Indices of Meat Ducks. **Chinese Journal of Animal Nutrition**. 1: 204-211.

PO-06-56

Various Levels of Milk By-products in Weaning Diet on Growth Performance, Blood Profiles and Intestinal Morphology

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INTRODUCTION

There are many challenges in recent Korea pork industry from FTA contract, disposal for livestock excretion, diseases, and production cost. Feed cost accounts for 50~60% of the production cost. Feed cost is especially high in Korea because Korea feed industry highly depends on importation for main feed ingredients. To stabilize and develop pig industry in Korea, production cost should be lowered but import dependence of feed ingredients fluctuates so it makes stabilizing cost difficult. Not only Korea but also other many countries try to find various ways to solve those problems. One of the solutions is reducing the expensive ingredients in feed. Generally, lactose and whey powder are main feed ingredients in weaning pigs diet in Korea. It is well known that using lactose and whey powder in weaning pig's diet can maintain an enhanced intestinal environment in pig (Wolter et al., 2003). Moreover, the ability to grow and to enhance feed intake can be achieved by supplementation of milk by-product in weaning pig diet (Mahan et al., 2004; Cromwell et al., 2008). However, international price trends for dairy products are severely unstable, and its price is also expensive relatively compared to grains (USDA, 2015). Therefore weaning pig diet containing high levels of milk by-products results in an expensive weaning pig diet and it causes an increasing cost of pork production. Supplementation of milk by-products in weaning pig diets helps feed intake and growth performance to increase (Lawrence et al., 1983). However lactase, the enzyme associated with the digestion of milk carbohydrates, is high level at birth, and reaches a peak at 2 weeks of age, and then rapidly decreases (Coffey et al., 2000). It can explain that why reducing lactose content has no negative effect on weaning pigs after 1-2 weeks after weaning when development of enzyme activation is almost finished. Therefore, this study was conducted to evaluate various levels of milk by-products in weaning pig diet on growth performance, blood profiles, intestinal morphology, muscle fiber size, diarrhea incidence and economical analysis in weaning pigs.

MATERIALS AND METHODS

A total of 160 crossbred ([Landrace x Yorkshire] x Duroc) piglets were used in this experiment. Weaning pigs (averaging 5.79 ± 1.527 kg initial body weight) were assigned to 4 treatments based on sex and initial body weight with 5 replicates of 8 pigs per pen according to randomized complete block design (RCBD). Diets were divided into two phases (d 0 to 14 and d 15 to 35). Treatments included: 1) A (corn-soybean meal based diet, levels of milk products, phase1 30%, phase2 15%), 2) B (levels of milk products, phase1 20%, phase2 10%), 3) C (, levels of milk products, phase1 10%, phase2 5%), 4) D (levels of milk products, phase1 5%, phase2 0%). The experimental pigs were housed in 1.54 x 1.96m^2 plastic floor. Feed and water were provided *ad-libitum* through feeder and nipple during whole experimental periods. The ambient temperature in the weaning house was maintained at 31 °C, and then gradually fallen to 27 °C at the end of the experiment. Body weights and feed intake were recorded at 0, 2 and 5 weeks to calculate average daily gains (ADG), average daily feed intakes (ADFI) and gain to feed ratio (G/F ratio). Blood samples were taken from the jugular vein of each pig for measuring blood urea nitrogen (BUN) concentration, IGF-1 concentration and serum immunoglobulins (IgA, IgG) when the body weights were recorded. Observation of diarrhea incidence was conducted every 8:00 am for 35 days. Data was recorded by each pen and divided into 2 phases (phase1 and phase2). Score of diarrhea incidence was given into 5 numbers by counting pigs with evidence of watery diarrhea (0=No evidence of water diarrhea, 1=2pigs show evidence of watery diarrhea, 2=4pigs, 3=6pigs and 4=all pigs show evidence watery diarrhea in the pen). Three pigs were selected from each treatment at 14^{th} and 35^{th} days of experiment. The selected pigs were moved to individual cage and fasted overnight. Feed was supplied 3 hrs before an atomy, the pigs were slaughtered after 30 minutes at the last feeding and small intestine was collected. Analysis of the experimental diets was conducted according to the methods of the AOAC (2000). Statistical analysis was carried out by least squares mean comparisons using General Linear Model (GLM) procedure in SAS (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

As shown in Table 1, there were significant differences in body weight ADG, ADFI and G/F ratio among treatments during 3-5 week and whole experimental period (linear, P<0.05). However, there was no significant difference in 0-2 weeks on growth performance. These results could be explained by secretion of digestive enzymes and utilization of dietary milk by-products. Result of serum creatinine concentration was shown in Table 2 and Table 3. The concentration of BUN, creatinine, IgA and IgG had no significant difference among treatments during whole experimental period. Therefore, these results indicated that protein utilization for pigs fed low levels of milk by-products did not show any problem compared to high milk by-products treatment. However, IGF-1 concentration had significant difference among treatments (quadratic response, P<0.05). In 5 week, IGF-1 concentration of C treatment was higher than other treatments. As shown in Table 4, in weaning period, diarrhea incidence had no significant difference by various levels of milk by-products in weaning pig diet. Table 5 showed villi height and crypt depth of small intestine and muscle fiber size. There was no effect of various levels of milk by-products in intestinal morphology and muscle cell size.

Table 1. Influence of various milk by-products levels in weaning pig diet on growth performance in weaning pigs[1)]

Criteria	Treatment[2)]				SEM[3)]	*P* - value	
	A	B	C	D		Linear	Quadratic
Body weight, kg							
Initial	5.97	5.97	5.97	5.97	-	-	
2 week	7.56	7.42	7.26	7.09	0.383	0.24	0.87
5 week	12.75^{a}	12.94^{a}	11.53^{ab}	10.46^{b}	0.653	<0.01	0.13
ADG, g							
0-2 week	114	103	92	80	9.2	0.24	0.86
3-5 week	247^{AB}	263^{A}	203^{BC}	160^{C}	15.4	<0.01	<0.05
0-5 week	194^{a}	199^{a}	159^{ab}	128^{b}	11.7	<0.01	0.13
ADFI, g							
0-2 week	201	192	187	171	6.9	0.18	0.74
3-5 week	539	553	517	451	22.3	0.04	0.11
0-5 week	398	405	375	336	15.7	0.04	0.16
G:F ratio							
0-2 week	0.551	0.519	0.486	0.447	0.0348	0.35	0.88
3-5 week	0.470	0.479	0.385	0.341	0.0220	0.02	0.25
0-5 week	0.489	0.493	0.418	0.366	0.0197	0.02	0.23

[1)] A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2)] A : Milk product 30%-15%, B : Milk product 20%-10%, C : Milk product 15% 10%, D : Milk product 5%-0%.
[3)] Standard error of mean.
[ABC] Means in a same row with different superscript letters significantly differ (P<0.01)
[ab] Means in a same row with different superscript letters significantly differ (P<0.05).

Table 2. Influence of various milk by-products levels in weaning pig diet on BUN, IGF-1 and creatinine in weaning pigs[1)]

Criteria	Treatment[2)]				SEM[3)]	*P* - value	
	A	B	C	D		Linear	Quadratic
BUN, mg/ml							
Initial	----------9.35----------				-		-
2 week	13.49	9.33	15.01	16.63	1.158	0.17	0.14
5 week	13.27	13.37	16.17	16.20	0.722	0.10	0.98
IGF-1, mg/ml							
Initial	----------52.98----------				-		-
2 week	72.16	80.47	73.02	62.03	5.381	0.50	0.37
5 week	57.66	67.03	69.20	47.23	5.451	0.95	0.05
Creatinine, mg/ml							
Initial	----------1.03----------				-		-
2 week	0.67	0.57	0.65	0.75	0.036	0.54	0.11
5 week	0.73	0.73	0.74	0.81	0.205	0.11	0.35

[1)] A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2)] A : Milk product 30%-15%, B : Milk product 20%-10%, C : Milk product 15% 10%, D : Milk product 5%-0%.
[3)] Standard error of mean.

Table 3. Influence of various milk by-products levels in weaning pig diet on IgA and IgG in weaning pigs[1)]

Criteria	Treatment[2)]				SEM[3)]	*P* - value	
	A	B	C	D		Linear	Quadratic
Serum IgA, mg/ml							
Initial	----------0.10----------				-		-
2 week	0.17	0.27	0.25	0.32	0.025	0.11	0.75
5 week	0.79	0.73	0.98	0.61	0.084	0.85	0.51
Serum IgG, mg/ml							
0-2 week	----------1.31----------				-		-
3-5 week	1.78	1.95	2.00	1.87	0.052	0.43	0.29
0-5 week	3.40	3.36	3.24	3.06	0.080	0.11	0.48

[1)] A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2)] A : Milk product 30%-15%, B : Milk product 20%-10%, C : Milk product 15% 10%, D : Milk product 5%-0%.
[3)] Standard error of mean.

Table 4. Influence of various milk by-products levels in weaning pig diet on diarrhea incidence in weaning pigs[1)]

Criteria	Treatment[2)]				SEM[3)]	*P* - value	
	A	B	C	D		Linear	Quadratic
Diarrhea scores[3)]							
0-2 week	1.29	1.26	1.32	1.20	0.055	0.66	0.73
3-5 week	1.06	0.84	0.92	1.00	0.054	0.64	0.09
0-5 week	1.15	1.01	1.08	1.07	0.049	0.60	0.30

[1)] A : Milk product 30%-15%, B : Milk product 20%-10%, C : Milk product 15% 10%, D : Milk product 5%-0%.
[2)] Standard error of mean.
[3)] 0: No signs of diarrhea in pen, 4: All pigs had signs of diarrhea in pen.

Table 5. Influence of various milk by-products levels in weaning pig diet on villous height and crypt depth of small intestine and muscle fiber size in weaning pigs[1)]

Item	Treatments[2)]				SEM[2)]	*P*-value	
	A	B	C	D		Linear	Quadratic
Weaning phase Ⅰ (2week), um							
Proximal SI							
Villi height	270.84	280.45	283.90	281.38	20.019	0.91	0.91
Crypt depth	184.00	242.93	220.61	225.21	18.012	0.76	0.76
Mid SI							
Villi height	261.85	245.76	258.28	252.43	10.810	0.96	0.98
Crypt depth	281.86	212.67	237.72	251.30	19.440	0.96	0.61
Distal SI							
Villi height	282.67	256.27	273.43	224.96	14.209	0.33	0.66
Crypt depth	259.30	251.29	258.29	229.45	20.197	0.81	0.83
Muscle fiber							
Size	19.30	17.27	18.63	13.90	4.187	0.23	0.55
Weaning phase II (5week), um							
Proximal SI							
Villi height	446.20	441.12	488.36	512.10	13.901	0.09	0.81
Crypt depth	304.75	321.31	292.49	337.66	8.917	0.39	0.27
Mid SI							
Villi height	344.15	350.52	389.46	379.89	10.717	0.23	0.37
Crypt depth	317.85	324.22	328.73	310.27	12.365	0.84	0.72
Distal SI							
Villi height	338.92	349.90	375.93	375.13	8.704	0.11	0.34
Crypt depth	290.05	307.78	281.46	283.34	6.556	0.39	0.79
Muscle fiber							
Size	24.06	22.11	22.71	27.29	0.817	0.09	0.15

[1)] A total of 160 crossbred pigs were fed from average initial body weight 5.97 ± 1.527 kg.
[2)] A : Milk product 30%-15%, B : Milk product 20%-10%, C : Milk product 15% 10%, D : Milk product 5%-0%.
[3)] Standard error of mean.

