ISSN-0126-4400 E-ISSN-2407-876X
Volume 46 (1)
February 2022

http://buletinpeternakan.fapet.ugm.ac.id/
Accredited No : 36a/E/KPT/2016

BULETIN PETERNAKAN

Bulletin of Animal Science

Bulletin of Animal Science is indexed in:

Published by:
Faculty of Animal Science
Universitas Gadjah Mada
In Collaboration with
ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

DAFTAR ISI

Buletin Peternakan 46 (1): 1-6, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.70339

The Effect of Protected Lemuru Fish Oil Supplementation on In Vivo Nutrient Digestibility and Sheep Blood Profile

Ratri Ratna Dewi[1], Kustantinah[2]*, and Muhlisin[2]

[1]The Graduate School of Animal Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Department of Animal Nutrition, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Article history
Submitted: 10 November 2021
Accepted: 6 January 2022

* Corresponding author:
E-mail: kustantinah@ugm.ac.id

ABSTRACT

This research was intended to observe the effect of protected lemuru fish (*Sardinella longiceps*) oil for ruminants, especially for sheep. This study aimed to evaluate the digested nutrients and blood profile of sheep. This study was conducted in September-October 2020 in Dumbira Farm, Kalasan, Yogyakarta, using 12 sheep divided into 3 treatments with 4 replications each. Treatment P0 was Total Mixed Ration (TMR) without protected Lemuru fish oil (control), treatment P1 was TMR with 5% protected Lemuru fish oil, and treatment P2 was TMR with 10% protected Lemuru fish oil. The data were statistically analyzed using one way analysis of variance and continued with Duncan new Multiple Range Test for significant results. The results of this study indicated that the addition of 10% protected Lemuru fish oil in TMR feed had a significant effect ($P<0,05$) on the increased value of in vivo digestion of crude fiber and crude fat, but did not affect the digestibility of dry matter, organic matter, and protein. The addition of protected Lemuru fish oil did not cause hematological disorders showed by the blood profiles were in the normal range. In conclusion, protected lemuru fish oil supplementation had a favorable influence on the production performance without affecting blood profile of sheep.

Keywords: Blood profile, Digestibility, Lemuru fish oil protected, Sheep

Introduction

Increasing energy intake in ruminants can be done by increasing feed energy using fat (oil), while protein intake can be increased by providing protected protein that undegradable by rumen microbes (Pramono *et al*., 2013). One of the potential energy sources, widely available, and affordable is Lemuru fish oil. Lemuru fish oil contains high unsaturated fatty acids. Supplementation of unsaturated fatty acids has been shown to increase energy efficiency through increasing energy density and supporting increased efficiency of protein tissue synthesis via increasing the flow of non-ammonia nitrogen into the duodenum (Johnson *et al*., 2002). Constraints found when Lemuru fish oil is given directly in the feed are (1) possibility of hydrogenation process in the rumen which converts unsaturated fats into saturated fats; (2) application of oil can interfere the cellulolytic microbial activity, thereby reducing the rate of fermentation in the rumen; (3) Lemuru fish oil has a fishy smell due to its trimethylamine oxide compounds, and may lead to low palatability if it mixes directly to the feed rations (Pramono *et al*., 2013). Therefore, protective treatment is needed to obtain tangible benefits from the supplementation of energy and/or protein sources in the feed. In unsaturated fatty acid supplementation, protection is needed to prevent unsaturated fatty acids from double bond biohydrogenation by rumen microbes (Ashes *et al*., 1995). Protection is also helpful in eliminating the negative impact of unsaturated fatty acid supplementation at high levels, in the form of decreasing fiber degradability (Aharoni *et al*., 2004). Calcium soap (Ca-soap) is one of the technologies to protect fat that has been developed recently. Calcium soap is a form of protected fat and is an effective source of fat in ruminant feed ingredients because it can make the rumen fermentation system remains normal, high fatty acid digestibility, and this soap can be easily mixed with various types of feed ingredients (Jenkins and Palmquist, 1984). Through the saponification method with calcium salt ($CaCl_2$), it is expected that the use of fat will not have a negative impact on the rumen microbial ecosystem. Increased digestion of fatty acid in Ca soap as a result of decreased ruminal biohydrogenation and hence larger concentrations of UFA in intestinal mucosa was also observed to improve extract ether (EE) and crude fiber (CF) digestibility with calcium salt supplementation.

Lipolysis and biohydrogenation are common in unprotected fat that travels through the rumen. As the unsaturation of a fatty acid increases, so does its digestibility. As a result, the increased digestibility of EE and CP in protected fat in the diets could be related to reduced ruminal biohydrogenation and the presence of a considerable proportion of long chain unsaturated fatty acids in the small intestine for absorption (Behan *et al*., 2019).

The decrease in sheep's performance might be caused by the not optimum metabolic processes in the sheep's body. Metabolic processes that are not optimal can disturb the physiological condition of livestock, and one of the indicators that can determine the physiological condition of livestock is the livestock's blood profile (Astuti *et al*., 2008). Lipid profile in blood is one of the parameters used to determine livestock's health condition and productivity. Blood has a complex role in the body's physiological processes (Gross *et al*., 2016). Fatty acids, especially omega-3 in fish oil and omega-6 in plant oil, can affect fat metabolism. Therefore, the level of fat components (cholesterol, triacylglycerol, HDL, and LDL) in the blood can be used to indicate the effectiveness of fat protection in ruminant rations (Adawiah *et al*., 2006). Fatty acids that pass from the rumen and into the duodenum are typically connected to meal particles or microorganisms. Fatty acids will dissolved by bile salts.

Phospholipase enzymes hydrolyze lecithin, a microbial phospholipid, to produce lysolecithin. Micelles will be formed from fatty acids, salt bile, and lysolecithin (small circles). These micelles aid in the absorption of fatty acids in the intestine (jejunum). Fatty acids are esterified in epithelial cells of the small intestine, and triacylglycerol and phospholipid bind to chyclomicron and very low density lipoprotein (VLDL) and are transported to the lymph glands (Wina and Susana, 2013).

This study aimed to evaluate the protection of Lemuru fish oil by saponification method as a feed supplement based on in vivo nutrient digestibility and blood profile in sheep.

Materials and Methods

Protected lemuru fish oil making process

Lemuru fish oil was obtained from the waste of sardine canning industry by PT Sumber Yalasamudra in Muncar, Banyuwangi, East Java. Other ingredients were distilled water, caustic soda (technical NaOH), technical $CaCl_2$, and starch flour as a capsule.

The oil was heated and mixed with different concentrations of technical NaOH solution (caustic soda) while stirring and adding starch solution until a soft and elastic paste (gel) was formed. The ratio of the volume of oil with a solution of NaOH and starch was 1:2:1. The formed paste was left overnight (12 hours) to solidify. After the solidification, the gel was made into thin plates, sliced with a knife, and then immersed in a saturated $CaCl_2$ solution until the gel plate hardened. Subsequently, the dough was crushed (still immersed in a saturated $CaCl_2$ solution) by pressing until small granules (crystals) were formed. After the granules were formed, it was still immersed in a saturated $CaCl_2$ solution for approximately 1 hour so that the granules harden, and then the granules were filtered. The remaining liquid carried by the granules was removed by pressing. The granules obtained were dried under the sun (10 to 20% moisture content) to form fatty acid soap from Lemuru fish oil (Setyaningrum *et al*., 2015). The soap that has been formed then later mix into the Total Mixed Ration (TMR).

In vivo experiments on sheep

This study used 12 ewes age less than one year with an average body weight of 18 kg. The research location was the Dumbira Farm, Tamanmartani, Kalasan, Yogyakarta. The basal ratio used in this study was dried kale, and concentrate was given using the Total Mixed Ration (TMR) method. The concentrate given was the one commonly used by breeders. The constituent ingredients of the concentrate were coffee husk, cocoa husk, copra meal, palm cake, and pollard. The ingredient of each experimental diets is presented in Table 1. Drinking water was provided ad libitum. The materials used for taking sheep blood were 5 ml sterile syringe, 3 ml EDTA tube, venoject, vacutainer, and cooler box.

The treatment was P0 (control = without Lemuru fish oil supplementation), P1 (TMR containing 5% protected Lemuru fish oil supplementation), and P2 (TMR containing 10% protected Lemuru fish oil supplementation). The composition of feed nutrients is presented in Table 2.

The experiment was carried out for 8 weeks with an adaptation period of one week. Feeding was done twice in the morning and evening. Nutrient digestibility was calculated based on the total collection method carried out for 14 days in the fifth and sixth weeks of the experiment. The fecal collection was carried out by collecting all sheep feces during the total collection period. Every day (24 hours), the feces were weighed, and then 200 grams of feces were taken for drying. Feces for 14 days in each experimental unit were composited for nutrient levels analysis. Blood samples were taken at the sixth week of rearing before the sheep was fed. Blood was taken from the jugular vein using a 5 ml sterile syringe, then put into a sterile tube containing EDTA anticoagulant. The samples were then analyzed for levels of blood metabolites, including cholesterol, triglycerides, LDL, HDL, and glucose.

Experimental design and data analysis

The study used a completely randomized design with 3 treatments and 4 replications. The data obtained were analyzed using One way ANOVA. The observed variables included the

Table 1. The ingredients of each experimental diets

Feed ingredients	Nutrient content							
	Dry matter	Ash	Organic matter	Crude protein	Crude fat	Crude fiber	Nitrogen free extract	Total digestible nutrient
Coffee husk	95.22	0.85	98.31	3.08	0.43	39.74	55.90	82.22
Cocoa husk	94.01	9.63	80.74	14.18	4.83	13.74	57.62	54.08
Copra meal	95.91	10.67	78.66	18.41	3.6	10.38	56.94	53.58
Palm cake	95.96	4.34	91.31	11.76	12.61	14.82	56.47	93.25
Pollard	95.71	5.74	88.52	11.98	3.82	8.33	70.13	66.95
Dried kale	91.97	11.67	76.66	7.2	2.1	23.32	55.71	53.69

Table 2. Nutrient composition of the total mixed ration (TMR)

Chemical composition (%)	Treatment		
	P0	P1	P2
Dry matter [1]	82.95	80.87	80.20
Organic matter[1]	89.57	87.94	87.12
Crude protein[1]	12.41	11.89	11.53
Crude fat[1]	4.33	5.12	6.14
Crude fiber[1]	25.62	28.17	26.55
Total Digestible Nutrient[2]	67.29	64.68	65.73

[1] Analysis Results from the Laboratory of Animal Feed Science, Faculty of Animal Science, UGM.
[2] Calculation results based on the formula of John (2005).

digestibility of the dry matter, organic matter, crude protein, crude fat, crude fiber, Nitrogen free extract (NFE), and TDN, and blood profiles consisting of cholesterol triglycerides, LDL, HDL, and glucose.

Results and Discussion

Nutrient digestibility

The average dry matter digestibility and nutrients of sheep are presented in Table 3. Dry matter digestibility, organic matter, crude protein, and TDN resulted from the three treatments showed no significant difference (P>0.05). The dry matter digestibility that did not show a difference was possible because of the protection of the oil. According to Tanuwiria *et al*. (2006), protection is a form of manipulation of feed in the rumen to maximize the nutrients intake. This causes regular microbial activity in the rumen due to the protected fat can go directly to the post-rumen. There was no difference between the amount of feed consumed and feed rate in the rumen between the three treatments due to the normal microbial activity. According to Abqoriyah *et al*. (2013), factors that affect dry matter digestibility include the ratio composition, the rate of travel through the digestive tract, and the physical form of the feed ingredients. The faster the flow rate of feed particles leaving the rumen causes a higher chance for feed ingredients to the shorter degradation, which leads to higher digestibility. The physical form of feed ingredients from P0, P1, and P2 in this study did not differ, so it was assumed that the feed flow rate in the rumen did not affect digestibility. According to the research of Kustantinah *et al*. (2007), the possible cause of the treatment did not provide a significant difference was the body weight and age of the livestock used in the study were almost the same, and there were not enough replications. The protection may prevent rumen microbial degradation, and the protection of lemuru oil will be ruptured post ruminally, so that it can be easily digested and absorbed post ruminally which in turn effects on nutrient digestibility.

Analysis of variance from the three treatments showed significantly different results (p<0.05) for digestibility of crude fat, crude fiber, and Nitrogen free extract (NFE). Fiber digestibility is related to the ability of rumen microbes to degrade fiber components. In this study, crude fiber digestibility of the ratio using protected fish oil was higher than the control one. This showed that the role of protection in the use of oil could maintain rumen microbial growth conditions. Protection can cover oil against feed particles so that microbial growth in rumen fluid is not inhibited and does not reduce fiber digestibility (Abqoriyah *et al*., 2013).

The treatment was P0 (control = without Lemuru fish oil supplementation), P1 (TMR containing 5% protected Lemuru fish oil supplementation), and P2 (TMR containing 10%

Table 3. The average in vivo nutrient digestibility of sheep

Digestibility	Treatment		
	P0	P1	P2
	----------%----------		
Dry matter	65.70±5.51	65.76±0.77	70.50±3.32
Organic matter	66.26±5.49	66.09±0.97	70.85±3.30
Crude protein	70.83±5.66	71.33±0.70	72.45±3.55
Crude fiber	53.70±9.02^{a}	61.83±2.00^{ab}	65.54±3.53^{b}
Crude fat	84.51±3.20^{a}	88.73±3.69^{ab}	92.06±2.09^{b}
Nitrogen free extract (NFE)	70.04±3.88^{a}	64.49±2.42^{b}	70.65±3.48^{a}
Total digestible nutrient (TDN)	64.09±5.07	64.20±1.34	69.19±3.21

Different superscript letters in the same line shows significantly different (p<0.05).
P0 (control=without protected Lemuru fish oil supplementation); P1 (TMR with 5% protected Lemuru fish oil supplementation) and P2 (TMR with 10% protected Lemuru fish oil supplementation).

protected Lemuru fish oil supplementation). The composition of feed nutrients is presented in Table 2.

Sheep blood profile

The average cholesterol, triglycerides, LDL, HDL, and blood glucose of sheep from the study are presented in Table 4.