REFERECES

Cromwell, G. L., G. L. Allee, and Mahan D. C. 2008. Assessment of lactose level in the mid- to late-nursery phase on performance of weanling pigs. J. Anim. Sci. 86:127–133.

Lawrence, N. J. and Maxwell, C. V. 1983. Effect of dietary fat source and level on the performance of neonatal and early weaned pig. J. Anim. Sci. 57:936–942.

Mahan, D. C., Fastinger, N. D. and Peters, J. C. 2004. Effects of diet complexity and dietary lactose levels during three starter phases on postweaning pig performance. J. Anim. Sci. 82:2790–2797.

Richard D. Coffey, Gary R. Parker, Kevin M. Laurent. 2000. Feeding and Managing The Weanling Pig. Department of Animal Sciences. ASC-149.

Wolter, B. F., Ellis, M., Corrigan, B. P., DeDecker, J. M., Curtis, S. E., Parr, E. N. and Webel, D. M. 2003. Impact of early postweaning growth rate as affected by diet complexity and space allocation on subsequent growth performance of pigs in a wean-to-finish production system. J. Anim. Sci. 81:353–359.

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Evaluation of Rapeseed Meal Supplementation on Growth Performance, Blood Profiles and Immune Response in Weaning Pigs

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INTRODUCTION

The main cost of pig production is affected by feed cost which impacts pork producers' profitability. Soybean meal (SBM) is the most widely used protein supplement throughout the world whereas increasing dietary supplementation level of SBM could be increased feed cost in pig diet (Patrick et al., 2010). Rapeseed meal (RSM) is a by-product of oil extraction from rapeseed and contains 33 to 35% protein, 10% crude fiber but energy is not high (Bell, 1984). RSM has been used growing-finishing pig diets because of glucosinolate (Gls) which cause thyroid hypertrophy to young animals. Erucic acid appears to have toxic effects on the heart at high enough doses, an association between the consumption of rapeseed oil. In addition, voluntary feed intake could be affected by inclusion of RSM in the diets because of decreased feed consumption due to high content of erucic acid, bitter taste of sinapine and astringent effect in the mouth by tannin (Mawson et al., 1994). Therefore, the objective of the present study was to evaluate the effect of dietary supplementation levels of RSM on growth performance, blood profiles and immune response of weaning pig.

MATERIALS AND METHODS

A total of 120 cross-bred weaning pigs ([Yorkshire × Landrace] × Duroc) with an initial body weight (BW) of 7.28 ± 863kg were allotted to 5 treatments in a randomized complete block (RCB) design. Five experimental diets containing different levels of RSM (0, 2, 4, 6, and 8%) were provided to weaning pigs. Feed and water were provided *ad libitum* through a feeder and a nipple installed in pen. Body weight and feed intake were recorded at 2nd, 3th and 6th week. Blood samples were taken from the jugular vein of randomly selected six pigs in each treatment for measuring blood urea nitrogen (BUN), serum immunoglobulins (IgA, IgG), HDL cholesterol, LDL cholesterol, total cholesterol, glucose. Data were analyzed by ANOVA for a completely randomized design using the GLM procedure of SAS (SAS Institute, 2009).

RESULTS AND DISCUSSION

Table 1 showed the effects of supplementation of rapeseed meal (RSM) on growth performance in weaning pigs. There was no significant difference on growth performance during the whole experimental period. In addition, glucose and total cholesterol, HDL cholesterol, LDL cholesterol were not affected by dietary RSM supplementation in weaning pig diets (Table 2). Thyroid hormone is an important modulator of intermediary metabolism by hypercholesterolemia of hypothyroidism and associated with altered lipoprotein metabolism and increase serum LDL cholesterol (Brent, 1994). However, there was no significant difference. The blood urea nitrogen (BUN) concentration was decreased as dietary RSM level increased in 6 week (linear response, $P<0.01$). Protein digestibility in RSM is influenced to a large extent by the presence of relatively anti-nutritional factors such as glucosinolate, erucic acid, tannins and sinapine (Bell, 1984). However, there was no clear evidence to fully describe the possible reasons of positive effects on BUN. Table 3 showed the effects of supplementation of rapeseed meal (RSM) on immune response in weaning pigs. there were no significant differences on IgG and IgA but IgA was decreased as dietary RSM level increased in 6 week (linear response, $P<0.05$). Consequently, RSM could be supplemented up to 8% in the weaning pig diets without a negative impact on the growth performance. However, further studies are needed to demonstrate the potential impact.

Table 1. Influence of rapeseed meal (RSM) supplementation on growth performance of weaning pigs

Item	Treatment[1]					SEM[2]	p-value	
	Con	A	B	C	D		Lin.	Quad.
Body weight, kg								
Initial	7.28	7.28	7.28	7.28	7.28			
3 week	10.75	11.04	10.68	10.55	10.24	0.333	0.27	0.98
6 week	22.18	22.90	22.40	21.91	21.41	0.566	0.20	0.93
ADG, g								
0-3 week	165	178	163	155	142	9.0	0.28	0.97
4-6 week	545	565	558	541	532	11.9	0.19	0.90
0-6 week	355	372	361	348	337	9.9	0.20	0.93
ADFI, g								
0-3 week	305	314	309	293	290	11.8	0.42	0.92
4-6 week	1,042	1,181	1,032	1,047	1,059	33.4	0.21	0.16
0-6 week	673	748	671	670	674	21.8	0.23	0.26
G:F ratio								
0-3 week	0.540	0.562	0.528	0.530	0.471	0.0147	0.16	0.66
4-6 week	0.524	0.479	0.541	0.525	0.508	0.0089	0.50	0.10
0-6 week	0.528	0.497	0.538	0.526	0.501	0.0072	0.96	0.10

[1] Con : basal diet, A: basal diet + 2 % RSM, B: basal diet + 4 % RSM, C: basal diet + 6 % RSM, D: basal diet + 8 % RSM.
[2] Standard error of mean.

Table 2. Influence of rapeseed meal (RSM) supplementation on blood profiles in weaning pigs

Item	Treatment[1]					SEM[2]	p-value	
	Con	A	B	C	D		Lin.	Quad.
BUN[3], mg/dL								
Initial	11.07	11.07	11.07	11.07	11.07			
3 week	14.35^{A}	13.08^{A}	11.80^{AB}	12.68^{A}	9.10^{B}	0.632	0.15	0.11
6 week	10.21^{a}	11.08^{a}	9.55^{ab}	7.47^{bc}	7.00^{c}	0.443	<0.01	0.63
Glucose, mg/dL								
Initial	84.50	84.50	84.50	84.50	84.50			
3 week	103.83	107.00	100.67	96.17	96.67	1.425	0.07	0.35
6 week	101.17	104.50	105.17	103.67	107.33	1.095	0.83	0.29
Total cholesterol, mg/dL								
Initial	161.00	161.00	161.00	161.00	161.00			
3 week	59.33	60.17	68.67	60.50	66.83	2.007	0.77	0.76
6 week	82.17	80.67	86.83	73.71	85.17	1.941	0.76	0.33
LDL cholesterol, mg/dL								
Initial	106.33	106.33	106.33	106.33	106.33			
3 week	29.17	31.00	33.33	31.50	33.83	2.394	0.85	0.68
6 week	45.67	44.17	45.67	40.00	43.50	3.790	0.88	0.71
HDL cholesterol, mg/dL								
Initial	52.50	52.50	52.50	52.50	52.50			
3 week	23.00	23.33	27.50	22.33	28.00	0.941	0.46	0.33
6 week	28.83	29.67	32.67	31.33	33.33	0.767	0.55	0.79

[1] Con : basal diet, A: basal diet + 2 % RSM, B: basal diet + 4 % RSM, C: basal diet + 6 % RSM, D: basal diet + 8 % RSM.
[2] Standard error of mean. [3]Blood urea nitrogen. [AB]Means in a same row with differnece superscript letters significantly differ (P<0.05). [ab]Means in a same row with differnece superscript letters significantly differ (P<0.01).

Table 3. Influence of rapeseed meal (RSM) supplementation on immune response in weaning pigs

Item	Treatment[1]					SEM[2]	p-value	
	Con	A	B	C	D		Lin.	Quad.
Serum IgG, mg/ml								
Initial	2.67	2.67	2.67	2.67	2.67			
3 week	2.67	2.50	1.97	2.37	2.54	0.088	0.23	0.32
6 week	2.78	3.23	2.73	3.38	3.25	0.180	0.94	0.65
Serum IgA, mg/ml								
Initial	1.39	1.39	1.39	1.39	1.39			
3 week	5.02	3.91	3.43	3.80	4.16	0.294	0.28	0.97
6 week	0.56	0.82	0.54	0.56	0.48	0.041	0.03	0.34

[1] Con : basal diet, A: basal diet + 2 % RSM, B: basal diet + 4 % RSM, C: basal diet + 6 % RSM, D: basal diet + 8 % RSM.
[2] Standard error of mean.

REFERENCES

Bell, J. M. 1984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58: 996-1010.

Brent, G. A. 1994. The molecular basis of thyroid hormone action. N. Engl. Med. 331:847-853.

Mawson, R., R. K. Heaney, Z. Zdunczyk and H. Kozlowska. 1994b. Rapeseed meal-glucosinolates and their antinutritional effects: 5. Animal reproduction. Nahrung. 38:588-598.

Patrick, M., C. O'Shea, S. Figat and J. V. O'Doherty. 2010. Influence of incrementally substituting dietary soya bean meal for rapeseed meal on nutrient digestibility, nitrogen excretion, growth performance and ammonia emissions from growing-finishing pigs. Arch. Anim. Nut. 64:412-424.