The cholesterol level in this study ranged from 52.95±8.66 to 66.68±16.69 mg/dl. This study has a lower cholesterol level than the study of Gagah *et al.* (2016), which was between 58 mg/dL–81 mg/dL. The results of the analysis of variance showed that the effect of treatment on the cholesterol level of sheep's blood was not significantly different (P>0.05), but descriptive calculations showed that there was a decrease in cholesterol level in the treatment that was given 10% protected Lemuru fish oil. The decrease in sheep blood cholesterol levels was caused by the inhibition of saturated fatty acids formation so that the production of unsaturated fatty acids increased. Fatty acids that pass to the small intestine will increase the production of bile. Increased bile fluid can indirectly reduce cholesterol in the blood (Sudarmi *et al*., 2012). Another possible reason for the insignificant blood profile level was the stress due to weighing, which was done once a week that may disturb the sheep's metabolic system. Cholesterol is a component of fat, and the otal cholesterol is a composition of many substances, including triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL) (Alam *et al*., 2010). Special receptors in peripheral tissues capture the cholesterol contained in LDL. Excess cholesterol in peripheral tissues is transported by HDL to the liver to be excreted through the bile ducts as bile acids (Cheng and Hardy, 2004). The triglyceride level in sheep in this study ranged from 23.68±5.64 to 33.40±7.02. This result was lower than the previous study by Hatta et al. (2018), ranged from 25,732 - 40.44 mg/dl. The low level of triglycerides in the blood was due to the body's energy needs being met. Soehardi (2004) stated that if cells need energy, the lipase enzyme will break down triglycerides into glycerol and fatty acids and release them into the blood vessels. Damron (2003) stated that the blood triglyceride level is influenced by the fat level digested from food or the amount of fat that enters from outside the body.

High-Density Lipoprotein (HDL) plays an essential role in binding the excess cholesterol and transporting it to the liver. High HDL level can prevent the risk of atherosclerosis. Hasanudin *et al.* (2013) stated that HDL is a lipoprotein that maintains the balance of cholesterol to not accumulate in cells by equalizing the sterol removed from the membrane and the cholesterol synthesized into the liver. Table 3 shows the HDL level of sheep blood ranged from 34.70 ± 1.97 mg/dl – 41.40 ± 6.65 mg/dl. The results of this study were almost the same as those of Faisal *et al.* (2017), which the HDL level ranged from 22.25 to 46.25 mg/dl, but lower than the study of Hatta *et al*. (2018) found that the HDL level in sheep serum ranged from 54.87 to 59.39 mg/dl.

Hasanudin *et al*. (2013) stated that LDL plays a role in providing cholesterol in body tissues because it is the primary carrier for cholesterol from the liver to body tissues. Table 3 shows the LDL level of sheep blood ranged from 14.40±2.61 – 19.22±5.43 mg/dl. This level was still lower than Prayitno and Heni (2021) research results, 31.78±1.16 – 43.94±15.36 mg/dl. Supported by Hasanudin *et al*. (2013) that there is a positive correlation between LDL and cholesterol. LDL plays a role in providing cholesterol in body tissues because it is the primary carrier for cholesterol from the liver to body tissues. The decrease in LDL was thought to be due to unsaturated fatty acids contained in protected Lemuru fish oil, which increased LDL catabolism's speed. This was supported by the statement of Pramitasari *et al.* (2012) that several hypotheses have explained the effect of unsaturated fatty acids in the form of stimulating cholesterol excretion into the intestine and stimulating the cholesterol oxidation becomes bile acids. LDL is often called bad fat, so its level must be lower than HDL.

The glucose levels in this study were still in the normal range, namely 55.22±11.09 to 54.65±8.19. According to Cynthia and Scott (2005), the normal level of sheep blood glucose is 44 – 81 mg/dL. Glucose serves as the fastest source of energy to be used as ATP for both major organs such as the brain and nervous system and other organs whose role cannot be replaced by other nutrients (Astuti *et al.,* 2006). The glucose level in this study was not affected by the addition of 5% and 10% protected Lemuru fish oil. The profile and blood metabolites level did not differ and were still in the normal range, a positive indication that the addition of 5% and 10% protected Lemuru fish oil did not affect the process of blood components formation and absorption of nutrients from metabolism, which indirectly also indicated that

Table 4. The average sheep blood profile levels

Blood profile	Treatment		
	P0	P1	P2
	----------mg/dl----------		
Cholesterol	63.73±4.92	66.68±16.69	52.95±8.66
Triglycerides (mg/dl)	23.68±5.64	24.95±4.20	33.40±7.02
HDL (mg/dl)	38.3±5.94	41.4±6.65	34.7±1.97
LDL (mg/dl)	17.45±0.34	19.22±5.43	14.40±2.61
Glucose	55.52±13.69	54.65±8.19	55.22±11.09

The analysis result from Laboratorium Penelitian dan Pengujian Terpadu (LPPT) UGM.

the health/physiological condition of sheep was not disturbed. This was in accordance with the statement of Astuti *et al.* (2008) that the hematological profile and blood metabolite status is one of the indicators that determine the physiological condition of livestock.

Conclusions

From this study, it can be concluded that 10% protected Lemuru fish oil supplementation in the TMR (P2) showed the best in vivo crude fiber digestibility and crude fat digestibility compared to P0 and P1 treatments. The addition of protected Lemuru fish oil in the TMR ration had no significant effect on the digestibility of the dry matter, organic matter, and crude protein. In general, the supplementation of protected Lemuru fish oil did not disturb the sheep's physiological condition, as reflected by the normal blood profile of the treated sheep.

Acknowledgement

The authors are thankful to the Universitas Gadjah Mada for providing research funds through the “Hibah Kompetisi Pascasarjana 2021 Fakultas Peternakan UGM”.

References

Abqoriyah, S. D. Widyawati, and Lutojo. 2013. Penggunaan minyak ikan lemuru (Sardinella longiceps) dan minyak kelapa sawit diproteksi dalam ransum domba lokal jantan terhadap daya guna pakan serat. Zoo Indonesia. 22: 39-46.

Adawiah, T. Sutardi, T. Toharmat, W. Manalu, and N. Ramli. 2006. Respons suplementasi sabun mineral dan mineral organik serta kacang kedelai sangrai pada kecernaan nutrien pakan dan leak serum domba. J. Indon. Trop. Anim. Agric. 31: 211-218.

Aharoni, Y., A. Orlov and A. Brosh. 2004. Effect of high-forage content and olseed supplementation of fattening diets on conjugated linoleic acid (CLA) and trans fatty acids profiles of beef lipid fractions. J. Anim. Sci. Techno. 117: 43-60.

Alam, M. K., Y. Ogata, Y. Sako, M. Al-Mamun and H. Sano. 2010. Intermediary metabolism of plasma acetic acid, glucose and protein in sheep fed a rice straw based diet. J. Anim. Sci. 23: 1333-1339.

Ashes, J. R., E. Fleck and T. W. Scott. 1995. Dietary manipulation of membrane lipids and its implications for their role in the production of second messenger. In: Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. W.V. Engerhardt, S.L. Marek, G. Breves and D. Giesecke. (eds) Ferdinand Enke Verlag. Stuttgart. 373-385.

Astuti, D., A Suprayogi, D. R. Marwah, and Suryani. 2006. Status nutrien dan gambaran darah pdomba lokal yang dipelihara di hutan pendidikan Gunung Walat Sukabumi. Seminar Nasional Teknologi Peternakan dan Veteriner. Fakultas Peternakan. Institut Pertanian Bogor.

Astuti, D. A., D. R. Ekastuti, Y. Sugiarti and Marwah. 2008. Profil darah dan nilai hematologi domba lokal yang dipelihara di Hutan Pendidikan Gunung Walat Sukabumi. Agripet. 8: 1-8.

Behan, A. A., T. C. Loh, S. Fakurazi, U. Kaka, A. Kaka, and A. A. Samsudin. 2019. Effect of supplementation of rumen protected fats on rumen ecology and digestibility of nutriens in sheep. Animals. 9: 1-18.

Cheng Z. J. and R. W. Hardy. 2004. Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, Litopenaeus vannamei (Boone). J. Anim. Sci. 82: 1136–1145.

Cynthia M. K. and L. Scott. 2005. The Merck Veterinary Manual. 9th edn. Kahn CM Merck & Co Inc, New Jersey.

Damron, W. S. 2003. Introduction to Animal Science: Biological, Industry Perspective. Prentice Hall, New Jersey.

Faisal, F., A. Rochana, and K. A. Kamil. 2017. Kajian kandungan kimia darah dan pertambahan bobot badan domba garut betina lepas sapih dengan imbangan protein dan energi yang berbeda. Jurnal Ilmu Ternak 17: 92-96.

Gagah, H. W., M. Yamin, H. Nuraini, and A. Esfandiari. 2016. Performans produksi dan profil metabolik darah domba garut dan jonggol yang diberi limbah tauge dan omega-3. Jurnal Veteriner 2: 246-256.

Gross, J. J., A. C. Schwinn, F. Schmitz-Hsu, F. Menzi, C. Drogemuller, C. Albrecht, and R. M. Bruckmaier. 2016. Rapid communiation: cholesterol deficiency associated APOB Impacts lipid metabolism in Holstein calves and breedingbulls. J. Anim. Sci. 94: 1761-1766.

Hasanudin, S., V. D. Yunianto and Tristiarti. 2013. Profil lemak darah pada ayam broiler yang diberi pakan step down protein dengan penambahan air perasan jeruk nipis sebagai acidifier. JITP 3: 11-17.

Hatta, M., R. Priyanto, M. S. Mas, and Kusumandari. 2018. Chemical characteristic and cholesterol level of local sheep with intensive fattening. 1st International Conference of Animal Science and Technology (ICAST).

Jenkins, T. C. and D. L. Palmquist. 1984. Effect of fatty acid or calcium soaps on rumen and total nutrient digestibility of dairy rations. J. Dairy Sci. 67: 978–986.

John, M. 2005. Tropical dairy farming : feeding management for small holder dairy farmers in the humid tropics. Landlinks Press.

Johnson, K. A., R. L. Kincald, H. H. Westberg, C. T. Gaski,/l/ns, B. K. Lamb and J. D.

Conrath. 2002. The effect of oilseed in diets of lactating cows on milk production and methane emissions. J. Dairy Sci. 85: 1509-1515.

Kustantinah, H. Hartadi, and R. S. Irwansyah. 2007. Pengaruh suplementasi pada pakan basal rumput raja terhadap kinerja kambing bligon yang dipelihara KWT Lestari, Dusun Kwarasan, Kecamatan Nglipar, Kabupaten Gunungkidul. Proceeding Seminar Nasional AINI VI. 455-461.

Pramono, A., Kustono, D. T. Widayati, P. P. Putro, E. Handayanta, and H. Hartadi. 2013. Evaluasi proteksi sabun kalsium sebagai pakan suplemen berdasarkan kecernaan bahan kering, kecernaan bahan organik dan pH in vitro di dalam rumen dan pasca rumen. Sains Peternakan. 11: 70-78.

Pramitasari, M. R., R. Riana, and M. Bahrudin. 2012. Pengaruh ekstrak bawang putih (*Allium Sativum L*) terhadap perbaikan profil lipid pada *Rattus norvegicus* strain wistar hiperkolesterolemia. Saintika Medika: Jurnal Ilmu Kesehatan dan Kedokteran Keluarga. 8: 85-96.

Prayitno, R. S. and N. Heni. 2021. Pengaruh pemberian limbah daun bawang merah (Allium ascalonicum L.) sebagai hijauan alternatif terhadap profil lemak darah domba. Jurnal Peternakan 18: 19-24.

Setyaningrum, A., Soeparno, L. M. Yusiati, and Kustantinah. 2015. Performance and meat quality of thin tailed sheep in supplementary feeding lemuru fish oil protected by saponification with different NaOH concentration. J. Anim. Prod. 17: 177-185.

Soehardi, S. 2004. Memelihara kesehatan jasmani melalui akanan. Penerbit Institut Teknologi Bandung, Bandung.

Sudarmi, N., S. N. O. Suwandyastuti, and M. Bata. 2012. Pengaruh penggunaan ampas bir terhadap konsumsi, profil lemak darah dan daging domba. Prosiding seminar nasional "pengembangan agribisnis peternakan menuju swasembada protein hewani Universitas Jenderal Soedirman. Pp.89-94.

Tanuwiria, U. H., D. C. Budinuryanto, S. Darodjah, and W. S. Putranto. 2006. Studi suplemen komplek mineral minyak dan mineralorganik dan pengaruhnya terhadap fermentabilitas dan kecernaan ransum invitro serta pertumbuhan pada domba jantan. Fakultas Peternakan Universitas Padjadjaran, Jatinangor. Jurnal Protein 14: 167-176.

Wina, E. and I. W. R. Susana. 2013. Manfaat lemak terproteksi untuk meningkatkan produksi dan reproduksi ternak ruminansia. Balai Penelitian Ternak, Bogor.

Buletin Peternakan 46 (1): 7-15, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.69852

Requirements of Energy and Protein for Arabic Chicken Hens During Late Egg Production Period

Syafwan*, Yatno, Ravido Multer Mahulae, Abraham Lincoln and Deby Isabela BR. Sembiring

Department of Animal Production, Faculty of Animal Science, University of Jambi, Jambi 36361, Indonesia

Article history
Submitted: 19 October 2021
Accepted: 4 January 2022

* Corresponding author:
Telp. +6285283274718
E-mail: syafwan@unja.ac.id

ABSTRACT

The present study aimed to estimate the metabolizable energy (ME) and protein (CP) requirements of Arabic chicken hens during the late egg production period reared under a semi-scavenging system with free-choice feeding. A total of 112 sixty-two-week-old Arabic chicken hens were used. The treatments were control and the choice diet consisted of 6 replicate pens. Control hens received a control diet (2750 kcal of ME/kg and 14.1% of CP) complying with the Hy-line Brown Commercial Management Guide 2011, whereas the choice hens offered control and three other diets (high energy-high protein [3006 and 17.3], high energy-low protein [3089 and 12.7], and low energy-high protein [2656 and 17.0] kcal of ME/kg and % of CP, respectively). Feed, ME, and CP intake, the concentration of dietary ME and CP, and egg production were recorded weekly. Data were analyzed using Proc Mixed of SAS. The feeding method influenced feed intake, CP concentration, and ME concentration but had no significant effect on CP intake, ME intake, and egg production. Weekly feed intake of choice hens was lower than that of control hens (514.03 vs. 551.18 g /hen/week; $P<0.03$). Dietary concentrations of ME and CP in the choice hens were higher than those in the control hens (2957 vs. 2750 kcal of ME/kg; $P<0.001$ and 150.6 vs. 14.1 g of CP/kg; $P<0.001$). Egg production of the choice hens was not significantly higher than that of the control hens (51.17% vs. 46.82%; $P>0.05$). Feed intake, CP intake, and ME intake decreased significantly at week 66 onward, while egg production decreased at week 65 onward. It can be concluded that Arabic chicken hens in the late egg production period were able to adjust their energy and protein requirements by consuming more from high dietary energy than from a high dietary protein. Based on the choice feeding, ME and CP requirements for Arabic chicken hens during the late egg production period in the semi-scavenging system were 2957 kcal/kg and 151 g/kg and higher than ME and CP contain in the control diet of 2750 kcal/kg and 141 g/kg to maintain egg production. The egg mass and feed conversion ratio were better in the choice hens group.