PO-06-59

Effects of Dietary Cashew Nut Testa Levels as Alternatives of Wheat Bran in Gestating Sow

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INTRODUCTION

Concerns over preserving the welfare and health of mono-gastric livestock in recent commercial production systems and tight profit margins have prompted livestock producers to seek alternative approaches to feeding their animals. As researchers and livestock producers have been seeking greater understandving of the role of fibrous feedstuffs in diets, some agro-industrial wastes were used as feed ingredients. These include cocoa pod husks, brewers spent grains, rice bran, groundnut skins, and wheat bran (Nelson et al., 2007). However, one by-product that has not been considered is cashew nut testa (skin), a by-product obtained from the processing of cashew nuts (Donkoh et al., 2012). Its utilization as animal feed even at relatively low concentrations will minimize its disposal problem as well as reduce the cost of feeding compare to wheat bran which is broadly used in sow gestating diet. Therefore, the objective of the present study was to evaluate the effect of dietary supplementation levels of cashew nut testa as alternatives of wheat bran on reproductive performance, blood profiles and milk composition of sows and the performance of their progeny.

MATERIALS AND METHODS

A total of 40 mixed-parity sows (Yorkshire × Landrace; average parity = 4.80) with an initial body weight (BW) of 211.53 kg were allotted to 1 of 4 dietary treatments in a completely randomized design (CRD). Four experimental diets containing different levels of cashew nut testa (0, 2, 4 and 6 %) were provided to sows during gestation. During lactation sows were fed a common diet after farrowing regardless of the gestation treatments. Sows were fed 2.4kg/d, twice a day during gestation, *ad libitum* during 21 d of lactation, and free access to water. Body weight and backfat thickness (P_2) of sows were measured at mating, d 110 of gestation, 24 h postpartum and d 21 of lactation. The numbers of total born and litter weight were recorded within 24 h postpartum and the number of pigs was recorded after cross-fostering and at 21 d of lactation. Weaning to estrus interval (WEI) was determined by monitoring for estrus from 3 to 10 d after weaning. Blood samples were collected from sows through jugular vein into serum separation tubes at d 110 of gestation as well as 24 h and 21 d postpartum and from nursing piglets through anterior vena cava at 24 h and 21 d after birth. Data were analyzed by ANOVA for a completely randomized design using the GLM procedure of SAS (SAS Institute, 2009).

RESULTS AND DISCUSSION

The body weight and backfat thickness at mating, d 35, d 70 and d 110 of gestation and during lactation were not affected by dietary cashew nut testa (CNT) supplementation in gestation diets. However, as level of CNT increased, feed intake during lactation showed a tendency to increase linearly (P=0.08). Adequate feed intake, especially during the first 7 to 10 d of lactation is important to replenish body reserves, and re-establish secretion of hormones which control subsequent reproductive performance (Kemp et al., 1995; Quesnel et al., 1998). There was a quadratic response in 2% CNS supplementation treatment on shorter weaning to estrus interval (P<0.02). Litter and reproductive performance were not affected by dietary CNT supplementation in gestation diets. There were quadratic response in milk at 24 h postpartum on fat, lactose and total solid (P<0.03, P<0.02 and P<0.03, respectively). In blood profiles, there was a linear effect on serum insulin concentration on d 70 of gestation (P<0.03). In addition, quadratic trend was observed on d 21 of lactation in serum glucose level. Consequently, the data from current experiment suggested that CNT could be supplemented up to 4% in the gestation diets without a negative impact on the reproductive performance.

Table 1. Effects of cashew nut testa level on body weight, back-fat thickness, WEI and ADFI in sows

Item	Cashew Nut Testa				SEM[1)]	p-value	
	C0	C2	C4	C6		Lin.	Quad.
No. of sows	10	10	10	10			
Body weight (kg) in gestating sows							
35 day	212.20	210.30	211.05	212.55	3.52	0.96	0.83
70 day	230.35	230.20	224.80	228.75	3.57	0.77	0.79
110 day	257.30	261.20	251.44	253.75	3.44	0.55	0.91
35-110 change	45.10	50.90	40.39	41.20	1.61	0.12	0.43
Body weight (kg) in lactating sows							
24h postpartum	230.05	233.85	223.25	227.20	3.73	0.61	0.99
21 d of lactation	210.30	218.70	212.69	215.75	3.66	0.77	0.74
Farrowing to 21 d	19.75	15.15	10.56	11.45	1.99	0.09	0.47
Backfat thickness (mm) in gestating sows							
35 day	17.35	17.90	19.75	17.65	0.86	0.74	0.48
70 day	16.95	17.40	19.45	16.35	0.88	0.98	0.34
110 day	19.80	18.15	18.94	18.20	0.95	0.67	0.83
35-110 change	2.45	0.25	-0.81	0.55	0.60	0.25	0.17
Backfat thickness (mm) in lactating sows							
24h postpartum	17.90	18.30	18.75	18.85	0.90	0.71	0.93
21 d of lactation	15.45	16.30	18.13	16.30	0.78	0.57	0.44
Farrowing to 21 d	2.45	2.00	0.62	2.55	0.56	0.84	0.32
Daily feed intake in lactation (kg/day)							
	4.20	5.14	5.02	5.14	0.18	0.09	0.24
Weaning to estrus interval (day)							
	6.00	4.93	5.30	5.70	0.16	0.69	0.02

[1)]Standard error of the means

Table 2. Effects of cashew nut testa level on reproductive and litter performance of sows

Item	Cashew Nut Testa				SEM[1)]	p-value	
	C0	C2	C4	C6		Lin.	Quad.
No. of sows	10	10	10	10	-	-	-
Reproductive performance							
Total born/litter	14.00	15.40	15.78	13.90	0.49	0.99	0.15
No. of born alive	13.00	13.80	14.25	13.10	0.37	0.40	0.49
No. of stillbirths	1.00	1.60	1.53	0.80	0.22	0.96	0.24
21 day of lactation	11.40	11.10	11.50	10.50	0.19	0.15	0.33
Litter weight, kg							
Litter birth weight	21.79	21.72	21.07	18.26	0.06	0.04	0.26
21 day of lactation	55.93	56.65	55.36	52.69	1.75	0.48	0.62
Litter weight gain	36.63	38.85	39.14	36.52	1.56	0.99	0.44
Piglet weight, kg							
Piglet birth weight	1.59	1.42	1.34	1.39	0.04	0.06	0.18
21 day of lactation	4.90	5.09	4.78	4.94	0.10	0.85	0.92
Piglet weight gain	3.28	3.59	3.41	3.57	0.09	0.41	0.68

[1)]Standard error of the means

Table 3. Effect of cashew nut testa level on colostrum and milk composition of sows

Item	Cashew Nut Testa				SEM[1)]	p-value	
	C0	C2	C4	C6		Lin.	Quad.
No. of sows	10	10	10	10			
Casein, %							
24 h postpartum	4.98	4.49	4.22	6.07	0.32	0.32	0.10
21 d of lactation	4.36	4.57	4.30	4.22	0.07	0.22	0.26
Fat, %							
24 h postpartum	7.62	5.27	7.11	8.24	0.48	0.25	0.03
21 d of lactation	6.18	7.35	6.44	6.29	0.20	0.68	0.06
Protein, %							
24 h postpartum	6.37	5.76	5.23	7.91	0.44	0.31	0.10
21 d of lactation	4.81	5.00	4.72	4.61	0.08	0.22	0.35
Lactose, %							
24 h postpartum	4.48	4.66	4.77	4.17	0.08	0.22	0.02
21 d of lactation	6.22	6.06	6.25	6.09	0.04	0.49	0.95
Total solid, %							
24 h postpartum	20.28	17.30	18.57	22.68	0.85	0.21	0.03
21 d of lactation	18.29	19.71	18.49	18.06	0.27	0.38	0.07
Solid not fat, %							
24 h postpartum	10.86	10.56	10.01	12.15	0.37	0.34	0.14
21 d of lactation	11.10	11.19	11.07	10.88	0.05	0.09	0.16

[1)]Standard error of the means.

Table 4. Effect of cashew nut testa level on blood profiles of sows

Item	Cashew Nut Testa				SEM[1)]	p-value	
	C0	C2	C4	C6		Lin.	Quad.
Insulin (ug/L)							
35 day	0.07	0.07	0.07	0.07	-	-	-
70 day	0.09	0.09	0.05	0.06	0.01	0.03	0.73
110 day	0.08	0.08	0.06	0.08	0.01	0.62	0.68
24 h postpartum	0.08	0.10	0.09	0.09	0.08	0.84	0.75
21 d of lactation	0.10	0.09	0.09	0.06	0.10	0.14	0.51
Glucose (mg/dL)							
35 day	71.17	71.17	71.17	71.17	-	-	-
70 day	66.50	68.50	67.25	67.25	1.26	0.92	0.65
110 day	79.75	77.00	76.25	74.75	1.32	0.28	0.84
24 h postpartum	84.75	95.50	83.00	89.75	2.25	0.95	0.81
21 d of lactation	82.67	74.50	74.25	78.25	3.47	0.34	0.07

[1)]Standard error of the means

REFERECES

Kemp, B., Soede, N. M., Helmond, F. A. and Bosch, M. W. 1995. Effects of energy source in the diet on reproductive hormones and insulin during lactation and subsequent estrus in multiparous sows. J. Anim. Sci. 73:3022–3029.

Quesnel, H., Pasquier, A., Mounier, A. M. and Prunier A. 1998. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J. Anim. Sci. 76:856–863.

Nelson, F. S., Ameleke, G. Y., Donkoh, A. and Lamptey, V. 2007. Use of locally available agro-industrial by-products (AIBPs) for egg production in the western region of Ghana. The results of an on-farm trial in Proceedings of the Ghana Society of Animal Production Symposium, vol. 15, pp. 103-107.

Donkoh, A., Attoh-Kotoku, V., Kwame, R. O. and Gascar, R. 2012. Evaluation of nutritional quality of dried cashew nut testa using laboratory rat as a model for pigs. Scientific World Journal. 2012:984249.

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Effects of Supplementation of Lysine Cell Mass as a Substitution for L-lysine?HCl on Growth performance and Blood Profiles in piglets

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INTRODUCTION

Feed prices are considered the most important part in Korean swine farms because approximately 50 to 70% of the feed prices accounts for total cost of pig production. Protein sources in animal feeds are one of the most expensive ingredients and the price of synthetic amino acid can be elevated dramatically. To decrease supplementation level of synthetic amino acid and save feed cost, various experiments were conducted for finding alternative protein sources and method. Lysine, which is regarded as the first limiting amino acid in monogastric animal and synthetic amino acid has been widely used in swine feed as feed additives in South Korea. Most commonly used synthetic Lysine is L-lysine·HCl, produced by industry in large amounts using bacteria. During the manufacturing process of lysine, several by-products can be produced. Condensed molasses solubles (CMS), for instance, has been evaluated in ruminant animals by many researchers (Kim et al., 1997; Ha et al., 1998; Lee et al., 1998; Broderick et al., 2000; Liu and McMeniman, 2001). However, CMS contains high non-protein-nitrogen (NPN) concentration and low Lysine level. Thus, availability of CMS to monogastric animals will be low. A lysine producing bacteria is Corynebacterium which is short-rod type, Gram positive, aerobic and typical amino acid-producing bacteria (Piao et al., 1998). Lysine cell mass is mainly composed of dried bacterial cell mass (Corynebacterium) and produced as a by-product of lysine fermentation. The bacterial cell mass from lysine fermentation is rich in amino acids, especially in lysine but not contain NPN. Thus, Lysine cell mass has a great potential to be used as a substitute for L-lysine·HCl to monogastric animal. There has been a lot of researches to evaluate the effect of Lysine cell mass as a protein source in broiler diets (Piao et al., 1998), in ruminant (Seo et al., 2008) and in Juvenile Israeli Crap diets (Kim et al., 2002). However, there was no attempt to investigate the potential value of Lysine cell mass as a protein source for swine. Therefore, this experiment was designed to evaluate the effect of Lysine cell mass as an alternative lysine source on growth performance and blood profiles in weaning pigs.

MATERIALS AND METHODS

For the feeding trial, a total of two hundred crossbred pigs ([Large White Yorkshire × Landrace] × Duroc) averaging 6.89 ± 1.043kg body weight (BW), weaned at 28 ± 3 days of age were segregated into one of 5 treatments in 4 replicates of 10 pigs per pen in a randomized completely block (RCB) design by BW and sex. The treatments were composed by dietary supplementation level of LCM (0, 0.25, 0.5, 0.75 and 1.0%) in weaning pigs' diet. The four experimental diets were fed during phase I (diet I, d 0 - 14), phase II (diet II, d 14 – 35), and were formulated to contain 20.56% and 18.88% crude protein respectively. Total lysine concentration in the control diets was 1.35% and 1.15% in diet I and diet II respectively, and the other basal diets of LCM25, LCM50, LCM75, LCM100 were supplemented with the same lysine concentration as Lysine cell mass to meet the requirement. Body weight and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio). In each treatment, 6 pigs near average body weight were bled through the anterior vena cava at 0, 2nd and 5th week for blood samples. Collected data was analyzed using the general linear model (GLM) procedure of SAS (2004).

RESULTS AND DISCUSSION

The effect of dietary lysine cell mass level on growth performance is presented in Table 1. During the whole experiment period there is no significant difference on BW, ADG, ADFI and G/F ratio. Growth performance of LCM100 (corn-soybean meal based diet supplemented with 1.0% lysine cell mass to experimental diet) showed the highest BW, ADG, ADFI and G/F ratio numerically. The effect of dietary lysine cell mass level on blood profiles is presented in Table 2. There was no significant difference in serum IGF-1 concentration and BUN concentration. However, in blood profiles, serum cortisol concentration of 5th week was significantly increased as dietary LCM level increased (linear response, $P<0.01$). A factor of lysine cell mass might influence serum cortisol concentration,

but the direct cause is not clear.

Table 1. Effect of dietary lysine cell mass levels on growth performance

Criteria	Treatment[1)]					SEM[2)]	P - value	
	CON	LCM25	LCM50	LCM75	LCM100		Linear	Quadratic
Body weight, kg								
Initial	6.89	6.89	6.89	6.89	6.89	0.229	-	-
2week	9.52	9.21	9.75	9.44	9.55	0.284	0.71	0.99
5week	18.01	18.11	18.34	17.85	**18.76**	0.467	0.56	0.72
ADG, g								
0-2week	203	179	220	195	205	7.9	0.72	0.98
3-5week	404	431	404	396	**438**	11.0	0.64	0.56
0-5week	327	331	337	321	**349**	8.6	0.56	0.71
ADFI, g								
0-2week	333	312	355	342	335	11.8	0.93	0.46
3-5week	791	798	772	749	**826**	19.8	0.88	0.38
0-5week	616	623	613	593	**638**	15.5	0.88	0.57
G:F ratio								
0-2week	0.608	0.518	0.625	0.575	0.611	0.015	0.45	0.33
3-5week	0.511	0.542	0.524	0.532	**0.530**	0.008	0.72	0.63
0-5week	0.531	0.531	0.552	0.544	**0.546**	0.007	0.44	0.61

[1)] CON : Corn-SBM based diet, LCM25 : Basal diet + 0.25% LCM, LCM50 : Basal diet + 0.5% LCM, LCM75 : Basal diet + 0.75% LCM, LCM100 : Basal diet + 1.0% LCM
[2)] Standard error of the mean.

Table 2. Effect of dietary lysine cell mass levels on blood profiles

Item[1)]	Treatment[2)]					SEM[3)]	P - value	
	CON	LCM25	LCM50	LCM75	LCM100		Linear	Quadratic
Cortisol, μg/dL								
Initial	2.20						-	-
2week	4.05	3.68	3.90	5.30	3.98	0.387	0.62	0.84
5week	3.32^{C}	8.90^{A}	5.73^{B}	7.08^{AB}	7.62^{AB}	0.485	0.01	0.07
IGF-1, mg/dL								
Initial	60.07							
2week	99.63	94.73	90.98	72.77	101.53	5.707	0.67	0.30
5week	108.70	96.33	98.65	137.47	123.18	7.024	0.17	0.58
BUN, mg/dL								
Initial	9.60							
2week	14.00	14.33	14.75	13.27	16.82	0.567	0.23	0.31
5week	17.65	13.05	16.72	13.92	13.63	0.799	0.21	0.74

[1)] The number of observations for each mean value was six (n=6).
[2)] CON : Corn-SBM based diet, LCM25 : Basal diet + 0.25% LCM, LCM50 : Basal diet + 0.5% LCM, LCM75 : Basal diet + 0.75% LCM, LCM100 : Basal diet + 1.0% LCM.
[3)] Standard error of mean.
[ABC] Means in a same row with different superscript letters significantly differ (P<0.01).

REFERENCES

Broderick, G. A., N. De Leon and Y. Nakamura. 2000. Potential of fermentation byproducts as nitrogen supplements for lactating dairy cows. J. Dairy Sci. 83:2548-2556

Ha, J. K., H. D. Kim, S. S. Shim, J. H. Lee, Y. G. Ko, B. O. Kwack, S. S. Lee, W. Y. Kim and I. K. Han. 1998. Studies on nutritive value of lysine fermentation by-product as a protein source for ruminants I. Kor. J. Anim. Sci. 22:371-380.

Kim, H. K., C. W. Kim, J. K. Ha and S. Y. Yang. 1997. Condensed molasses fermentation solubles from monosodium glutamate production as a liquid protein supplement for ruminants 1. Chemical composition and effects of different levels of CMS on the nutrient digestibility and rumen fermentation in sheep. Kor. J. Anim. Nutr. Feed 21:207-218. Davis, S. S. 1990. Phospholipids stabilized emulsions for parenteral nutrition and drug delivery, Edited by I. Hanin and G. Pepeu (phospholipids: biochemical, pharmaceutical, and analytical considerations), Plenum press, New York and London.

Kim, K. K., Xiaojie Wang, Sungchul C. BAI. 2002. Effects of dietary supplementation of lysine cell mass (LCM) in Juvenile Israeli Carp, Cyprinus carpio. J. Korean Fish. 35(4), 226-341.

Lee, J. H., B. O. Kwack, H. D. Kim, S. S. Shim, Y. G. Ko, S. S. Lee, W. Y. Kim, J. K. Ha and I. K. Han. 1998. Studies on nutritive value of lysine fermentation by-product as a protein source for ruminants II. Kor. J. Anim. Sci. 22:381-390.

Liu, Z. J. and N. P. McMeniman. 2001. Effect of supplementation with a by-product of molasses fermentation or a non-protein nitrogen/mineral mix on feed intake and microbial protein supply in sheep consuming chopped oat (Avena sativa) hay. Small Rumin. Res. 41:229-233.

Piao, X. S., Y. K. Han, S. H. Bae, H. Lee, In K. Han. 1998. Evaluation of CM (cell mass from lysine fermentation) as an alternative protein source in broiler diets. Asian-Aust. J. Anim. Sci. Vol.11, No.5 : 550-558.

Seo, S., H. J. Kim, S. Y. Lee, Jong K. Ha. 2008. Ruminal Protein degradation characteristics of cell mass from lysine production. Asian-Aust. J. Anim. Sci. Vol.21, No.3 : 364-370.

PO-06-64

EFFECT OF DIETARY SUPPLEMENTATION OF BAKER'S YEAST ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN BROILER CHICKENS

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Introduction

Saccharomyces cerevisiae also known "baker's yeast" is one of the most widely used commercially species and is rich in crude protein (40-45%) and also rich in vitamin B complex, biotin, niacin, pantothenic acid and thiamin and its biological value is high (Reed and Nagodawithana, 1999). Mmany studies have been conducted using yeasts as a probiotic in ruminants but little work has been done in poultry. Whole yeast or yeast cell wall components have been shown to improve growth and affect the physiology, morphology and microbiology of the intestinal tract of turkeys(Bradley *et al.*, 1993; Hooge, 2004a; Huff *et al.*, 2010)and broiler chicks (Hooge, 2004b; Morales-Lopez *et al.*, 2009). The objective of this study was to determine the effects of dietary supplementation of baker's yeast on growth performance, nutrient digestibility and carcass characteristics of broiler chickens.

Materials and Methods

Two hundred day-old broiler chicks were obtained from a commercial hatchery and originating from the same breeder flock. The chicks were randomly divided into four treatment groups, with five replicates and each replicate consisting 10 chicks per replicate. The chicks were raised in stainless steel battery cages and fed commercial starter ration from day 1 till day 21. From day 22 to day 42 they fed the dietary treatments,namely: T1 (control, basal diet), T2 (basal diet + 0.1% yeast), T3 (basal diet + 0.2% yeast), T4 (basal diet + 0.4% yeast), T5 (basal diet + 0.8% yeast). The basal diet was based on corn (60%) and soyabean meal (31%) and contained 20.2 % crude protein and 17 ME MJ/kg energy on dry matter basis. Other ingredients include fishmeal (3 %), palm oil (2.45%), limestone (1.3%), dicalcium phosphate (0.65%), common salt (0.4%), choline chloride (0.2%), lysine (0.1%), DL-methionine (0.4%) and vitamin-mineral premix (0.2%). Feed intake, body weights and feed conversion ration (FCR) were measured on a weekly basis.