Key words: Arabic chicken, Choice feeding, Egg production, ME and CP requirements

Introduction

Arabic chicken as an Indonesian local chicken strain has claimed that has been producing more egg (Husmaini and Sabrina, 2006; Hartawan and Dharmayanti, 2016) and will contribute to meet the demand for poultry egg in the future and to increase the contribution to national egg production. Therefore, the potential of Arabic chicken hens especially in the tropics area like Indonesia to produce more eggs should be improved. However, there are two important factors influencing egg production in the tropics, including temperature and nutrient requirements.

Laying hens' diets must contain sufficient quantities of all nutrients needed, particularly crude protein (CP) and metabolizable energy (ME). It is generally believed that feeding with a high dietary CP level to animals causes a low-protein utilization and high-heat production while limiting protein consumption and adding synthetic DL-methionine and L-lysine can improve the efficiency of protein utilization and productive performance of the hens (Poosuwan *et al.*, 2010). On the other hand, reducing the dietary CP resulted in lowering egg production as compared with the hens fed the normal-CP diets although lowered the NH_3 emission from laying-hen manure as a result of lowering the manure pH (Roberts *et al.*, 2007). However, different dietary levels of CP and amino acid contents had significantly improved feed conversion and a better protein utilization if the broiler fed with a high energy level at an ambient temperature ranging from 21.1℃ to 35℃ (Syafwan *et al.*, 2011).

Many farmers in tropical and subtropical countries raise laying hens in an open-housed system and the temperature inside the house is

high most of the day in the yearly round. The hens exposed to high environmental temperature reduced feed intake, egg production, egg mass, and eggshell quality to maintain body homeostasis (Deng *et al.*, 2012). Syafwan *et al.*(2012) also demonstrated that high environmental temperatures decreased feed intake and growth of broiler. Thus, the high ambient temperature in the poultry house brings economic loss from production. Therefore, the nutrient requirements studied for commercial egg-laying hens in the temperate environment may not be matched to the nutrient requirements of egg-laying hens in tropical regions. One way to get an appropriate requirement of ME and CP for Arabic chicken hens is to allow the hens to select from the various quality of feed. To be effective in choice by the hens, the feeds need to be nutritionally distinct (Fanatico *et al.*, 2016). Free-choice feeding is an alternative feeding method that allows the hens to adjust intake as a function of nutrient requirements such as energy, protein, minerals, or other nutrients (Syafwan *et al.*, 2012; Fanatico *et al.*, 2013). Offering choices of the diet with various quality of ME (2638 to 3133 kcal/kg) and CP (14.6 to 23.4%) in Arabic chicken hens during early egg production resulted in a high ME and CP concentration in the diet consumed and produced more eggs than the control diet (Syafwan and Noferdiman, 2020). However, they use five feeds in the choice feeding group including an energy-protein poor diet. Thus, the hens need more time in choosing to balance the ME and CP needs. We do not know what kind of diet the Arabic chicken hens during late lay production would select without an energy-protein poor diet to compose their CP and ME requirements that are suitable for their egg production capacity in the late egg production period.

The objectives of the present study were (1) to determine Arabic chicken hens aged at late egg production period can compose an adequate ration from different diets that varying in energy and protein contents with a choice feeding; (2) to calculate the CP and ME requirements of Arabic chicken hens in the age of late egg production period, and (3) to compare the egg production of Arabic chicken hens when given a free choice diet with a standard layer ration.

Materials and Methods

Animal care, birds, and housing

All experiments were approved by the Animal Science Faculty Ethical Clearance Committee number 002/UN21.7/ECC/2021. A total of 112 sixty-two-week-old silver Arabic chicken hens were used in this research. This chicken is a specific breed of native chicken for layer purposes (Hartawan and Dharmayanti, 2016). They were assigned to 12 pens with 9 to10 hens each. The pen dimension was 2 m inside the house and 3 m outside the house with similar wide (1.75 m) and high (2 Am). The pen was separated with netted nylon. The pen floor inside the house was covered with sand and the pen floor outside the house was ground. The house was open-sided and the hens could access the yard freely.

Each diet was served in separate feeders in each pen. Feeders' positions in each pen were changed every day randomly to avoid the habituation of the hens. One bell-shaped drinker was filled in when necessary and the wooden perch with rounded angles was fixed about 1 m above the floor in each pen. The nest was provided in each pen on the floor.

The temperature and humidity cycle were recorded 3 times daily (07:00 am, 12:00, and 5:00 pm) by using a maximum-minimum thermo-hygrometer. An emergency light was placed for every two pens to guarantee 16-hour light and 8-hour dark every day when the electricity went off.

Experimental design and treatments

The experiment was conducted as a completely randomized design with two treatments and six replicate pens. The no-choice hens received a control diet containing 2750 kcal of ME/kg and 14.10% of CP for the late laying period [>-60 weeks of age] as recommended by Hyline Brown Commercial Management Guide (HyLine, 2011). The choice hens were fed with a control diet [ME: 2750 kcal/kg and CP: 14.10%]; high energy-high protein diet, **HMEHCP** [ME: 3006 kcal/kg and CP: 17%], high energy-low protein diet, **HMELCP** [ME: 3089 kcal/kg and CP: 12.70%], and low energy-high protein diet, **LMEHCP** [ME: 2656 kcal/kg and CP: 17%]. Each diet was supplied as a mash form and adapted for one week. Rice bran, yellow corn, and palm oil were energy sources, and soybean meal and fish meal were protein sources. Dietary compositions of the diets are presented in Table 1, and the nutrients contents of the diets are presented in Table 2. The only big differences in the nutrient content of dietary treatments were protein and energy. The other nutrients' contents such as Ca, NPP, and Na were almost identical. Thereby, the hens are directed only to meet the protein and energy requirements.

Traits measured

Feed intake (FI) per pen was recorded by weighing the feed offered and feed residues weekly (g/bird/week). Energy and protein intake were calculated from the intake of each of the four diets times the content of ME and CP in each diet then divided by 1000 (g/kg). The concentrations of ME and CP in the diet intake were calculated from the ME and CP intake divided by FI times 1000 (g/kg) (Syafwan *et al.*, 2012; Syafwan and Noferdiman, 2020).

Data of FI, ME intake, CP intake, the concentration of dietary ME and CP were recorded weekly. Egg productions were recorded daily. The percentage of hen day egg production (% HDP) was calculated from the total number of eggs laid divided by the total number of live hens

Table 1. The ingredients (%) composition of dietary treatments

Ingredients	Control (> 60 weeks)	High energy-high protein diet (HMEHCP)	High energy- low protein diet (HMELCP)	Low energy-High protein diet (LMEHCP)
Rice bran	12.05	5.54	7.23	15.32
Maize	52.64	38.52	52.00	41.19
Soybean meal	19.19	28.90	14.00	27.00
Fish meal	0.00	1.00	3.00	0.50
Salt	0.37	0.38	0.33	0.37
Top Mix[1]	1.30	1.30	1.30	1.33
Dicalcium phosphate	0.99	0.83	0.63	0.83
Calcium carbonate	9.35	9.47	9.42	9.40
DL-Methionine	0.11	0.06	0.09	0.06
Palm oil	4.00	14.00	12.00	4.00
Total	100.00	100.00	100.00	100.00

[1]Composition of 1 kg Top Mix: vitamin A (retinyl acetate), 12,000 IU; vitamin D_3 (cholecalciferol), 2,000 IU; vitamin E (dl-α-tocopherol), 8.0 mg; vitamin K, 2.0 mg; vitamin B_1 (thiamin), 2.0 mg; vitamin B_2 (riboflavin), 5.0 mg; vitamin B_6 (pyridoxine-HCl), 0.5 mg, vitamin B_{12} (cyanocobalamin), 12 mg; vitamin C, 25 mg; niacin, 40 mg; vitamin B_5 (d-pantothenic acid), 6.0 mg; choline chloride, 10 mg; methionine, 30 mg; lysine, 30 mg; iron, 20 mg; copper, 4 mg; manganese, 120 mg; zinc, 100 mg; cobalt, 0.2 mg; iodine, 0.2; and santoquin (antioxidant), 10 mg.

Table 2. Calculated nutrients content (/kg) of dietary treatments

Calculated nutrients content	Control diet (> 60 weeks)*	High energy-high protein diet (HMEHCP)	High energy- low protein diet (HMELCP)	Low energy-High protein diet (LMEHCP)
Dry matter (%)	87.69	78.37	80.53	87.65
Energy (Kcal ME/Kg)[1]	2750.00	3006.00	3089.00	2656.00
Crude protein (%)	14.10	17.00	12.70	17.00
Crude fat (%)	9.75	17.80	16.86	9.85
Crude fiber (%)	8.76	5.92	6.50	8.56
Lysine (%)	0.73	0.99	0.71	0.96
Methionine (%)	0.35	0.35	0.35	0.35
Met+Cys (%)	0.50	0.58	0.49	0.59
Ca (%)	4.40	4.40	4.41	4.40
Total P (%)	0.51	0.50	0.46	0.54
NPP (%)	0.33	0.33	0.33	0.33
Na (%)	0.17	0.17	0.17	0.17

*Recommended by Hyline Brown Commercial Management Guide, Australia (2011).

[1]Metabolizable energy was calculated by determining (combustion) gross energy of the entire diet multiplied with a ME to GE-conversion factor (0.725).

per day (Khawajaa *et al.*, 2012; Syafwan and Noferdiman, 2020). Egg mass (EM) was calculated from average egg weight multiplied with egg production percentage, and FC ratio by dividing FI by EM (Bigge *et al.*, 2018).

Statistical analysis

Data were analyzed according to the method described (Syafwan *et al.,* 2012; Syafwan and Noferdiman, 2020) by using PROC MIXED in SAS. The completely randomized design was used to analyze data for a mixed model, with dietary treatment as the main effect and week as a repeated measurement. Since the data were taken repeatedly every week on the same animals, a mixed model was used to determine the covariance structure among repeated observations (Littell *et al.*, 1998; Wang and Goonewardene, 2004; Walter *et al.*, 2018) with Mixed Procedure in SAS. In the analysis, the week was used as the time factor, and the pen was considered as an additional random effect.

A probability level of ≤ 5% was considered to be statistically significant. Means were compared by pairwise comparison using the Least Significant Difference when the main effects or their interactions were significant. Means of significant effects were separated using the PDIFF option with PDMIX800 SAS macro at the $p<0.05$ level (Syafwan *et al.*, 2012; Naseem and King, 2020; Syafwan and Noferdiman, 2020). The Kenward-Roger method was used for computing the denominator df for the tests of main effects. The best covariance structure was based on the corrected Akaike Information Criteria (AICC). The autoregressive covariance structure [AR(1)] was the best fit for FI, CP intake, and ME concentration. The heterogeneous autoregressive covariance structure [ARH(1)] was the best fit for ME intake. The first-order ante-dependence covariance structure [ANTE(1)] was the best fit for CP concentration. The unstructured covariance structure [UN] was the best fit for egg production.

Results and Discussion

Environmental condition

Ambient temperature (Ta) and relative humidity (RH) are given as the average ± SD for each time recorded. During the period of this experiment, the average Ta and RH in the morning (07.00) were 25.0±1.0°C and 84±12%. On the day (12.00), the average Ta and RH were 32.2±0.9°C and 66±7.0%. In the afternoon (17.00), the average Ta and RH were 31.0±1.6°C and 69±9%. In the night (22.00), the average Ta and RH were 26.6±1.2°C and 86±6%.

The house used in this experiment was open-sided and there was no effort to control the Ta and RH. Therefore, the changes of Ta and RH both in the roofed pen and in the yard relayed on the natural conditions. The increasing and decreasing of Ta in the house were followed by decreasing and increasing of RH.

The cyclical temperature and relative humidity in the house during the experiment were dependent on the environmental climate conditions. When the temperature outside the house rose, then the temperature inside the house also rose because there was no effort made to control the house temperature. When the temperature inside the house rose, the relative humidity fell, and vise versa. The hens were changing their behavior and panting when the temperature rose above 28°C and generally happens between 12:00 to 17:00 h. Panting activity is an indicator of heat stress (Sugiharto *et al.*, 2017; Wang *et al.*, 2018) and it helps to release the extra heat to the environment. Therefore, the environmental temperature conditions indicate that the birds experienced heat stress during the time hot period of the day. The birds spent more time panting and drinking and less time walking and feeding during heat stress (He *et al.*, 2018). High ambient temperature harms body weight and feed intake of laying hens (He *et al.*, 2018) and broilers (Syafwan *et al.*, 2011; Syafwan *et al.*, 2012; Wang *et al.*, 2018).

Hens performance

The results showed that there was an effect of treatments on FI of the hens (P<0.03; Table 3). Overall, FI was lower in choice-fed hens than that in control-fed hens (Table 3), although it was similar between treatments each week (Table 4). The treatments did not affect protein and energy intake (P>0.05; Table 3). Feed intake, protein intake, and energy intake were affected by week (P<0.01; Table 3) and they decreased from 66th to 68th week of age and then did not increase significantly until the 70th week of age (Figure 1, 2 and 3). There were no interaction effects between dietary treatments and week on FI, protein intake, and energy intake (P>0.05; Table 3). CP and ME concentrations and crude fat intake were affected by dietary treatments (P<0.001; Table 3). CP and ME concentrations and crude fat intake were significantly higher in choice-fed hens than control-fed hens every week (P<0.001; Table 4). However, CP and ME concentrations were not affected by week and there were no interaction effects between dietary treatments and week on CP and ME concentrations (P>0.05; Table 3). Crude fat intake was affected by week (P<0.001; Table 3) and decreased from 65th to 68th week of age and then did not increase significantly until the 70th week of age (Figure 4). There was no interaction effect between dietary treatments and week on crude fat intake (P>0.05).