Digestibility Trial

A digestibility trial was conducted on the birds from day 36 to day 42. Faeces from each cage were collected beginning day 37 for six days. Each day, the feces from each treatment were collected, weighed and then mixed. Samples of faeces were collected and dried in a forced draught oven set at 65 C for 72 hours to determine their dry matter. The dried feces samples kept in PVC bottles until analysed for their nutrient contents. Proximate Analysis was conducted on the samples to determine the dry matter, ash, crude protein, crude fiber, crude fat, and gross energy.

Statistical Analysis

Data were analyzed using SAS (Statistical Analysis Software). The data in this study was presented as mean ± SD of four replicates and analyzed by one-way ANOVA. All differences among means were considered significant at $P \leq 0.05$ using Duncan's test.

Results and Discussion

Growth Performance

The result on feed intake, body weight gains and FCR are shown in Table 1. There were significant differences ($p<0.05$) between birds in the control diet and diets supplemented with yeast, in terms of body weight gain, feed conversion ratio, and body weight gain. However, there were no significant differences between diets T3, T4, and T5. Adebiyi *et al.*, (2012) reported that, there were no significant differences in the performance of birds fed with 0.125% and 0.15% of yeast. In terms of body weight gain, birds fed T1 had the lowest value compared with other treatments. Yeast culture contains yeast cells as well as metabolites such as peptides, organic acids, oligosaccharides, amino acids, flavor, and aroma substances, and possibly some unidentified growth factors, which have been proposed to produce beneficial performance responses in animal production. Zhang et al (2005) observed that yeasts supplementation had positive effects on growth performance of broilers. On the

contrary, Bradley et al (1993) reported that yeast products had no effect on performance in turkey poults and also in early weaned pigs(White *et al*., 2002). Differences in animal response may be related to differences in product formulations: yeast products are interchangeably classified as active dried yeast, live YC, or fermented YC as reported by(Gao *et al*., 2008).Several authors have indicated that the different results of using yeast as feed additive in broiler chicken depends on many factors like the physical state of yeast added into fed broiler chicks (dry, wet and fermented yeast), applied methods in feed or drinking water, age of birds and level of biosecurity (Perreten, 2003; Stanley *et al*., 2004; Gao *et al*., 2008).

In terms of nutrient digestibility (Table 2), no significant differences were observed in the values of the dry matter digestibility, and metabolizable energy. However, there were signidficant differences in the digestibility of crude protein, ether extract and organic matter. Reed and Nagodawithana (1999) stated that yeast also one of the effective adsorbents which is rich in crude protein (40-45%) and contribute to the high protein digestibility.

Carcass Characteristics

There was no significant change in the carcass dressing percentage of birds fed different level of yeast (Table 3). This is consistent with published results of Abaza, (2008) who showed that the carcass dressing percentage was not affected by yeast supplementation. In terms of meat to bone ratio, there were significant ($p<0.05$) differences observed between the treatment groups. T4 and T5 showed no significant differences between them but in T5, the value of meat to bone ratio was higher compared to T4. For meat to fat ratio, there were significant differences observed between the supplemented baker's yeast and the control treatment and T5 had the greatest ratio and the control group had the lowest ratio.

Conclusion

The results of this investigation showed that addition of baker's yeast to feed a broiler improved body weight gain and feed conversion ratio, although feed intake appeared not to be affected. The carcass dressing rates were not affected by using various levels of yeast in feed. However, the meat bone fat ratio of the broiler chicken improved with yeast supplementation.The nutrient absorption of the broiler improved by feeding with baker's yeast supplement. It can therefore be concluded that adding supplemental yeast to the diets of broiler birds 0.4% will improve the growth performance, nutrient digestibility, meat fat bone ratio.

Table 1: Initial body weight, final body weight, weight gain, feed intake and feed conversion ratio (mean ± standard error) in broiler chickens fed diets supplemented with baker's yeast.

Treatments	T1	T2	T3	T4	T5
Initial body weight (g) 21 d	851.36±5.60^{a}	848.17±2.54^{a}	847.88±0.70^{a}	844±0.60^{a}	843.81±0.77^{a}
Final body weight (g) 42 d	2249±5.18^{c}	2330±1.72^{b}	2332±0.4^{b}	2340±0.26^{a}	2342±0.30^{a}
Weight gain (g/day) 22-42 d	66.59±0.5^{c}	70.57±0.07^{b}	70.7±0.04^{ab}	71.26±0.03^{a}	71.36±0.03^{a}
Feed intake (g/day) 22-42 d	139±4.86^{a}	138±1.97^{a}	131±1.83^{a}	132±5.78^{a}	130±5.23^{a}
FCR 22-42 d	2.07±0.08^{a}	1.96±0.03^{ab}	1.86±0.03^{b}	1.86±0.08^{b}	1.82±0.07^{b}

Key: T1 (without yeast), T2 (0.1% yeast), T3 (0.2% yeast), T4 (0.4% yeast), T5 (0.8% yeast)

**a, b, c, values within column with different superscripts are significantly different ($p<0.05$)*

Table 2: Digestibility (%) of dry matter, crude protein, ether extract,metabolizable energy and organic matter in broiler chickens (mean ± standard error)

Treatment	T1	T2	T3	T4	T5
Dry Matter	61.12±0.30^{a}	60.94±0.48^{a}	61.40±0.14^{a}	61.51±0.24^{a}	60.84±0.19^{a}
Organic Matter	96.00±0.08	96.15±0.31^{a}	96.33±0.21^{a}	96.58±0.19^{a}	95.10±0.42^{b}
Crude Protein	95.21±0.12^{d}	95.25±0.003^{d}	95.44±0.01^{c}	95.65±0.01^{b}	96.81±0.004^{a}
Ether Extract	85.81±0.34^{b}	88.03±0.15^{a}	87.40±0.13^{a}	87.52±0.24^{a}	87.42±0.17^{a}
Metab Energy	16.14±0.04^{a}	16.08±0.08^{a}	16.13±0.05^{a}	16.18±0.02^{a}	16.17±0.05^{a}

Key: T1 (Control, basal diet), T2 (0.1% yeast), T3 (0.2% yeast), T4 (0.4% yeast), T5 (0.8% yeast)

**a, b, c, values within column with different superscripts are significantly different (p<0.05)*

able 3: Carcass characteristics of broilers fed diets supplemented with yeast(mean ± standard)

Parameters	T1	T2	T3	T4	T5
Dressing (%)	73.32±0.69^{a}	74.55±0.20^{a}	76.25±1.04^{a}	76.84±2.55^{a}	76.93±1.88^{a}
Meat : bone	2.66±0.09^{c}	2.84±0.004^{b}	2.93±0.01^{ab}	3.03±0.003^{a}	3.04±0.006^{a}
Meat : fat	11.65±1.54^{b}	14.48±0.12^{ab}	15.23±0.02^{ab}	13.72±1.94^{ab}	15.71±0.03^{a}
Bone : fat	4.95±0.23^{a}	5.09±0.05^{a}	5.19±0.02^{a}	5.18±0.01^{a}	5.17±0.01^{a}

Key: T1 (without yeast), T2 (0.1% yeast), T3 (0.2% yeast), T4 (0.4% yeast), T5 (0.8% yeast)

**a, b, c, values within column with different superscripts are significantly different (p<0.05)*

References

ABAZA IM, S. M., SHOIED MS, HASSAN II (2008) Evaluation of some natural feed additives in growing chick's diet *International Journal of Poultry Science,* 7 (9): 872-879.

ADEBIYI, O., MAKANJUOLA, B., BANKOLE, T. & ADEYORI, A. (2012) Yeast culture (Saccharomyces cerevisae) supplementation: Effect on the performance and gut morphology of broiler birds. *Global Journal of Science Frontier Research,* 12.

BRADLEY, G. L., SAVAGE, T. F. & TIMM, K. I. (1993) The Effects of Supplementing Diets with Saccharomyces cerevisiae var. boulardii on Male Poult Performance and Ileal Morphology. *Poultry Science,* 73: 1766-1770.

GAO, J., ZHANG, H. J., YU, S. H., WU, S. G., YOON, I., QUIGLEY, J., GAO, Y. P. & QI, G. H. (2008) Effects of Yeast Culture in Broiler Diets on Performance and Immunomodulatory Functions. *Poultry Science,* 87: 1377-1384.

HOOGE, D. M. (2004a) Turkey Pen Trials with Dietary Mannan Oligosaccharide: Meta-analysis, 1993-2003. *International Journal of Poultry Science,* 3: 179-188.

HOOGE, D. M. (2004b) Meta-analysis of Broiler Chicken Pen Trials Evaluating Dietary Mannan Oligosaccharide, 1993-2003. *International Journal of Poultry Science,* 3: 163-174.

HUFF, G. R., HUFF, W. E., FARNELL, M. B., RATH, N. C., SOLIS DE LOS SANTOS, F. & DONOGHUE, A. M. (2010) Bacterial clearance, heterophil function, and hematological parameters of transport-stressed turkey poults supplemented with dietary yeast extract. *Poultry Science,* 89: 447-456.

MORALES-LOPEZ, R., AUCLAIR, E., GARCIA, F., ESTEVE-GARCIA, E. & BRUFAU, J. (2009) Use of yeast cell walls; Î²-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. *Poultry Science,* 88: 601-607.

PERRETEN, V. (2003) Use of antimicrobials in food-producing animals in Switzerland and the European Union (EU). *Mitt Lebensm Hyg,* 94: 155-163.

REED, G., AND NAGODAWITHANA TW (1999). Yeast Technology. 2nd Edition, Van Nostrand Reinhold, New York.

STANLEY, V. G., GRAY, C., DALEY, M., KRUEGER, W. F. & SEFTON, A. E. (2004) An Alternative to Antibiotic-Based Drugs in Feed for Enhancing Performance of Broilers Grown on Eimeria Spp.-Infected Litter. *Poultry Science,* 83: 39-44.

WHITE, L. A., NEWMAN, M. C., CROMWELL, G. L. & LINDEMANN, M. D. (2002) Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *Journal of Animal Science,* 80: 2619-2628.

PO-06-69

Expression of Hsp72, Hsp73 and Their Co-chaperone in the Cattle with Different Thermo-tolerance Capacity

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Introduction

Heat shock proteins 70 (HSP70s) function as "molecular chaperones," respond to heat stress by refolding, preventing the aggregation of denatured protein (Sonna et al., 2002; Kregel, 2002). The most extensively studied of HSP70 family are Hsp72 (inducible form) and Hsp73 (constitutively form), and as chaperone proteins they were unable to function alone. The chaperone function of Hsp70s required co-chaperone proteins such as proteins responsible for re-folding denature protein which requires N-terminal adenosine triphosphatase (ATPase) domain to regulate C-terminal peptide-binding domain of Hsp72 or Hsp73 with hydrophobic peptide sequences of denatured proteins (Palleros et al., 1991). Alteration of ATP hydrolysis and ADP/ATP exchange is regulated by co-chaperones including Hsp40 (Mayer and Bukau, 2005) and BAG domain proteins (Tzankov et al., 2008). Obviously, the effective expression and function of HSP70 family, both Hsp72 and Hsp73, required other proteins. Since the functions of HSP70s respond to heat elevation, it is possible that expression of Hsp72 and Hsp73 and co-chaperone will be related to the thermo-tolerance capability (Basirico et al., 2011). There were several studies on HSP70s in the cattle but the association of expression of Hsp72 and Hsp73 and thermo-tolerant capability in cattle were limited (Camargo et al. 2007; Guerriero and Raynes, 1990). This study investigated the expression of Hsp72 (inductive protein), Hsp73 (constitutive protein), and co-chaperone proteins during heat elevation in the cattle with distinct thermo-tolerant capability.

Materials and Methods

The distinct heat-tolerant capability cattle employed in this study includes Sahiwal (*Bos indicus*), 100% Holstein Friesian (*Bos taurus*) and their crossbred of 50% Holstein Friesian, 87.5-97.7% Holstein Friesian (>87.5%). The study was conducted during summer in Thailand. Ambient temperature and relative humidity were measured and expressed as temperature-humidity index (THI). Leukocytes were collected at 0600 (THI=72), 0900 (THI=80), 1200 (THI=90), and 1500 (THI=94). The level of Hsp72 and Hsp73 was determined by western blot analysis using HSP70/HSP72 monoclonal antibody (SPA-810, Assay Designs, USA) and HSC70/HSP73 polyclonal antibody (SPA-816, Assay Designs, USA). Identification of proteins and their relation to the expression of Hsp72 and Hsp73 were analyzed by LC-MS/MS and the Search Tool for the Retrieval of Interacting Genes (STRING).

Result and Discussion

The expression of Hsp72 of HF and crossbred cattle increased steeply with the elevation of THI while the expression of Hsp72 in Sahiwal was slowly increase at low THI and sharply increase at THI more than 90 (Figure 1). It implied that the THI threshold of Hsp72 production in Sahiwal was more than 90. In contrast, this investigation found that elevated ambient temperature was unable to induce Hsp73 expression. However, the significant finding is that Sahiwal has higher level of Hsp73 than other cattle (Figure 1). Therefore, the high level of Hsp73 may indicate the capability on thermo-tolerant of Sahiwal.

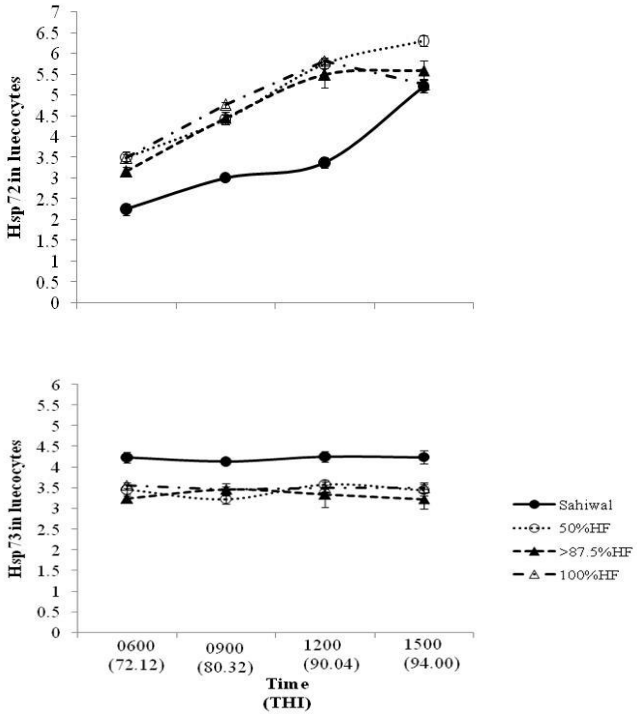

Figure 1. Hsp72 and Hsp73 expression in leukocytes of Sahiwal, 50%HF, >87.5%HF, and 100%HF under different THI

Beyond the distinct expression of Hsp72 and Hsp73, there were 8 proteins, by STRING, that showed relation to pathway of Hsp72 and Hsp73 expression (Figure 2). There were Heat shock factor protein 1 (HSF-1), Heat shock protein 105 kDa (Hsp105), DnaJ homolog subfamily A member 3, DNAJC2 protein, DnaJ (Hsp40) homolog (Hsp40), BAG family molecular chaperone regulator 1(BAG-1), Mitogen-activated protein kinase 10 (MAPK-10), and SUMO-activating enzyme subunit 10 (SUMO-10). These proteins were expressed differently among the distinct thermo-tolerant capability cattle (Figure 3).

In conclusion, the main findings are that the expression level of Hsp72, Hsp73, and their co-chaperone proteins were dissimilar among the distinct thermo-tolerant capability cattle. It is implicated that protein expression profile in bovine leukocytes was related to thermo-tolerant capability.

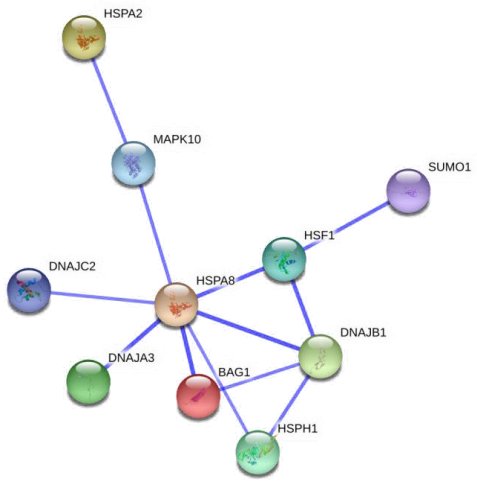

Figure 2. The protein-protein interaction pathway associated with Hsp72 (*HSPA2 gene*) and Hsp73 (*HSPA8 gene*) expression. Differentially expressed proteins identified with Mascot software were used as input data for STRINGDB. Proteins biological association networks were performed base on String database

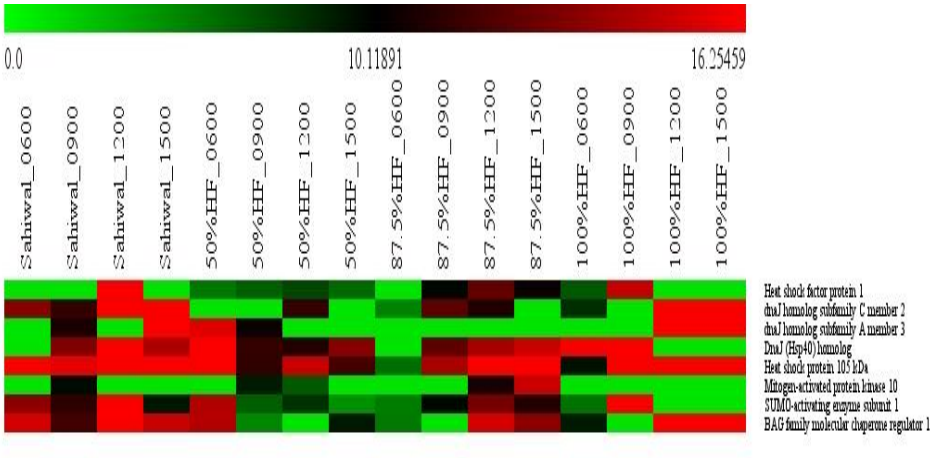

Figure 3. Differentially expressed protein related to Hsp72 and Hsp73 pathway. The heatmap shows the expression level of proteins related to Hsp72 and Hsp73 in Sahiwal, 50%HF, >87.5% HF, and 100% HF (green: low expression, red: high expression)

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References

Basirico L, Morera P, Primi V, Lacetera N, Nardone A, Bernabucci U. (2011). Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. *Cell Stress Chap.* 16: 441-448.

Camargo LSA, Viana JHM, Ramos AA, Serapião RV, de Sa WF, Ferreira AM, et al. (2007). Developmental competence and expression of the Hsp 70.1gene in oocytes obtained from *Bos indicus* and *Bos taurus* dairy cows in a tropical environment. *Theriogenology* 68: 626-632.

Guerriero JR, Raynes DA. (1990). Synthesis of heat stress proteins in lymphocytes from livestock. *J. Anim. Sci.* 68: 2779-2783.

Kregel KC. (2002). Molecular biology of thermotolerance invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J. Appl. Physiol.* 92, 12177-2186.

Mayer MP, Bukau B. (2005). Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol. Life Sci.*62: 670–684.

Palleros DR, Welch WJ, Fink AL. (1991). Interaction of hsp70 with unfolded proteins: effects of temperature and nucleotides on the kinetics of binding. *Proc. Natl. Acad. Sci. USA* 88: 5719–5723.

Sonna LA, Fujita J, Gaffin SL, Lilly CM. (2002). Molecular biology of thermotolerance invited review: effects of heat and cold stress on mammalian gene expression. *J. Appl. Physiol.*92: 1725-1742.

Tzankov S, Wong MJH, Shi K, Nassif C, Young JC. (2008). Functional divergence between co-chaperones of Hsc70. *J Biol Chem* 283: 27100–27109.

PO-06-76

Stimulatory effect of methionine on chicken lymphocyte cell line proliferation

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Introduction

Methionine is the first limiting essential amino acid which improve growth performance and carcass quality of poultry (Ribeiro *et al.,* 2005). Methionine deficiency could depress body weight gain, food intake and efficiency of food utilization in chicks (Sekiz *et al.,* 1975). Apart from these functions, Methionine is also involved in avian immune functions. There are some reports that high methionine supplement promote a good health for poultry. For example, supplementation improved cellular immune response and humoral immune response (Shini and Bryden, 2005), increase blood serum total protein, albumin, globulin and antibody response to Newcastle disease virus (Bhargava *et al.,* 1970). The supplementation of methionine increased total antibody, IgG, and response to the mitogen phytohemagglutinin (PHA) which might be related to the helping of T-lymphocyte (Tsiagbe *et al.,* 1987). Also, methionine deficiency can result in decreased humoral and nonspecific immunocompetence (serum lysozyme activity and phagocytosis of neutral red of peripheral blood lymphocytes) of broilers (Doug and Kirk, 2004). Therefore, this study aimed to examine in vitro effect of methionine on chicken lymphocyte cell line proliferation.

Materials and Methods

Cell line

A chicken monocytic cell, Con A-C1-Vick, was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were grown in RPMI 1640 medium containing 25 mM HEPES, 2mM L-glutamine, 10% heat inactivated Fetal Bovine Serum (FBS), penicillin (10 U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C incubator.