The choice-fed hens in the present study consumed a much lower amount of feed than the control-fed hens and overall they consumed about 6.74% lower of feed than the control-fed hens. The lower feed intake in choice-fed hens might be related to a higher concentration of CP and ME in the feed consumed (Table 4). This is in agreement with another study in Arabic chicken during the growing period that feed intake decreased with increased protein and energy concentration in the diet consumed (Syafwan *et al.*, 2021). Increasing protein concentrations in the diet reduced feed intake (Liu *et al.*, 2016) to avoid the increase in body temperature due to higher heat increment from protein metabolism (Syafwan *et al.*, 2011) and high environmental temperature.

Table 3. Probability values of main effects and interaction between dietary treatments[1] and week for different traits

Main Effect	No Choice	Choice	Feed	Week	Feed*Week
Feed intake (g/bird/wk)	551.18	514.03	**0.033**	**0.003**	0.979
CP intake (g/bird /wk)	77.72	77.41	0.898	**0.007**	0.965
ME intake (kcal/kg/bird/wk)	1515.75	1519.82	0.929	**0.003**	0.976
CP concentration (g/kg)	141.00	150.57	**<0.001**	0.371	0.371
ME concentration (Kcal/kg)	2750.00	2957.24	**<0.001**	0.430	0.430
Egg production (%)	46.82	51.17	0.349	**0.043**	0.969
Crude fat Intake (g/bird /wk)	53.74	78.85	**<0.001**	0.001	0.761
Egg mass (g/wk)	21.28	23.47	**0.01**	**<0.001**	0.999
FCR	3.90	3.33	**<0.001**	**0.01**	0.962

[1]No-choice: control diet [ME: 2750 kcal/kg and CP: 14.1%]; Choice: a) control diet [ME: 2750 kcal/kg and CP: 14.1%]. b) high energy-high protein diet. HMEHCP [ME:3006 kcal/kg and CP:17.0%]. c) high energy-low protein diet. HMELCP [ME:3089 kcal/kg and CP:12.7%]. and d) low energy-high protein diet. LMEHCP [ME:2656 kcal/kg and CP:17.0%].

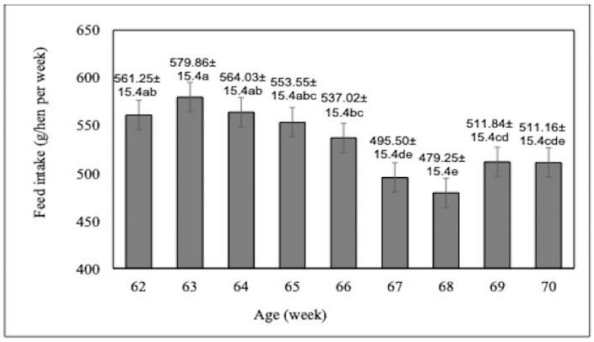


Figure 1. Least square means for feed intake that effected by week in the late laying period. Means without a common letter (a-e) differ significantly (P<0.05).

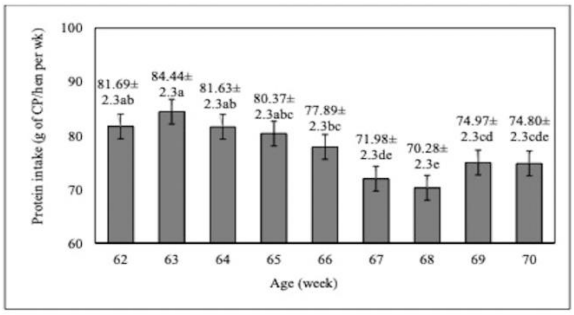


Figure 2. Least square means for protein intake that effected by week in the late laying period. Means without a common letter (a-e) differ significantly (P<0.05).

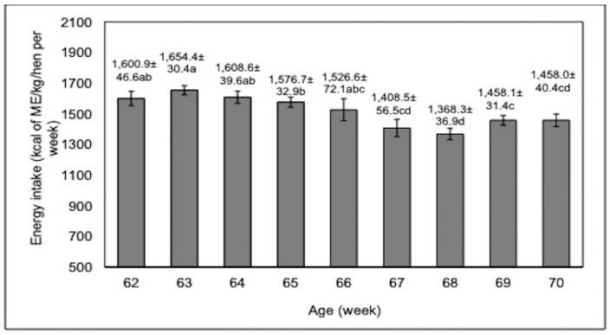


Figure 3. Least square means for energy intake that effected by week in the late laying period. Means without a common letter (a-d) differ significantly (P<0.05).

Furthermore, intraluminal infusion with lipids causes gastrointestinal delays in gastric emptying by lowering the gastric cycle frequency, increasing duodenogastric refluxes, and elongating the migrating myoelectric complex. Therefore, higher lipid concentration reduces feed intake (Khoddami *et al.*, 2018).

The average energy and protein consumption in the hens offered the free-choice diet were similar to those provided by the standard diet (1520 vs. 1516 kcal of ME/hens/week and 77.41 vs. 77.72 g/hens/week; P>0.05; Tabel 3). To meet the ME and CP requirements during this experiment, the hens in the choice group consumed HMEHCP, HMELCP, Control, and LMEHCP diets about 41.17%, 30.07%, 19.45%, and 7.31%, respectively (Figure 5). This distinct preference suggests that Arabic chicken hens during the late egg production period can fine-tune their energy and protein requirements from different diet contents independently. The shifted preference to a high-energy diet was also observed in Arabic chicken hens during the early egg production period (Syafwan and Noferdiman, 2020).

Based on these choices (Figure 5), energy and protein concentrations in the diet consumed were significantly higher in the choice group (Table 3). These preferences show that hens were capable of choosing a diet that contains nutrients for their needs. The capability of Arabic chicken hens to adjust their energy and protein requirements by selecting several diets during the early egg production period has been

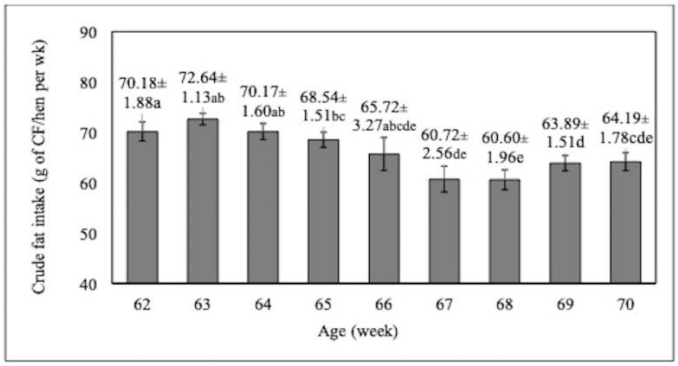


Figure 4. Least square means for crude fat intake that effected by week in the late laying period. Means without a common letter (a-e) differ significantly (P<0.05).

Table 4. Least square means of performance parameter in Arabian hens as affected by dietary treatments[1]

Parameters	Week									
	62	63	64	65	66	67	68	69	70	Average
Feed Intake (g/bird/week)										
No Choice	573.88	595.91	590.12	573.13	550.28	521.29	501.41	522.77	531.86	551.18
Choice	548.61	563.80	537.95	533.96	523.77	469.72	457.09	500.92	490.45	514.03
SEM	21.75	21.75	21.75	21.75	21.75	21.75	21.75	21.75	21.75	11.31
Probability	0.41	0.30	0.10	0.21	0.39	0.10	0.16	0.48	0.18	**0.03**
Crude Protein Intake (g/bird/week)										
No Choice	80.92	84.02	83.21	80.81	77.59	73.50	70.70	73.71	74.99	77.72
Choice	82.47	84.85	80.04	79.93	78.20	70.45	69.87	76.24	74.62	77.41
SEM	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	3.26	1.68
Probability	0.74	0.86	0.49	0.85	0.90	0.51	0.86	0.58	0.94	0.90
Energy Intake (kcal of ME/kg/bird/week)										
No Choice	1578.17	1638.75	1622.83	1576.11	1513.26	1433.54	1378.87	1437.61	1462.62	1516
Choice	1623.60	1669.99	1594.42	1577.22	1539.92	1383.42	1357.80	1478.67	1453.39	1520
SEM	65.86	42.98	56.06	46.66	101.94	79.98	52.22	44.40	57.18	36.10
Probability	0.63	0.62	0.73	0.99	0.86	0.67	0.78	0.52	0.91	0.94
Crude fat Intake (g/kg)										
No Choice	55.95	58.10	57.54	55.88	53.65	50.83	48.89	50.97	51.86	53.74
Choice	84.41	87.18	82.80	81.20	77.79	70.62	72.31	76.81	76.52	78.85
SEM	2.67	1.60	2.26	2.14	4.63	3.62	2.78	2.14	2.51	1.69
Probability	<0.001	<0.001	<0.001	<0.001	0.005	0.003	<0.001	<0.001	<0.001	<0.001
CP concentration (g/kg)										
No Choice	141.00	141.00	141.00	141.00	141.00	141.00	141.00	141.00	141.00	141.00
Choice	150.25	150.50	148.78	149.63	149.34	149.77	152.60	152.17	152.12	150.57
SEM	0.62	0.91	1.29	0.78	0.87	0.85	0.78	0.61	1.13	0.37
Probability	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
ME Concentration (kcal/kg)										
No Choice	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00
Choice	2959.71	2962.72	2964.57	2954.72	2941.94	2945.82	2970.68	2952.14	2962.87	2957.24
SEM	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	7.41	3.40
Probability	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Egg Production (%)										
No Choice	58.0	54.8	53.4	50.1	46.0	41.3	39.9	37.1	40.8	46.8
Choice	59.4	59.8	57.2	56.0	49.8	46.1	44.7	42.7	44.7	51.2
SEM	4.44	3.19	3.90	4.33	3.63	3.60	3.47	3.44	4.66	3.13
Probability	0.82	0.29	0.50	0.35	0.48	0.37	0.35	0.27	0.57	0.35
Egg mass (g/wk)										
No Choice	26.50	24.46	24.62	22.57	20.95	18.75	18.42	16.81	18.48	21.28
Choice	27.17	27.48	26.35	25.50	23.22	21.00	20.39	19.63	20.45	23.47
SEM	1.86	1.86	1.86	1.86	1.86	1.86	1.86	1.86	1.86	0.62
Probability	0.80	0.25	0.51	0.27	0.39	0.39	0.45	0.28	0.45	**0.01**
FCR										
No Choice	3.30	3.42	3.46	3.68	3.85	4.12	4.08	4.65	4.53	3.90
Choice	3.02	2.99	2.95	3.09	3.36	3.20	3.39	4.42	3.54	3.33
SEM	0.22	0.17	0.22	0.25	0.24	0.27	0.27	0.44	0.52	0.10
Probability	0.38	0.11	0.14	0.12	0.18	**0.04**	0.10	0.72	0.20	**<0.001**

[1]No-choice: control diet [ME: 2750 kcal/kg and CP: 14.1%]; Choice: a) control diet [ME: 2750 kcal/kg and CP: 14.1%]. b) high energy-high protein diet. HMEHCP [ME:3006 kcal/kg and CP:17.0%]. c) high energy-low protein diet. HMELCP [ME:3089 kcal/kg and CP:12.7%]. and d) low energy-high protein diet. LMEHCP [ME:2656 kcal/kg and CP:17.0%].

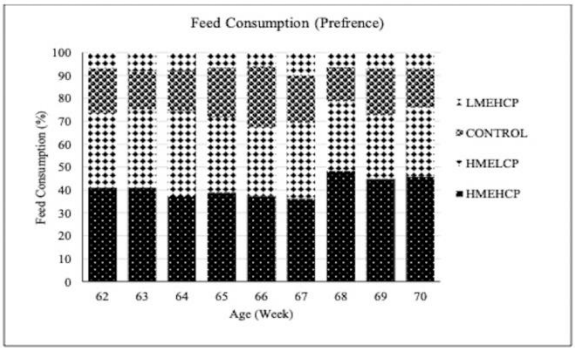


Figure 5. Intake of high energy-high protein diet (**HMEHCP),** high energy-low protein diet (**HMELCP**), control diet, and low energy-high protein diet (**LMEHCP)** as a proportion of total feed intake of dietary treament[1].
[1] a) control diet [ME: 2750 kcal/kg and CP: 14.1%]. b) high energy-high protein diet. HMEHCP [ME:3006 kcal/kg and CP:17.0%]. c) high energy-low protein diet. HMELCP [ME:3089 kcal/kg and CP:12.7%]. and d) low energy-high protein diet. LMEHCP [ME:2656 kcal/kg and CP:17.0%].

reported by Syafwan and Noferdiman (2020). These results suggested that energy and protein needs for Arabic chicken hens during the late egg production phase are higher than the energy and protein in the control diet. The higher energy requirement of Arabic chicken hens in this study was more likely due to the hens need more energy to walk in and out to the scavenging area and to release body heat to the environment. Behura *et al.* (2016) reported that

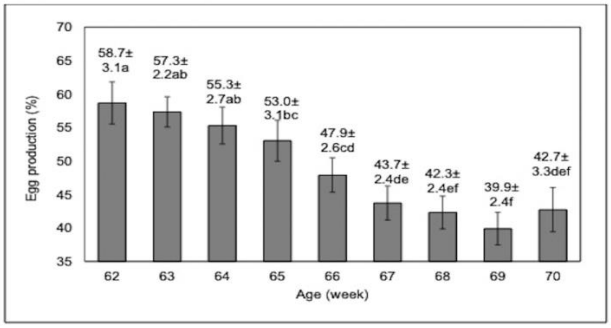


Figure 6. Least square means for egg production that effected by week in the late laying period. Means without a common letter (a-f) differ significantly (P<0.05).

the coefficient correlation between a climatic variable and ME requirement for maintenance (MEm) for broiler breeder pullet was positive and MEm increased when the temperature-humidity index increased in the hot and humid climatic condition during summertime. This higher energy concentration under high temperature reflects the higher demand for energy to release the heat load due to birds showing panting activity during summertime (Behura *et al.*, 2016). Panting is the common way for the bird to release body heat at high temperatures. On the other hand, an energy-rich diet may be favorable for the hens than a protein-rich diet under high environmental temperature because protein produces more heat load per kilojoules than do fat and carbohydrate (Syafwan *et al.*, 2011).