MTT assay

MTT assay was developed by Mosmann in 1983, using the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT). This assay determined the mitochondrial function which indicates activity of viable cells. Mitochondrial dehydrogenase of viable cells cleave the tetrazolium ring of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, leading to the formation of purple crystals (formazan), which are insoluble in aqueous solutions. Con A-C1-Vick were cultured with or without methionine in different concentration for 72 h. DMSO was added to dissolve formazan dye and the absorbance of this purple solution was measured at 540 nm in a Wallac Victor 1420 automatic microplate reader (Perkin Elmer).

Statistical analysis

Data from at least five individual experiments were analyzed and presented as mean + SD Statistical significance was determined by using one-way ANOVA and student Newman-Keuls, with a value of P.

Results and Discussion

Results showed that DL-methionine and L-methionine at concentration of 0.001-0.1 µg/ml has no proliferative effect on chicken lymphocyte cell line. Interestingly, DL-methionine at concentration of 1-100 µg/ml significantly increase chicken lymphocyte cell line proliferation from 105.3 ± 0.8% to 107.4 ± 1.0% ($p<0.05$) as shown in Figure 1.

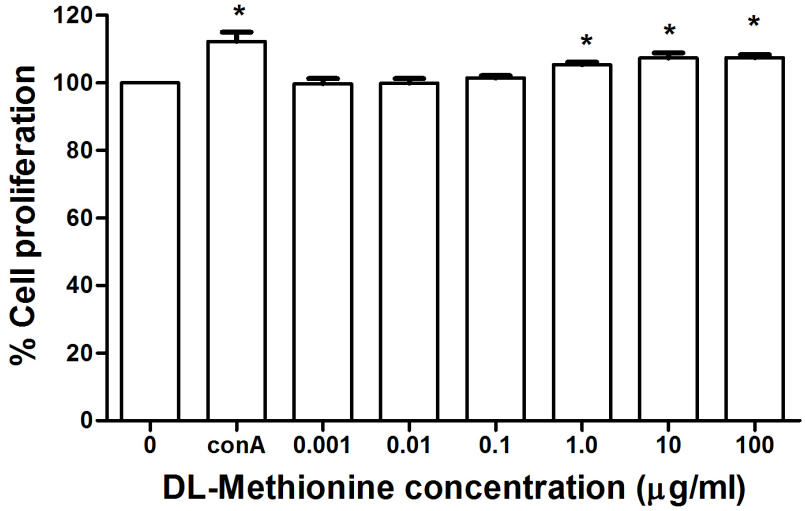

Figure 1. Effect of DL-Methionine on chicken lymphocyte cell line proliferation. Con A-C1-Vick (1 x 10^6 cells) was incubated for 72 h with different concentration of DL-Methionine(0.001-100 µg/ml). (*) Statistically significant difference in cell proliferation (P<0.05), as compared with control (0 µg/ml). ConA serve as a positive control in this study.

In addition, L-methionine at concentration of 1-100 µg/ml significantly increase chicken lymphocyte cell line proliferation from 105.7 ± 1.1 to 108.8 ± 0.9 (p<0.05) as shown in figure 2. However, the increasing level of cell proliferation did not greater than the effect of ConA (10 µg/ml) stimulated lymphocyte cell line (112.3 ± 2.7) which is served as a positive control.

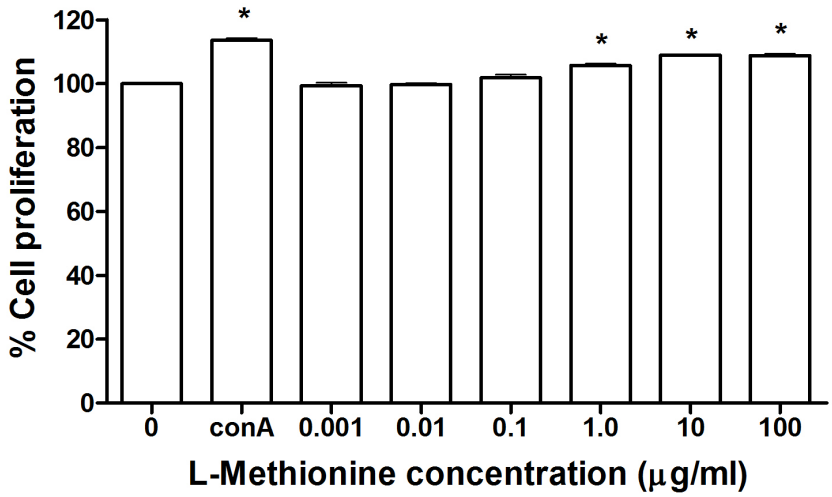

Figure 2. Effect of L-Methionine on chicken lymphocyte cell line proliferation. Con A-C1-Vick (1 x 10^6 cells) was incubated for 72 h with different concentration of L-Methionine(0.001-100 µg/ml). (*) Statistically significant difference in cell proliferation (P<0.05), as compared with control (0 µg/ml). ConA serve as a positive control in this study.

It has been reported that methionine plays an important role in humoral and cellular immune responses (Swain and Johri, 2000; Shini *et al.,* 2005). Its one of the mechanisms proposed to explain methionine interference in the immune system is based on the proliferation of immune cells, which are sensitive to intracellular variations in glutathione and cysteine levels, compounds which also participate in the metabolism of methionine (Shini *et al.,* 2005). In conclusion, it is possible that one mechanism of methionine that can enhance immune response in chicken should be due to the stimulation of lymphocyte cell proliferation.

References

Bhargava, K.K., R.P. Hanson and M.L. Sunde. 1970. Effect of methionine and valine on antibody production in chickens infected with Newcastle disease virus. J. Nutr. 100: 241-248.

Doug, K. and K. Kirk. 2004. Influence of nutrition on immune status of the bird. Proceedings of the 24th Technical Turkey conference. 43 p.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 65(1-2): 55-63.

Ribeiro, A.M., L.F. Dahlke and A.M. Kessler. 2005. Methionine source does not affect performance and carcass yield of broilers submitted to cyclic heat stress. Braz. J. Poult. Sci. 7: 159– 164.

Sekiz, S.S., M.L. Scott and M.C. Nesheim. 1975. The effect of methionine deficiency on body weight, food and energy utilization in the chick. Poult. Sci. 54: 1184–1188.

Shini, S., X. Li and W.L. Bryden. 2005. Methionine requirement and cell-mediated immunity in chicks. Brit. J. Nutr. 94: 746-752.

Swain, B.K. and T.S. Johri. 2000. Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. Br. Poult. Sci. 41: 83-88.

Tsiagbe, V.K., M.E. Cook, A.E. Harper and M.L. Sunde. 1987. Efficacy of cysteine in replacing methionine in the immune responses of broiler chicks. Poult. Sci. 66: 1138–1146.

PO-06-77

Effect of methionine on IL-2 release from chicken lymphocyte cell line

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Introduction

The nutrient deficiencies are particularly deleterious to the immune system when they occur early in life during the development of the primary lymphoid organs and the maturation of immune system (Kirk, 1997). Methionine is essential or limiting amino acid in poultry feed. Methionine is usually first limiting amino acid in maize and soybean meal based diet (Swick *et al*., 1990). Addition of methionine over and above the recommended requirement of broilers improves their performance of body weight gain and food conversion efficiency. Methionine is essential for various vital functions in body such as: protein synthesis, regulation of cell division, methyl donor, reduces reactive oxygen species and increased antibody level whereas others found that deficiency might lead to decrease in the antibody titer (Doug and Kirk, 2004).

Interleukin-2 (IL-2) exercises an array of biological effects on many cells including the functional activation of cells of the innate immune response (Michael Kogut *et al.*, 2002). IL-2 is one such cytokine that is being investigated in both tumor and infectious disease immunology because it plays an active role in the activation and maintenance of both acquired and innate immune defenses. IL-2 has been shown to activate human monocytes and neutrophils to generate tumoricidal and microbicidal activities, produce an oxidative burst, and secrete several cytokines and bioactive lipids. The aim of the present study was to examine in vitro effect of DL-methionine and L-methionine on IL-2 release from chicken lymphocyte cell line (Con A-C1-Vick).

Materials and Method

Cell line

The chicken lymphocyte cell line, Con A-C1-Vick, was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were grown in RPMI 1640 medium containing 25 mM HEPES, 2mML L-glutamine, 10% heat inactivated Fetal Bovine Serum (FBS), penicillin (10 U/mL) and streptomycin (100 g/mL) in 5% CO2 incubator 37 ° C.

IL-2 Assay

Lymphocyte cell line ($1x10^6$ cell/ml) were culture with different concentration of methionine or 10 µg/ml of Concanavalin A (ConA) for 72 h. The levels of IL-2 release in cultured medium were determined by Chicken IL-2 ELISA Kit (USCN)

Statistical analysis

Data from at least five individual experiments were analyzed and presented as mean + SD Statistical significance was determined by using one-way ANOVA and student Newman-Keuls, with a value of P

Results and Discussion

The effect of DL-methionine and L-methionine at concentration of 0.001-0.1 µg/ml has no effect on IL-2 release from chicken lymphocyte cell line (Figure 1 and 2). On the other hand, DL-methionine at concentration of 1-100 µg/ml significantly stimulated IL-2 release from chicken lymphocyte cell line from 54.4 ± 7.9 pg/ml to 134.8 ± 9.0 pg/ml ($p<0.05$).

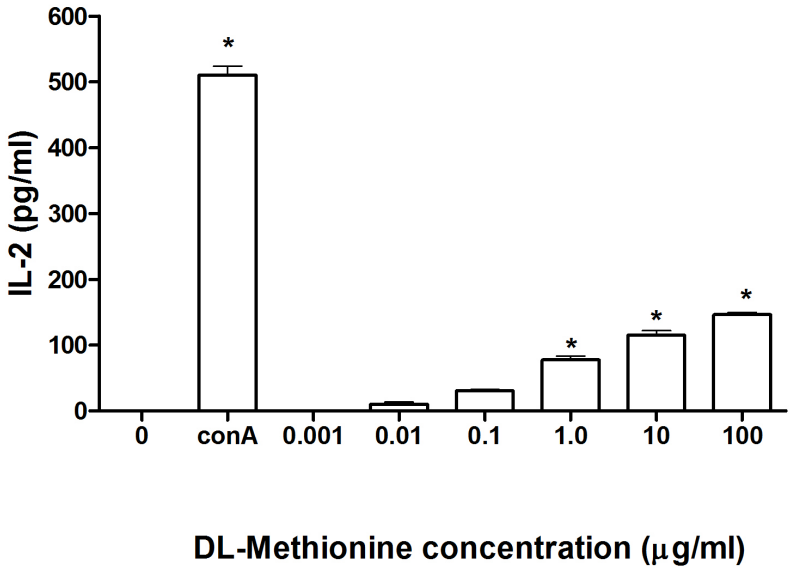

Figure 1. Effect of DL-Methionine on chicken lymphocyte cell line on IL-2 release. Con A-C1-Vick (1 x 10^6 cells) was incubated for 72 h with different concentration of DL-Methionine (0.001-100 μg/ml). (*) Statistically significant difference in cell proliferation (P<0.05), as compared with control (0 μg/ml). ConA serve as a positive control in this study.