There was no effect of treatments on egg production of the hens (P>0.05; Table 3). Egg production was influenced by week (Table 3) and egg production decreased significantly from the 65th week of age onward (Table 4, Figure 6). Egg mass and feed conversion ratio were affected by treatments and week (Table 3). Egg mass decreased significantly from the 66th week of age onward (Figure 7) and feed conversion ratio increased significantly after 68th week of age (Figure 8).

The increasing CP concentration in the choice hens compared with the control hens (150.57 vs. 141.00 g/kg, P<0.001; Table 3) did not increase egg production significantly in this experiment. Torki *et al.*(2014) reported that the egg production of Lohmann Selected Leghorn (LSL-Lite) laying hens did not increase by

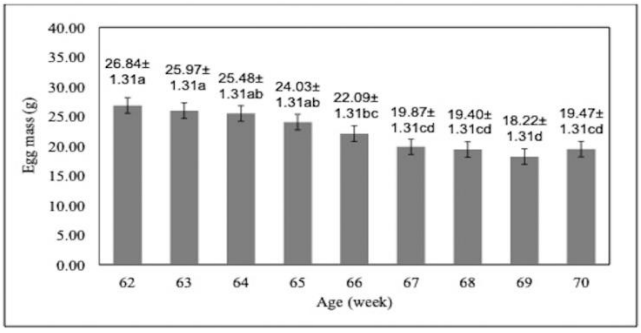


Figure 7. Least square means for egg mass that effected by week in the late laying period. Means without a common letter (a-d) differ significantly (P<0.05).

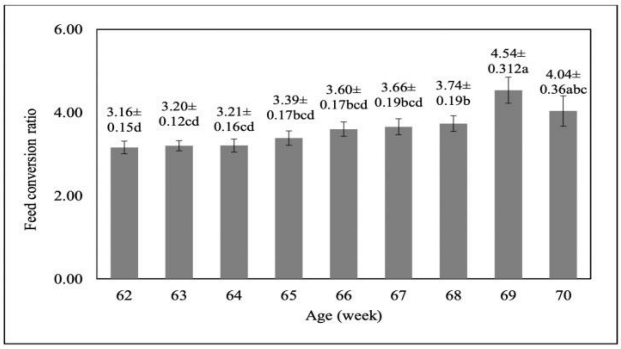


Figure 8. Least square means for feed conversion ratio that affected by week in the late laying period. Means without a common letter (a-df) differ significantly (P<0.05)

increasing CP level in the diet from 12.0 to 16.5% at an ambient temperature of 30.5±2.14°C and RH of 46.5±1.88% during 52 to 60 week of age. Limited information is available regarding the response of laying hens-fed diets with choice feeding under high-temperature conditions. In our previous study showed that egg production during the early egg production period was higher in the choice-fed hens with higher CP (2.87%; P<0.001) and ME (8.38%; P<0.001) dietary concentration in the diet consumed than the control diet (Syafwan and Noferdiman, 2020). The non-significant higher egg production in this experiment might be contributed by the ME concentration in the diet consumed by the choice hens (7.01% higher ME concentration than control diet; P<0.001) used more to release the heat load due to accumulation of heat in the body coming from protein metabolism (6.36% higher CP concentration than control diet; P<0.001) and environment and consequently less available of ME to increase egg production significantly. The Arabic chicken hens were probably more important to fulfill the nutrient requirements to balance the heat production rather than to increase egg production when they have a choice during the late egg production age under high temperatures. In normal environmental conditions (22°C), the commercial laying hens fed with a high protein diet showed a higher body weight gain, FI, and hen day egg production than that with medium and low protein diet for every phase of egg production (Shim *et al.*, 2013).

Feed intake was significantly lower and the egg mass was significantly higher and leading to a significantly lower feed conversion ratio in the choice hens group (3.33; Table 3) compared to the control group (3,90; Table 3). The feed cost for the control diet (14,1% of CP and 2750 of kcal ME/kg) was about IDR. 8,464/kg and the feed cost for the amount of CP and ME consumed in the choice hens group (15,1% of CP and 2975 of kcal ME/kg) was about IDR. 9,063/kg. By assuming the egg price was IDR. 40,000/kg, the income over feed cost was 21% and 32% for the control hens and choice hens group respectively.

Conclusions

Arabic chicken hens in the late egg production period were able to adjust their energy and protein requirements by consuming more from high dietary energy than from high dietary protein. Based on the choice feeding, ME and CP requirements for Arabic chicken hens during the late egg production period in the semi-scavenging system were 2957 kcal/kg and 151 g/kg and higher than ME and CP contain in the control diet of 2750 kcal/kg and 141 g/kg to maintain egg production. The egg mass and feed conversion ratio were better in the choice hens group.

Acknowledgement

The authors gratefully acknowledge the funding of the University of Jambi. We also would like to thank the Faculty of Animal Science, the University of Jambi to facilitate the experiment.

References

Behura, N. C., F. Kumar, L. Samal, K. Sethy, and K. Behera. 2016. Use of Temperature-Humidity Index (THI) in energy modeling for broiler breeder pullets in hot and humid climatic conditions. J. Livest. Sci. 7:75–83. Available from: http://livestockscience.in/wp-content/uploads/brolerodisa.pdf

Bigge, A. E., S. Purdum, and K. Hanford. 2018. Effect of xylanase on the performance of laying hens fed a low energy corn and soybean diet. Poult. Sci. 97:3183–3187. doi:10.3382/ps/pey200. Available from: http://dx.doi.org/10.3382/ps/pey200

Deng, W., X. F. Dong, J. M. Tong, and Q. Zhang. 2012. The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poult. Sci. 91:575–582. doi:10.3382/ps.2010-01293. Available from: http://dx.doi.org/10.3382/ps.2010-01293

Fanatico, A. C., V. B. Brewer, C. M. Owens-Hanning, D. J. Donoghue, and A. M. Donoghue. 2013. Free-choice feeding of free-range meat chickens. J. Appl. Poult. Res. 22:750–758. doi:10.3382/japr.2012-00687. Available from: http://dx.doi.org/10.3382/japr.2012-00687

Fanatico, A. C., C. M. Owens-Hanning, V. B. Gunsaulis, and A. M. Donoghue. 2016. Choice feeding of protein concentrate and grain to organic meat chickens. J. Appl. Poult. Res. 25:156–164. doi:10.3382/japr/pfv076. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1056617119302193

Hartawan, R., and N. L. P. I. Dharmayanti. 2016. The Meq Gene Molecular Profile of Marek's Disease Virus Serotype 1 From Kampung and Arabic Chicken Farms in Sukabumi, West Java, Indonesia. HAYATI J. Biosci. 23:160–167. doi:10.1016/j.hjb.2016.12.004.

He, S. P., M. A. Arowolo, R. F. Medrano, S. Li, Q. F. Yu, J. Y. Chen, and J. H. He. 2018. Impact of heat stress and nutritional interventions on poultry production. Worlds. Poult. Sci. J. 74:647–664. doi:10.1017/S0043933918000727. Available from: https://www.tandfonline.com/doi/full/10.1017/S0043933918000727

Husmaini, H., and S. Sabrina. 2006. Egg production performance of offspring (F1) crossbred between Arabic and kampung chickens fed a diet with different level of protein. Indones. J. Anim. Sci. 11:18–24.

HyLine. 2011. Hy-line Brown Commercial

Management Guide. Hy-Line, Australia.

Khawajaa, T., S. H. Khanb, N. Mukhtara, M. A. Ali, T. Ahmed, and A. Ghafar. 2012. Comparative study of growth performance, egg production, egg characteristics and haemato-biochemical parameters of Desi, Fayoumi and Rhode Island Red chicken. J. Appl. Anim. Res. 40:273–283. doi:10.1080/09712119.2012.672310. Available from: https://www.tandfonline.com/doi/full/10.1080/09712119.2012.672310

Khoddami, A., P. V. Chrystal, P. H. Selle, and S. Y. Liu. 2018. Dietary starch to lipid ratios influence growth performance, nutrient utilisation and carcass traits in broiler chickens offered diets with different energy densities. C. Óvilo, editor. PLoS One. 13:e0205272. doi:10.1371/journal.pone.0205272. Available from: http://dx.plos.org/10.1371/journal.pone.0205272

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216. doi:10.2527/1998.7641216x. Available from: https://academic.oup.com/jas/article/76/4/1216-1231/4625238

Liu, S. Y., P. H. Selle, D. Raubenheimer, D. J. Cadogan, S. J. Simpson, and A. J. Cowieson. 2016. An assessment of the influence of macronutrients on growth performance and nutrient utilisation in broiler chickens by nutritional geometry. Br. J. Nutr. 116:2129–2138. doi:10.1017/S0007114516004190.

Naseem, S., and A. J. King. 2020. Effect of Lactobacilli on production and selected compounds in blood, the liver, and manure of laying hens. J. Appl. Poult. ResApplied Poult. R. 29:339–351.

Poosuwan, K., C. Bunchasak, and C. Kaewtapee. 2010. Long-term feeding effects of dietary protein levels on egg production, immunocompetence and plasma amino acids of laying hens in subtropical condition. J Anim Physiol Anim Nutr. 94:186–195.

Roberts, S. A., H. Xin, B. J. Kerr, J. R. Russell, and K. Bregendahl. 2007. Effects of dietary fiber and reduced crude protein on ammonia emission from laying-hen manure. Poult. Sci. 86:1625–1632. doi:10.1093/ps/86.8.1625.

Shim, M. Y., E. Song, L. Billard, S. E. Aggrey, G. M. Pesti, and P. Sodsee. 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92:2687–2696. Available from: https://doi.org/10.3382/ps.2012-02569

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, and E. Kusumanti. 2017. Dietary supplementation of probiotics in poultry exposed to heat stress - A review. Ann. Anim. Sci. 17:591–604. doi:10.1515/aoas-2016-0062.

Syafwan, Noferdiman, S. Zubaida, T. M. Pasaribu, and Adrizal. 2021. Estimation of energy and protein requirements of Arabic hens during rearing period by free choice feeding. Trop. Anim. Sci. J. 44:462–477. doi:10.5398/tasj.2021.44.4.462.

Syafwan, S., R. P. Kwakkel, and M. W. A. Verstegen. 2011. Heat stress and feeding strategies in meat-type chickens. Worlds. Poult. Sci. J. 67:653–674. doi:10.1017/S0043933911000742. Available from: https://www.tandfonline.com/doi/full/10.1017/S0043933911000742

Syafwan, S., and N. Noferdiman. 2020. Requirements of Energy and Protein for Arabic Chicken During Early Egg Production. Trop. Anim. Sci. J. 43:339–346. doi:10.5398/tasj.2020.43.4.339.

Syafwan, S., G. J. D. Wermink, R. P. Kwakkel, and M. W. A. Verstegen. 2012. Dietary self-selection by broilers at normal and high temperature changes feed intake behavior, nutrient intake, and performance. Poult. Sci. 91:537–549. doi:10.3382/ps.2011-01559. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0032579119402162

Torki, M., A. Mohebbifar, H. A. Ghasemi, and A. Zardast. 2014. Response of laying hens to feeding low-protein amino acid-supplemented diets under high ambient temperature: performance, egg quality, leukocyte profile, blood lipids, and excreta pH. Int. J. Biometeorol. 59:575–584. doi:10.1007/s00484-014-0870-0.

Walter, W. S., A. M. George, A. C. Elizabeth, and D. W. Russel. 2018. SAS for Mixed Models: Introduction and Basic Applications. Cary, NC: SAS Institute Inc.

Wang, W. C., F. F. Yan, J. Y. Hu, O. A. Amen, and H. W. Cheng. 2018. Supplementation of Bacillus subtilis-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens. J. Anim. Sci. 96:1654–1666. Available from: https://doi.org/10.1093/jas/sky092

Wang, Z., and L. A. Goonewardene. 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. Can. J. Anim. Sci. 84:1–11. doi:10.4141/a03-123.

Buletin Peternakan 46 (1): 16-22, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.70685

The Effect of Complete Feed Containing Protected Soybean Groats on the Production of Javanese Thin-Tailed Male Sheep Carcasses

Yahya Nor Arif, Nurul Fatimah Isnaniyah, Ahmad Pramono, and Joko Riyanto*

Animal Husbandry Study Program, Faculty of Agriculture, Sebelas Maret University, Surakarta 57126, Indonesia

Article history
Submitted: 24 November 2021
Accepted: 11 January 2022

* Corresponding author:
E-mail: jokoriyanto@staff.uns.ac.id

ABSTRACT

The present study aims to determine the impact of complete feed containing protected soybean meal on the production of male thin-tailed lamb carcasses. A total of 15 (23.43 ± 1.40 kg) 12 months old male Thin-Tailed Sheep (TTL) were given 3 treatments and 5 replicates in a completely randomized design. The ration consisted of complete feed and formaldehyde-protected soybean groat (PSG). The treatments includes 100% complete feed (F1), 90% complete feed + PSG 10% (F2), and 80% complete feed + PSG 20% (F3). Data were analyzed using ANOVA and differences between treatments were further tested using Duncan's New Multiple Range Test. The results indicated that the use of PSG containing complete feed did not improve the characteristics of carcasses (weight and percentage of carcass components, half-cuts and commercial cuts of carcass, fleshing index and meat bone ratio) of male TTLs ($P>0.05$). However, differences were found in the rib eye muscle area. The use of complete feed containing 20% PSM (F3) resulted the highest rib eye muscle area figure compared to those of F2 and F1 (35.17 vs 27.00 and 26.33; $P<0.05$). Our study revealed that the use of complete feed containing 20% protected soybean groat resulted in higher rib eye muscle area by 25.14% compared to those containing no protected soybean groat. However, no differences in carcass characteristics were found in view of weight and percentage of carcass components, half cut and commercial carcass, fleshing index and meat bone ration in thin-tailed sheep.

Keywords: Complete feed, Male thin-tailed lamb, Protected soybean groat, Carcass production

Introduction

Thin-tailed sheep (TTL) breeding practices in Indonesia have been implemented traditionally based on people's husbandry. Common problems faced by the breeders includes limited stock of high-quality feed. In addition, the meals that the breeders fed to their livestock remain in the form of forage and concentrate which is given separately. When sheeps chooses their own meal according to their palatability, this may reduce the feeding efficiency. Genetic as well as environmental factors such as fodders play a critical role in affecting the productivity of thin-tailed sheep (Çilek and Tekin, 2005).