Moreover, L-methionine at concentration of 1-100 μg/ml significantly stimulated IL-2 release from chicken lymphocyte cell line from 77.5 ± 10.2 pg/ml to 146.2 ± 5.9 pg/ml (p<0.05). However, the level of IL-2 release was not greater than IL-2 released from ConA (10 μg/ml) stimulated lymphocyte cell line which is served as a positive control (498.0 ± 36.3 pg/ml).

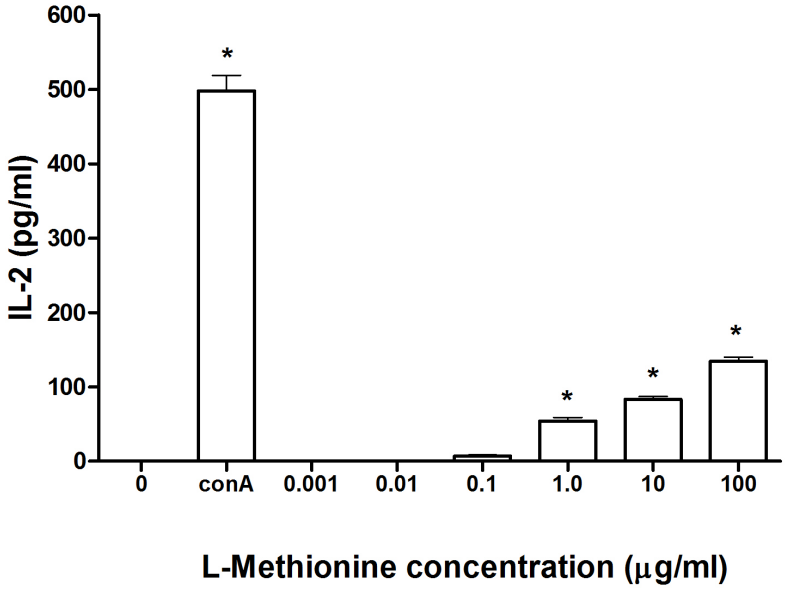

Figure 2. Effect of L-Methionine on chicken lymphocyte cell line on IL-2 release. Con A-C1-Vick (1 x 10^6 cells) was incubated for 72 h with different concentration of L-Methionine (0.001-100 μg/ml). (*) Statistically significant difference in cell proliferation (P<0.05), as compared with control (0 μg/ml). ConA serve as a positive control in this study.

The better immune response could be obtained with adequate supplementation of methionine which have been identified to be in marginal quantities in poultry feed (Ali A.S. Al-Mayah, 2006). The methionine deficiency could cause pathological and ultrastructural changes of thymus, reduce the T-cell population, serum IL-2 contents and the proliferation function of T cells, and induce increased percentage of apoptotic cells. The cellular immune

function was finally impaired in broilers (WU Bang-yuan *et al*., 2012). In conclusion, the possibly mechanism for methionine that can enhance immune function in chicken may be due to the stimulation of IL-2 release which is possess T-lymphocyte growth activity.

References

Ali A.S. Al-Mayah., 2006. Immune Response of Broiler Chicks to DL-Methionine Supplementation at Different Ages. Int J Poult Sci, 5(2), 169-172.

Doug, K. and K. Kirk, 2004. Influence of nutrition on immune status of the bird. Proceedings of the 24th Technical Turkey conference, 43.

Kirk, C.K., 1997. Interaction between nutrition and of poultry. 10th Ed., 73-74.

Michael Koguta, Lisa Rothwellb and Pete Kaiserb., 2002. Differential effects of age on chicken heterophil functional activation by recombinant chicken interleukin-2. Developmental & Comparative Immunology, 26(9), 817–830.

Swick, R.A., D.C. Creswell, J.J. Dibner and F.J. Ivey, 1990. Impact of methionine sources on performance of broilers growing under warm and humid conditions. Poult. Sci., 69, 194.

V.H. Kalbande1, K. Ravikanth2, S. Maini2 and D.S. Rekhe2., 2009. Methionine Supplementation Options in Poultry. Int J Poult Sci, 8(6), 588-591.

WU Bang-yuan1, 2, CUI Heng-min1, 2, PENGXi1, 2, FANG Jing1, 2, CUIWei1, 2 and LIUXiao-dong1, 2., 2012. Effect of Methionine Deficiency on the Thymus and the Subsets and Proliferation of Peripheral Blood T-Cell, and Serum IL-2 Contents in Broilers. Journal of Integrative Agriculture, 11(6), 1009-1019.

PO-06-78

DIFFERENTIAL LOCALIZATION OF CLAUDIN-1 IN INTESTINAL EPITHELIAL SEGMENTS OF KU BETONG AND THAI NATIVE CHICKENS

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Introduction

Intestinal tight junctions (TJs) play an important role in the epithelial integrity of intestine barrier. TJs are located at the apical side of the intestinal epithelial cells and then form a cell–cell adhesion. The components of TJs compose of a complex protein including transmembrane proteins, cytoplasmic plaque proteins, signaling proteins and adapter-linking actins (Günzel and Yu, 2013). TJs have the ability to specifically regulate paracellular transport in the aspects of the paracellular barrier function and the paracellular selective-channel function. Claudins contribute towards a forming paracellular pore and barrier. Claudins are in a family of proteins that have been found a critical role in maintaining the integrity of epithelial and endothelial tight junctions (Furuse et al., 2002). In particular, claudin-1 is a major of cell adhesion molecules that localizes at TJs and expresses in the intestinal tract in animals and humans. In rat and mouse, expression of claudin-1 has been present throughout the intestinal tracts (Holme et al., 2006). The localization of claudin-1 protein is little known its expression in the intestine of chicken. Moreover, the integrity of the intestinal epithelium of chickens might require TJs for a prevention of pathogenic bacteria and toxic substances. Objective of this study, we investigated the morphology and localization of claudin-1 expression in the intestinal epithelium of two different chicken breeds in Thailand.

Materials and Methods

Twenty chickens of each KU betong and Thai native chickens were obtained from Kasetsart University poultry farm and approved under the Animal Ethics Committee of Kasetsart University. All chickens were feed and water ad libitum, and lighting was continuous. They were euthanized at 6-weeks. Intestinal segments of duodenum, jejunum and ileum were kept in 10 % of the neutral buffered formalin for 24 hours. Histological morphology of the intestinal mucosa of KU Betong and Thai native chickens were evaluated. The tissue sections were cut in 3–5 μm and stained with the Hematoxylin and Eosin (H&E).

For immunohistochemistry, the tissue sections on positive-charged slides were incubated in citrate buffered pH 6.0 at 95°C for antigen retrieval process. The blocking endogenous peroxidase activity was used 3% H_2O_2 and incubated with 2%BSA at 37°C for blocking non-specific background. The primary rabbit anti-claudin-1 antibody (Cell marque, USA) was applied on slides at 4°C overnight. The secondary antibody, polymer Poly-HRP IgG Envision (DAKO, Denmark) was incubated on slides at RT for 1 hr. The visualization was developed with diaminobenzidine chromogen (DAB, Invitrogen, USA) as a substrate. Intestinal morphology and claudin-1 localization of intestinal epithelium was evaluated by Olympus FSX-100 microscope (Olympus, Japan). The digital image analysis Cell-D program (Olympus, Japan) was used for intensity analysis. The positive areas and the intensity of immunochemistry staining were calculated an intensity score [Theerawatanasirikul et al., 2016].

Statistical analysis used the student's T test for comparison of intestinal morphology between two breeds. Mann-Whitney test was used for comparison of the intensity score of claudin-1 between two breeds. The *P*-value at 0.05 was considered as a statistic significant.

Results

Morphology of intestine, the villus height of different intestinal segments was the highest in duodenum of both breeds. The villus height of ileum of Thai native chickens was smallest when compare with that from KU betong chickens ($P<0.05$). There was no significant different of crypt depth between two breeds. Immunohistochemistry of claudin-1 represents the TJ barrier of each breed. The intensity of claudin-1 was calculated as intensity score. The ileum segment of Thai native chickens was observed the highest intensity ($P<0.05$), whereas the duodenum segment of KU betong was the lowest intensity (Figure 1).

Localization of claudin-1 showed significant difference in the apical and basolateral site of intestinal cells. The distribution was faintly sparse granules between the intestinal cells, but not within the goblet cells. We also found

that the intestinal epithelial cells of both two breeds had more expressed the claudin-1 in the ileum (Figure 2).

Discussions

The present study demonstrated an intestinal morphology and a localization of TJ barrier, claudin-1, of two Thai chicken breeds. The villus height and crypt depth are widely use as an indicator of dietary supplement and strengthen of intestinal barrier in the broilers and piglets (Xiao et al., 2013). The epithelial TJs form a morphological and function gate, influencing the function of paracellular permeability. It is also as the protection of other pathogenic invasion as well as the regulation of TJ barrier (Pelicano et al., 2005; Kotler et al., 2013). The morphologic analysis on the use of probiotic and prebiotic diet has been evaluated all segments of the small intestine (Pelicano et al., 2005). In this study, immunohistochemistry was used to determine the claudin-1 expression in intestinal mucosa. Claudin-1 was expressed in the majority of specialized epithelial tissues during embryogenesis. It was observed in the lining of esophagus, gizzard, small intestine and cloaca (Simard et al., 2005). In adult, the claudin-1 highly presented in the ileum and the jejunum, which declined permeability of the intestinal barrier for an antigenic and toxic substances and microbial infections across the intestinal mucosa (Shao et al., 2013). The pattern of expression revealed along the basolateral membranes (Holmes et al., 2006), which corresponded to our study. It is also use as marker to determine gut barrier function of piglets (Xiao et al., 2013). However, it has not been performed in all segments (Pelicano et al, 2005; Shao et al, 2013). We found that the intestinal epithelial cell of both breeds more expressed the claudin-1 in the ileum in particular Thai chicken breed. In conclusion, it should be suggested that claudin-1 might be a useful marker as a gut TJ barrier along with the intestinal mucosa morphology. It could use for further intestinal barrier experiments, such as dietary enhancing-gut barrier. Moreover, the results might provide information for an animal breeding of Thai poultry industry.

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