Complete feed serves as an alternative for sheep breeders to provide nutritionally balanced food for their animals and offer higher feed efficiency (Beigh *et al.*, 2017). Other advantages includes stable rumen environment for fermentation, release of ammonia, better utilization of non-protein nitrogen, acetate-propionate ratio stabilization that favor normal fat synthesis, and improved utilization of low quality fiber (Konka *et al.*, 2016) (Lailer *et al.*, 2005). In addition, some food ingredients such as soybean meal and fish powder can be added to optimize the TTL performance (Lailer *et al.*, 2005).

Riyanto *et al.* (2020) reported that soybean meals constitute is one of potential fodders with protein content higher than 30% and can be used as feed ingredients for large and small ruminants. However, proteins are quick to degrade in the rumen (about 65%) according to NRC (2007), causing efficiency losses. Lei *et al.* (2008) have conducted degradation test of soybean meal in the rumen of Cashmere goats using an in-vitro technique. The degradation of dry soybean meals after 24 and 12 hour incubation achieved 64.75% and 80.57%, respectively. Several chemical methods that have been tried to protect proteins from rumen microbial activity include the use of sodium hydroxide (Mir *et al.*, 1984), acetic acid (Waltz Loerch, 1986), and formaldehyde (Ferguson *et al.*, 1967), while physical method was carried out by heat treatment (Stern *et al.*, 1985).

Many studies have proposed formaldehyde because it can improve the nutritional status of livestock through its ability to reduce protein

degradation in the rumen. Formaldehyde protection of soybean meal in the ration can increase protein and fatty acid levels of omega 3 and 6 and reduces cholesterol levels of local male lamb (Riyanto and Sudibya, 2018). In addition, the use of 0.5% and 1% formaldehyde, performed in vitro, protected soybean groats was able to reduce degradation levels by 37.6 and 54.4%, respectively (Suhartanto *et al.*, 2014). Studies that use formaldehyde-protected soybean groat in complete feed have been conducted on goat farming. Adiwinarti *et al.* (2019) studied the impact of 1% formaldehyde-protected soybean groats in the complete feed on the production of Kacang goat meat. The results showed that the Kacang goats in this research relatively dont like formaldehyde-protected soybean groat, despite the fact that the latter improve the efficiency in their carcass production. Because studies on protected soybean groat have never been conducted on lamb, the current study aims to determine the impact of formaldehyde-protected soybean groat in complete feed on the carcass characteristics of TTL.

Materials and Methods

Duration and place of the study

The study was completed in 4 months from September to December 2020 in Jatikuwung Experimental Farm, Animal Husbandry Study Program, Faculty of Agriculture, Sebelas Maret University, Surakarta.

Research design and ration

This research was conducted experimentally using a completely randomized design (CRD). A total of 15 thin-tailed sheep (TTL) in this research were approximately 12 months old with incisors have appeared in pairs, and average weight of 23.43 ± 1.4 kg, fed with complete feed "Semar OMEGA" from Sekar Mendho Farm, Wonogiri, Central Java. The TTLs were assigned to 3 treatments with 5 sheep each. The treatment rations are as follows:

F1: 100% complete feed served as a control.
F2: 90% complete feed + 10% protected soybean groat (PSG).
F3: 80% complete feed + 20% protected soybean groat (PSG).

The rations were fed at 08.00 AM and 04.00 AM and water was given ad libitum. The adaptation period lasted for 1 month and after that the sheep were given treatment feed for 3 months. The nutrient content of the complete feed and PSG are presented in Table 1; meanwhile, the composition and nutrient contents of the treatment feed are presented in Table 2.

Making protected soybean groat (PSG)

The PSG is made through several stages, which starts from drying the soybean groats, milling, to spraying the aldehyde from 37% formaldehyde and then mix them with water in a ratio of 1:5 until evenly distributed. The PSG is cured for 24 hours and fed to DET after being aerated for 8 hours (Riyanto *et al.*, 2017).

Carcass data collection

After being reared for 4 months (1 month for adaptation and 3 months for treatment), TTLs were slaughtered in order to collect carcass data. Slaughtered TTLs were then weighed to determine their dead weight. The slaughtering was performed in three days with 3 sheep that represent each treatment on the first day, and 3 sheep each on the next two days. The slaughtering procedure is in compliance with the MUI halal certification (2009) and the cuttings of lamb carcass follow the procedures proposed by Khotijah *et al.* (2019). The lamb carcass has four major primal cuts; the joints of atlas bone, jugular vein, esophagus and trachea.

Before being skinned, the lamb head was removed from the body, in the exact area of the atlanto-occipital joint. The forelegs and hind limbs were cut at the carpometacarpal and tarsometatarsal joints. The skinning started by incision of the skin from the anus to the neck, and then from hind legs and forelegs to the first incision, and finally the whole part of the carcass. The lamb innards and plucks were taken out from the abdomen and chest, the rectum is separated and tied to prevent the discharge of feces. Carcass weight can be obtained after the removal of innards, plucks, omental fat, liver, and tail.

Carcass length was measured from the front end of the shoulder to the end of the hip bone (os pubis) (Hafid *et al.*, 2019). Carcass half cutting was made between the 12th and 13th rib (BSN, 2020). Rib eye muscle area was measured across the front-half of the carcass. There are 8 commercial cuts of lamb carcass: neck, shoulder, shank, rack, breast, loin, leg, and flank (Jatnika *et al.*, 2019). Those commercial cuts were then weighed and dissected between bone, muscle and fat tissue to obtain carcass composition.

The variables observed in this study includes slaughter weight, carcass weight, carcass percentage, flashing index, rib eye muscle area, and meat bone ratio.

Data analysis

The data collected in this study were analyzed using analysis of variance based on a completely randomized design (CRD) with a unidirectional pattern. When differences exist ($P<0.05$), then we can proceed with the test for difference of means, namely the Duncan's Multiple Range Test (DMRT) (Susilawati, 2015).

Results and Discussion

Carcass weight and percentage

Both weight and percentage of carcass are presented in Table 3. Slaughter weight and carcass weight have no difference between treatments ($P>0.05$). This means that PSG content, both 10% and 20%, in the complete feed

Table 1 Nutrient content in feed ingredients that make up the ration

Feed ingredient	BK[a]	PK[a]	LK[a]	SK[a]	ABU[a]	BO[b]	BETN[b]	TDN[c]
	BK (%)							
Complete feed	87.47	15.12	2.55	20.91	12.84	87.16	48.58	58.20
Protected soybean groat	86.41	36.39	11.65	7.01	7.51	92.49	37.44	93.93

[a] The results of proximate analysis conducted at the Biochemistry Laboratory of Nutrition and Animal Feed, Faculty of Animal Husbandry, Gajah Mada University, Yogyakarta 2021.
[b] The results of calculations by Sutardi (2001).
[c] The results of calculations by Tilman *et al.* (1998).

Table 2 Composition and nutrient content of the treatment ration

Feed ingredient	F1	F2	F3
		--%--	
Complete feed	100	90	80
Protected soybean groat	0	10	20
Total	100	100	100
	Nutrient content (%)		
Crude protein	15.12	15.72	16.53
Coarse fiber	20.91	19.52	18.13
Crude fat	2.55	3.46	4.37
Organic ingredient	87.16	87.69	88.23
Nitrogen-free extract	48.58	48.99	49.20
Ash	12.84	12.31	11.77
Total digestible nutrient	58.20	61.23	64.32

Calculated based on the nutrient content of feed ingredient in Table 1.

did not affect the slaughter weight and carcass weight of TTLs in all treatments.

Slaughter weights between treatments in this research are relatively similar. This can be related to the protein and energy retention in muscles. Baracos (2005) stated that the amount of tissue stored is largely determined by the level of protein consumption and energy availability. When viewed from the perspective of protein content in the treatment rations, the F1, F2, and F3 were relatively not different, i.e. 15.12%, 15.72%, and 16.53%, respectively. Baihaqi *et al.* (2013) reported that the total of dry matter (DM) and crude protein (CP), coarse fiber (CF) and total digestible nutrient (TDN) intake that are relatively similar have led to a relatively similar carcass tissues of TTLs. In addition, the sheep breed in this research also affects the resulting slaughter weight. Whereas being fed with similar formaldehyde-protected soybean groats, Malpua sheep in Bhatt *et al.* (2017) have much greater slaughter weight than the sheep in this research (38.5 vs 34.51 and 37.24).

Comparable to those of slaughter weight and carcass weight, the carcass percentage and carcass composition (bone, meat, and fat) have relatively similar figures. Carcass percentage and composition in all treatments showed relatively similar figures (P>0.05). The PSG content in the complete feed in this research did not affect the TTL carcass percentage and composition.

The PSG content in the complete feed also has no effect on carcass weight in this study. Carcass weights in this study are relatively comparable; 17.26, 16.75, and 18.14 for F1, F2 and F3, respectively. The results may be related to the relatively similar slaughter weights. Hwangbo *et al.* (2009) reported that carcass weight was affected by slaughter weight, slaughter age, and livestock breed. However, the carcass weight in this study was still higher than that Dentinho *et al.* (2020). The carcasses in this study have the weight range of 13.5-14.5. Detinho used Merino Branco sheep fed with tannin protected soybean groats of Cistus ladanifer L.

Both percentage and composition of carcass (meat, bone, and fat) are relatively similar between treatments. Carcass percentage in this study has the range of 47.39–48.56%. The percentages of meat, bone, and fat have the ranges of 57.18–59.98, 16.08–16.17, and 23.93–25.71, respectively. Bhatt *et al.* (2017) showed different results, the effect of PSG on carcass, meat, bone and fat manifested after Malpua sheep were reared for 90 days. The sheep that have been fed with PSG experienced an increase in carcass, meat, and fat percentage and decrease in bone percentage compared to those of control. In line with our results, soybean groat protection by Detinho *et al.* (2020) using tannin from Cistus ladanifer L has not been able to affect the percentage of Merino Branco sheep carcass. The percentages of carcass, meat, bone and fat are relatively similar between treatments. The fact that PSG in the complete feed has no effect on carcass, meat, bone and fat percentages in this study is a consequence of similar carcass weight and slaughter weight between treatments. Never (2015) reported that the slaughter weight is directly proportional to fat percentage; however, it inversely proportional to the percentages of bone and meat. It is also presumed that the sheep capacity to develop muscles has reached the optimum level, despite being fed with higher protein diets. Soeparno (2015) reported that the limit of muscle development cannot be exceeded, even though the sheep were fed with high quality feed.

Half cuts and commercial cuts of carcass

No differences were found (P>0.05) in weight and percentage of TTL carcass halves in this study. Both 10% and 20% PSG content in the complete feed do not affect the commercial cuts of carcass. Commercial cuts of carcass are those that usually sold in the market and already familiar to consumers. Production of TTL carcass in all treatments is presented in Table 4.

National Standardization Agency of Indonesia (2020) has divided sheep carcass cuts into three classes. Class I consists of tenderloin and loin, class II consists of leg, shoulder, and rack, and class III consists of breast, flank and shank. In this study, the weight and percentage of TTL legs ranged from 4.37 to 5.01 kg and 26.08 to 27.64%, respectively. In accordance with the statement of Mawati *et al.* (2004), legs are parts of carcass that grows faster in early development because they are the primary muscle involved in walking and moving. On a sequential basis, the largest and the smallest commercial cuts of carcass consist of leg, shoulder, loin, shank, neck breast, rack and flank. However, carcass cuts in Class I, II and II indicate no differences between treatments. This was mainly due to the fact that carcass weight between treatments did not show any difference. Carcass weight serves as a comparison in determining the percentage of each half cut and commercial cut of carcass. We found that formaldehyde PSG in the complete feed—F1, F2, and F3--produces daily weight gain that relatively equal; 95.98; 110.45; and 127.08 g/lamb/day. This result indicate that TTLs between treatments have reached similar growth rate and possibly similar ration efficiency that subsequently leading to equal slaughter weight and carcass weight between treatments.

Quality of produced carcass

The quality of produced male TTL carcass that includes the percentage of flashing index (FI), rib eye muscle area, and meat-bone ratio is presented in Table 5. No difference was found between FI percentage and meat-bone ratio (P>0.05). Differences were found in the rib eye muscle area (P<0.05). The use of complete feed containing 20% PSG contributes to the largest rib eye muscle area compared to F1 and F2 (35.17 vs 27.00 and 26.33).

The quality of produced carcass observed in this study includes the percentage of fleshing index (FI), rib eye muscle area, and meat bone ratio. The FI were similar between treatments. This is because the carcass weights that is relatively similar. FI is affected by the weight and length of carcass. The greater the weight of carcass, the higher the FI percentage will be (Soeparno, 2015) (Mawati *et al.*, 2004). FI is used to determine the proportion of meat to the lamb carcass length.

In addition, the rib eye muscle area indicated a significant difference. The DMRT suggests that F3 treatment is significantly different from F1 and F2, while no significant difference was found between F1 and F2. The rib eye

Table 3. Mean slaughter weight and carcass production

Variable	Treatment			P-Value
	F1	F2	F3	
Weight (kg)				
Slaughter weight	36.41±0.74	34.51±1.06	37.24±1.93	0.096
Carcass weight	17.26±0.65	16.75±0.24	18.14±1.09	0.150
Percentage (%)				
Carcass percentage	47.39±1.19	48.56±0.83	48.69±0.46	0.216
Bone	16.17±0.50	16.16±1.42	16.08±1.42	0.995
Meat	58.12±1.87	57.18±0.49	59.98±0.81	0.464
Fat	25.71±1.49	26.65±5.41	23.93±2.22	0.646

Table 4. Mean weight and percentage of half cuts and commercial cuts of carcass

Variable	Treatment			P-Value
	F1	F2	F3	
Weight (kg)				
Front-half carcass	9.52±0.46	9.18±0.36	10.04±0.80	0.254
Rear-half carcass	7.74±0.21	7.58±0.33	8.10±0.45	0.239
Neck	1.78±0.11	1.77±0.22	2.03±0.06	0.126
Shank	1.82±0.21	1.77±0.23	2.01±0.21	0.426
Shoulder	3.42±0.17	3.12±0.25	3.24±0.42	0.491
Rack	1.07±0.26	1.13±0.03	1.25±0.07	0.404
Breast	1.43±0.11	1.39±0.17	1.51±0.15	0.588
Loin	2.56±0.17	2.71±0.88	2.45±0.15	0.836
Flank	0.48±0.16	0.50±0.13	0.64±0.14	0.385
Leg	4.69±0.08	4.37±0.66	5.01±0.30	0.275
Percentage (%)				
Front-half carcass	55.15±0.68	54.77±1.92	55.30±1.81	0.915
Rear-half carcass	44.85±0.68	45.23±1.92	44.70±1.81	0.915
Shank	10.55±0.89	10.55±1.27	11.06±0.57	0.759
Shoulder	19.84±1.15	18.64±1.44	17.86±1.36	0.244
Rack	6.22±1.30	6.74±0.15	6.91±0.18	0.518
Breast	8.27±0.38	8.28±0.88	8.32±0.35	0.994
Loin	14.83±1.60	16.16±5.45	13.50±0.90	0.634
Flank	2.80±0.84	2.98±0.73	3.55±0.63	0.479
Leg	27.20±0.50	26.08±3.66	27.64±1.58	0.698

Table 5. Quality of produced carcass

Variable	Treatment			P-Value
	F1	F2	F3	
Fleshing index (%)	25.14±0.03	26.01±0.02	26.79±1.15	0.397
Rib eye muscle area (cm^2)	26.33±1.53[b]	27.00±4.36[b]	35.17±2.02[a]	0.017
Meat bone ratio	3.60±0.22	3.54±0.14	3.75±0.27	0.538

[a,b] superscripts that differ in the same line indicate a significant difference (P<0.05).

muscle area of F3 treatment is greater than those of F2 and F1. The rib eye muscle areas between treatments have increased by 25.14% for the F1 to F3 treatment, and 23.23% for F2 to F3 treatment. Rib eye muscle area can serve as an indicator for assessing livestock productivity because it signifies the total meat production. Yurleni *et al.* (2016) described that rib eye muscle area illustrates the proportion of carcass meat; the greater the rib eye muscle area, the larger the carcass meat proportion will be. Fed with fermented rice straw and supplemented with vitamin A, the rib eye muscle area in this study has a range of 5.1-9.8 cm2, which is greater than that of Jarmani and Haryanto (2007). This may be because this research used protected soybean groat with higher PK content in F2 and F3 treatments by 15.72% and 16.53%, respectively.

On the other hand, the meat bone ratios in this study indicated no differences between treatments. This may be due to the relatively similar effect of feed nutrient. Animal with efficient dietary protein intake will experience protein deposition and therefore improvement in carcass quality. The use of formaldehyde-protected PSG in the complete feed in this study did not affect the characteristics of TTL carcass. Principally, formaldehyde will develop hard complexes of proteins and these complexes will prevent proteolitic enzyme from digesting the protein below ruminal pH. This effect will be canceled at acidic pH in the abomasum and the protected protein will be digested in the abomasum (Riyanto *et al.*, 2017). Broadly speaking, the actions of formaldehyde in protecting these proteins include: 1) forming a methylol group at the amino terminal of the protein chain group and an epsilon or lysine amino group and 2) condensing these groups with the primary amides of asparagine and glutamine groups, and the guanindyl groups of arginine. The condensation result forms intermolecular and intramolecular methylene bridges. These bridges are cleaved in abomasum acid medium with the liberation of formaldehyde (Purawisastra and Sahara, 2011).

Another factor that optimizes the protection of soybean groats is the use of formaldehyde on DET carcass characteristics. Formaldehyde solution is the most commonly used substance in the livestock diets with 40% formaldehyde. The use of this solution must be adapted to the type of feed and protein content. The treatment with higher dose of formaldehyde can have an adverse effect such as overprotection which prevents protein from being digested in the lower tract and can hardly supply RDN for rumen microbial growth (Kumar *et al.*, 2015). Thus, the level of formaldehyde is very important and must be optimal because of its criticality.

Conclusions

The descriptions presented earlier lead us to conclude that the use of complete feed containing 20% protected soybean groats generates higher rib eye muscle area percentage (25.14%) than those without protected soybean groat content. However, no difference was found in carcass characteristic in terms of weight and percentage of carcass components, half cuts and commercial cuts of carcass, fleshing index and meat bone ratio in thin-tailed sheep.

Acknowledgements

This research was funded by the P2M Non-APBN Universitas Sebelas Maret scheme Penelitian Unggulan Terapan (PUT-UNS) 2020.

References

Adiwinarti, R., I. G. S. Budisatria, K. Kustantinah, R. Rusman, and E. Indarto. 2019. Effects of rations containing formaldehyde-protected soybean meal on meat production in Kacang goats. Vet. World. 12: 890-895. doi: 10.14202/ vetworld.2019.890-895

Badan Standardisasi Nasional (BSN) Indonesia. 2020. Mutu karkas dan daging kambing/domba [Internet]. 2020 [cited 2020 Apr 1]. Available from: https://bsn.go.id/

Baihaqi, M., R. Rahayu, Duldjaman, and N. Nurmalasari. 2013. Komposisi jaringan karkas Domba Ekor Tipis yang diberi pakan hijauan pada periode akhir penggemukan. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan. 1: 8–14.

Baracos, V. E. 2005. Whole animal and tissue proteolysis in growing animals. Biology of Growing Animals. Elsevier. 3: 69-82. Available from: https://doi.org/10. 1016/S1877-1823(09)70010-4

Beigh, Y. A., A. M. Ganai, and H. A. Ahmad. 2017. Prospects of complete feed system in ruminant feeding: A review. Vet. World. 10: 424–437. doi: 10.14202/vetworld. 2017.424-437

Bhatt, R. S., A. Sahoo, L. K. Soni, and Y. P. Gadekar. 2017. Effect of protected fat as Ca-Soap and formaldehyde-treated full-fat soybean in the finisher diet of lambs on

growth performance, carcass traits and fatty acid profile. Agric. Res. 6: 427–435.

Çilek. S. and M. E. Tekin. 2005. Environmental factors affecting milk yield and fertility traits of Simmental cows raised at the Kazova state farm and phenotypic correlations between these traits. Turkish J. Vet. Anim. Sci. 29: 987–993.

Dentinho, M. T. P., K. Paulos, A. Francisco, A. T. Belo, E. Jerónimo, and J. Almeida. 2020. Effect of soybean meal treatment with Cistus ladanifer condensed tannins in growth performance, carcass and meat quality of lambs. Liv. Sci. 236: 104-021. https://doi.org/10.1016/ j.livsci.2020.104021

Ferguson, K., J. Henslely, and P. Reis. 1967. Nutrition on wool growth: The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. 30: 215–222.

Hafid, H., P. Patriani, and R. Aka. 2019. Indeks perdagingan sapi bali jantan dan betina dari pemeliharaan tradisional di sulawesi tenggara. Prosiding Seminar Nasional Tek. Pet. Vet. Pp. 86-94.

Hwangbo, S., S. H. Choi, S. W. Kim, D. S. Son, H. S. Park, and S. H. Lee. 2009. Effects of crude protein levels in total mixed rations on growth performance and meat quality in growing Korean black goats. Asian-Australasian J. Anim. Sci. 22: 1133–1139. https://doi.org/10.5713/ajas.2009.80527

Jarmani, S. N. and B. Haryanto. 2007. Penampilan karkas domba pada tatalaksana pemberian pakan berbasis jerami padi fermentasi dengan suplementasi vitamin A. Seminar Nasional Teknologi Peternakan dan Veteriner.

Jatnika, A. R., M. Yamin, R. Priyanto, and L. Abdullah. 2019. Komposisi dan karateristik jaringan karkas Domba Ekor Tipis yang diberi ransum berbasis indigofera zollingeriana pada sistem pemeliharaan yang berbeda. J. Ilmu. Pro. Tek. Pet. 7: 111–119. https://doi.org/10. 29244/jipthp.7.3.111-119

Khotijah, L., T. Suryati, and M. Fandi 2019. Karakteristik dan potongan komersial karkas domba muda umur lima bulan yang diberi ransum komplit mengandung minyak biji bunga matahari. Jurnal Ilmu Nutrisi Teknologi Pakan 17: 78–82. https://doi.org/10.29244/jintp.17.3.78-82

Konka, R., D. Kumar, J. Ramana, A. Ravi, and E. Rao. 2016. Fermentation pattern in Murrah buffalo bulls fed crop residue based complete rations vis-a-vis conventional feeding system. Anim. Nutr. Feed Tech. 16: 171–179. http://dx.doi.org/10.5958/ 0974-181X.2016.00016.0

Kumar, S., R. Kumari, K. Kumar, and T. K. Walli. 2015. Roasting and formaldehyde method to make bypass protein for ruminants and its importance: A review. Indian J. Anim. Sci. 85: 223–230.

Lailer, P., S. Dahiya, and T. Chauhan. 2005. Complete feed for livestock concept, present status and future trend: A review. Indian J. Anim Sci. 75: 84–91.

Lei, Y. G., X. Y. Li, Y. Y. Wang, Z. Z. Li, Y. L. Chen, and Y. X. Yang. 2018. Determination of ruminal dry matter and crude protein degradability and degradation kinetics of several concentrate feed ingredients in cashmere goat. J. Appl. Anim. Rese. 46: 134-140. https://doi.org/10.1080/09712119.2016.127 6916

Mawati, S., F. Warastuty, and A. Purnomoadi. 2004. Pengaruh pemberian ampas tahu terhadap potongan komersial karkas domba lokal jantan. J. Ind. Trop. Anim. Agric. 29: 172–176.

Mir, Z., G. MacLeod, J. Buchanan-Smith, D. Grieve, and W. Grovum. 1984. Methods for protecting soybean and canola proteins from degradation in the rumen. Canadian J. Anim. Sci. 64: 855–865. https://doi.org/10.4141/cjas84-099

MUI. 2009. Fatwa MUI Nomor 12 tahun 2009 tentang Standar Sertifikasi Penyembelihan Halal. MUI Pusat Bidang POM dan IPTEK, Jakarta.

NRC (National Research Council). 2007. Nutrient requirements of sheep and goats. National Academy Press, Washington DC.

Never, A. 2015. Review article: Some major factors affecting carcass composition in goats. Scientific J. Anim. Sci. 4: 81–88. doi: 10.14196/sjas.v4i7.1923

Purawisastra, S. and E. Sahara. 2011. Penyerapan formalin oleh beberapa jenis bahan makanan serta penghilangannya melalui perendaman dalam air panas [The adsorption of formaldehyde by some foodstuffs and its elimination by soaking them in hot water]. J. Nut. Food. Res. 34: 63–74. https://dx.doi.org/10.22435/ pgm.v34i1.3112

Riyanto, J., S. D. Widyawati, A. Pramono, L. Lutojo, and R. Riyanti. 2017. Penampilan produksi penggemukan feedlot sapi persilangan simental-ongole jantan diberi ransum menir kedelai-minyak ikan lemuru terproteksi. J. Pen. Ilmu. Pet. 15: 22-28. http://dx.doi.org/10.20961/sainspet.v15i1.5 694

Riyanto, J., S. D. Widyawati, and S. Sudibya. 2020. Pengaruh perbedaan rasio menir kedelai proteksi dan tanpa proteksi terhadap konsumsi, kecernaan dan nilai nutrien pakan Domba Ekor Gemuk. Liv. Anim. Res. 18: 240-245. https://doi.org/10.20961/lar.v18i3.45995

Riyanto, J. and S. Sudibya. 2018. Evaluation of feed for thin-tailed sheep fattening with supplemented protected and unprotected aldehide. IOP Conf. Ser: Earth Environ.

Sci. 119. p. 012020. doi :10.1088/1755-1315/119/1/012020

Soeparno. 2015. Ilmu dan teknologi daging. Gadjah Mada University Press, Yogyakarta.

Stern, M. O., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. J. Dairy. Sci. 68: 45–56. https://doi.org/10.3168/jds.S0022-0302(85)80796-7

Suhartanto, B., R. Utomo, Kustantinah, I. G. S. Budisatria, L. M. Yusiati, and B. P. Widyobroto. 2014. Pengaruh penambahan formaldehid pada pembuatan undegraded protein aktivitas mikrobia rumen secara in vitro. Buletin Peternakan 38: 141–149. https://doi.org/10.21059/buletinpeternak.v38i3.5249

Susilawati, M. 2015. Perancangan percobaan. Bahan Ajar Mata Kuliah. Universitas Udayana, Denpasar.

Sutardi, T. 2001. Revitalisasi peternakan sapi perah melalui penggunaan ransum berbasis limbah perkebunan dan suplementasi mineral organik. Laporan Akhir RUT VIII 1. Kantor Kementrian Negara Riset dan Teknologi dan LIPI.

Tillman, A. D., H. Hartadi, S. Prawirokusumo, S. Reksohadiprodjo, and S. Lebdosoekojo. 1998. Ilmu makanan ternak dasar. 7th edn. Gadjah Mada University Press, Yogyakarta.

Waltz, D. M. and S. C. Loerch. 1986. Effect of acid and alkali treatment of soybean meal on nitrogen utilization by ruminants. J. Anim. Sci. 63: 879–887. https://doi.org/10.2527/jas1986.633879x

Yurleni, R. Priyanto, and G. W Komang. 2016. Pengaruh penambahan asam lemak terhadap kualitas karkas dan irisan komersial karkas potong ternak. Jurnal Ilmu Peternakan 16: 35-45. https://doi.org/10.22437/jiiip.v19i1.3107

Buletin Peternakan 46 (1): 23-30, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.67045

Comparison of Productivity of Sentul and Kampung Chickens until the Age of 3 Months in the First Generation Selection Population (G1)

Wahyuni, Depison*, and Gushariyanto

Animal Science Study Program, Faculty of Animal Science, University of Jambi, Jambi, 36361, Indonesia

Article history
Submitted: 14 October 2021
Accepted: 29 December 2021

* Corresponding author:
Telp. +62 812 7446 999
E-mail: depison.nasution@unja.ac.id

ABSTRACT

This study aimed to compare the productivity of Sentul chickens and first-generation Kampung chickens (G1) until the age of 3 months. The research material were Sentul chicken and Kampung chicken. The method used was an experiment method or direct observation with a sample of 174 chickens from each strain. The data collected were egg weight, doc weight, body weight, body weight gain, body measurements, and selection response. Data on egg weight, body weight, and body measurements were analyzed using the average difference test (t-test), while the average value vector of body measurements Sentul chicken and Kampung chicken were analyzed using the T2-Hotelling statistical test. To identify the body size and body shape characteristics of Sentul chickens and Kampung chickens were analyzed using principal component analysis. Data processing used the statistical software Minitab version 18. The results of this study showed that egg weight, body weight at the age of DOC-3 months and body measurements of Sentul chickens were significantly different ($P<0.05$) higher than the Kampung chickens. This study concludes that the egg weight, body weight, body weight gain, and body sizes of Sentul chickens are higher than Kampung chickens. The size characteristic of Sentul and Kampung chickens is chest circumference. The characteristic of the shape of the Sentul chicken is the length of the wings, while the shape of the native chicken is the width of the chest. The selection response and the heritability value of the Sentul chickens was higher than Kampung chikens.

Keywords: Kampung chickens, Productivity, Selection response, and Sentul Chickens

Introduction

Indonesia has the world's fourth largest population after China, India, and America, so the availability of plant protein and animal protein food is critical. Chicken is one of the livestock that can be used as a source of animal protein. Chicken is one of the livestock that contributes enough protein to meet the needs of rural communities, so many are kept and used as a source of income. Local chicken is one of the potential chickens to be developed.

Local Indonesia chicken is germplasm native to Indonesia that has adapted and breeds well in the Indonesia. Local chicken has several advantages, including disease resistance, adaptability to various environmental conditions, easy maintenance, a more delicious and savory taste, and a higher selling price (Nuraini *et al.*, 2018). Sentul chicken and Kampung chicken are two of the many types of local chickens found in Indonesia.

Sentul chicken is a type of poultry that is unique to the Ciamis Regency livestock sector and the original local chicken of Ciamis, as confirmed by the Minister of Agriculture of the Republic of Indonesia's Decree No. 689/Kpts.PD40/2/013 recognizing the Sentul chicken family as a local Indonesian family chicken from Ciamis (Minister of Agriculture, 2013). The kampung chicken (*Gallus domesticus*) is a source of genetic wealth for local livestock, and its distribution is nearly uniform throughout Indonesia (Amlia, 2016).

The productivity of Sentul and Kampung chickens is still relatively low when compared to purebred chickens. One of the efforts that can be done to increase the productivity of Sentul and Kampung chickens is through selection. Selection can be done through egg weight, DOC weight, body weight, body weight gain, and body measurements (Tarigan, 2015).

Egg weight is the value used as the selection criteria for DOC weight. DOC weight is the weight obtained from weighing chicks that hatch after the chicks' feathers are dry (Lestari *et al.*, 2013), the higher the egg weight, the higher the hatching weight is expected. Bodyweight is the value obtained from weighing at a certain time. Bodyweight gain is the difference between the

final weight and the initial weight with the length of maintenance (Kurnia, 2019). Body measurements are one of the quantitative traits described by: beak length, beak width, head length, head circumference, head height, neck length, neck circumference, wing length, back length, chest circumference, carrying body length, back height, chest-length, chest width, shank length, shank circumference, tibia length, tibia circumference, third finger length, pubic bone distance (Ashifudin *et al.*, 2017).

The high and low productivity of livestock can be seen from the selection response. Selection response is a change in the average generation of offspring due to the selection of the parent population (Hardjosubroto, 1994) obtained from the difference between the first generation (G1) and the basic population (G0). The First Generation (G1) is the generation obtained from the selection of Generation Zero (G0) at the age of 3 months.

Until now, data regarding egg weight, DOC weight, body weight, body weight gain, and body sizes of Sentul chickens is necessary, therefore it is necessary to research the comparison of the productivity of Sentul chickens and first-generation Kampung chickens (G1) until the age of 3 months.

Materials and Methods

Materials

The research material was 315 eggs and 174 birds from each strain of Sentul and Kampung chickens obtained from the parents (G0). The feed used was Japfa Comfeed production with BR 1 energy composition (kcal/kg): 4,100, protein (%): 21, fat (%): 3 – 7, calcium (%): 0.9 - 1.1, phosphorus (%): 0.6 - 0.9, and BR2 energy (kcal/kg): 4,100, protein (%): 19, fat (%): 3 – 8, calcium (%): 0.9 - 1.1, phosphorus (%): 0.6 - 0.9. (Br 1 for DOC-1 month and Br 2 for 1-3 months) vaccines, and medicines.

The equipment used was writing instruments, digital calipers, digital scales with a capacity of 3 kg with an accuracy of 0.1 g, digital cameras, measuring tapes, incandescent lamps, feeders, drinking places, and incubators.

Methods

This research used an experimental method through direct observation. The data collected were egg weight, doc weight, body weight, body weight gain, body measurements, selection response, sentul and kampung chiken from first generation. The process of formated of the first generation (G1) was by selecting the G0 broodstock by 20% males and 36% females, so that 10 males and 36 females were obtained. Chickens that have been selected were reared until they produce eggs with a male to female ratio of 1:6. Eggs were collected for 12 days, every 6 days, the collected eggs were weighed and then marked (A, B, C) using a marker to facilitate the process of turning the eggs when incubated and put into the incubator, eggs were incubated for ± 21 days. The first observation was carried out on the 5th day to determine fertile and infertile eggs. Fertile eggs were indicated by the presence of cobweb-like blood vessels with a spot in the middle, while infertile eggs were removed from the incubator. From 315 Sentul and kampung chicken eggs that were hatched, 261 and 255 fertile eggs were obtained and 183 and 176 eggs hatched.

The process of formated of the first generation (G1) was by mating in strains (Sentul x Sentul and Kampung x Kampung) selecting the G0 broodstock by 20% males and 36% females, so that 10 males and 36 females were obtained. Chickens that have been selected were reared until they produce eggs with a male to female ratio of 1:6. The hatched DOC were marked using label paper and weighed after the DOC feathers were dry (Okatama *et al.*, 2018) to obtain hatch weights. Chickens are reared from DOC until the age of 3 months. The size of the cage used is 4x3x1.8 m. for 100 chickens equipped with a feeder, a drinking place, and a lamp for lighting. Feed and water are provided continuously (ad libitum). Body weight measurements were carried out every week and body measurements were carried out every month, where the measured chickens were marked on the legs aged 0-1 months and on the wings aged 1-3 months.

The variables observed in this study were: Egg weight, DOC weight, body weight at 1 month until 3 month of age, body weight gain, and body measurements. Variables measured include beak length (BeLe), beak width (BeW), length head (LeH), head height (HeHe), head circumference (HeCc), neck length (NeLe), neck circumference (NeCc), wing length (WLe), Upper body length (UBLe), lower body length (LBLe), back height (BHe), chest circumference (CCc), chest length (ChLe), chest width (CW), shank length (ShLe), shank circumference (ShCc), tibia length (TLe), tibia circumference (TCc), third finger length (TFLe), pubic bone distance (PuBD).

Data analysis

t-test. The t-test is the average difference test used to see the difference between egg weight, DOC weight, body weight at the age of 1 months to 3 months, body weight gain, and body measurements between Sentul chicken and Kampung chicken**.**

T^2-Hotelling.T^2-Hoteling was used to analize vector values of the average body sizes of Sentul and Kampung chickens whice include, beak length (BeLe), beak width (BeW), length head (LeH), head circumference (HeCc), head height (HeHe), neck length (NeLe), neck circumference (NeCc), wing length (WLe), Upper body length (UBLe), chest circumference (CCc), lower body lengte (LBLe), back height (BHe), chest length (ChLe), chest width (CW), shank length (ShLe), shank circumference (ShCc), tibia length (TLe), tibia circumference (TCc), third finger length (TFLe), pubic bone distance (PuBD).

Empirically, it is proven that if there are differences between Sentul chickens and

Kampung chickens through the T2-Hotelling test, then data processing is continued with Main Component Analysis (MCA) (Gaspersz, 2006). If the linear measurements of the body surface between the two lines were the same, the two groups were combined and analyzed as one group.

Principal component analysis. Principal component analysis is a statistical technique used on a set of correlated data (Gaspersz, 2006). Principal component analysis is used to see differences in size or shape characteristics between Sentul chickens and Kampung chickens. The goal is to find a number of coherent variables in the subgroups, which are relatively independent of the others. The equation of size and shape is derived from the covariance matrix.

Selection response. The Selection response was calculated by comparing the average body weight of G1 at the age of 3 months with the average weight of G0. Heritability is obtained from the selection response divided by the selection differential. The selection differential was obtained from the average weight of 3 months of age, the body weight of the selected broodstock, minus the entire population of G0. Data processing is assisted by using statistical software, namely Minitab version 18.

Operational limits. The operating limits below are in accordance with the research of Putri *et al.* (2020).

Results and Discussion

Egg weight and body weight of Sentul chicken and Kampung chicken

The average egg weight and body weight of Sentul chickens and Kampung chickens at the age of DOC-3 months are presented in Table 1. Based on Table 1, it can be seen that the average egg weight, DOC weight, body weight at 1 month, 2 months, and 3 months of Sentul chickens were 50,12±2,76 g, 35,98±26,98 g, 400,63±26,82 g, 781,63±75,28 g, and 1281,49±56,96 g.

The average egg weight in this study was higher than the results of Irianti and Hartoyo (2019), which states that the average egg weight of Sentul chickens was 43.63±3.18 g. The body weight of Sentul chickens at the age of DOC-3 months in this study where higher than the result of Muhlishah *et al.* (2016), which states that the DOC weight of Sentul chickens was 30.19 g, the results of Puteri *et al.* (2020), which states that the average body weight of Sentul chicken at the age of 1 month was 217.06±35.10 g, the results of Mariandayani *et al.* (2013), which states that the average body weight of Sentul chichen at the age of 2 months was 632.88±85.10 g, and the results of Sartika and Iskandar (2019), which states that the average body weight of Sentul chicken at the age of 3 months was 532.1 g.

The average egg weight, DOC weight, body weight at 1 month, 2 months, and 3 months of Kampung chickens were 44,64±1,97 g, 31,48±1,30 g, 336,91±20,73 g, 726,16±25,62 g, and 1114,56±68,78 g.The average egg weight of Kampung chickens in this study were higher than the results of Wardono *et al.* (2014), which states that the average egg weight of Kampung chickens was 42.49 g. The body weight of Kampung chickens at the age of DOC-3 months in this study where higher than the result of Irmaya *et al.* (2021), which states that the DOC weight of Kampung chickens was 26.38 ± 1.78 g aged 1 month 224.68 g, age 2 months 605.53 g, age 3 months 1030.30 g.

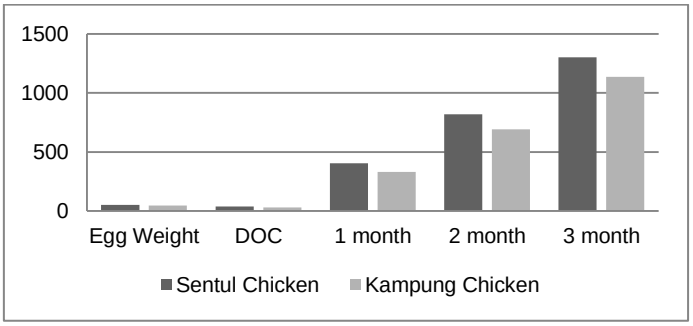
The results of this study showed that the average egg weight and the average body weight of Sentul chickens and Kampung chickens at the age of DOC-3 months of Sentul chickens and Kampung chickens were higher than some previous studies. This difference thought to due to genetic differences and the rearing environment. This is in accordance with the statement of Subekti and Arlina (2011), which states that differences in body weight of chickens can be caused by environmental conditions of different seed origin and different rearing environments, as well as genetic factors.

The results of the average difference test (t-test) showed that the average egg weight, body weight at the age of DOC-3 months of Sentul chickens were significantly different ($P<0.05$) higher than the Kampung chickens. This is presumably due to the difference in strains, this is in accordance with the statement of Susanti and Sri (2015), which states that the potential for weight growth is influenced by strain factors. Besides being influenced by strain, differences in body weight are also influenced by genetics. This is in accordance with the statement of Pagala *et al.* (2018), which states that there are differences in the increase in chicken growth caused by the influence of genetic factors. This means that the average egg weight, DOC weight, body weight age 1 month, 2 months, 3 months Sentul chickens are better than Kampung chickens.

Table 1. The average egg weight, body weight, Sentul chicken, and Kampung chicken

The weight/g	Sentul chicken	Kampung chicken
Egg	50.12±2.76[a]	44.64 ± 1.97[b]
DOC	35.98±26.98[a]	31.48±1.30[b]
1month	400.63±26.82[a]	336.91±20.73[b]
2month	781.63±75.28[a]	726.16±25.62[b]
3month	1281.49±56.96[a]	1113.56±68.79[b]

[a, b] Different letter superscripts on the same row are significantly different ($P<0.05$).



Graph 1. The average egg weight, DOC weight, body weight of Sentul and Kampung chickens.

Body weight gain of Sentul chicken and Kampung chicken

The body weight gain of Sentul chickens and Kampung chickens from the age of DOC-1 month, 1-2 months, 2-3 months is presented in Table 2. Based on Table 2 Showed that the body weight gain of DOC-1 month, 1-2 months, 2-3 months Sentul chickens, respectively, were 364,65±23,94 g, 381,00±54,00 g, and 499,86±45,03 g. The average body weight gain of Sentul chickens the age of DOC-1 month and 2-3 months, were higher than the results of Puteri *et al.* (2020), which stated that the body weight gain of Sentul chickens aged DOC-1 month and 2-3 months were 184.31±38.88 g, and 374.87±90.56 g, while aged 2-3 months was lower than the results of Puteri *et al.* (2020), which stated that the body weight gain of Sentul chickens aged 2-3 months was 411.12±78.60 g.

The body weight gain of DOC-1 month, 1-2 months, 2-3 months Kampung chickens, respectively were 364,65±23,94 g, 381,00±54,00 g, and 499,86±45,03 g. The average body weight gain of Kampung chickens from the age of DOC-1 month, 1-2 months, 2-3 months this study was higher than the results of Kestaria *et al.* (2016), which states that the increase in the average body weight gain of Kampung chickens aged DOC-1 month was 2250.30 g and 1-2 months old was 322.93 g, and the results of Irmaya *et al.* (2021), which stated that the average body weight gain of Kampung chickens aged 2-3 months was 372.87±76.31 g.

The average body weight gain from the age of DOC-1 month, 1-2 months, 2-3 months in this study was better than some previous studies. This is presumably due to differences in the research environment, this is in line with the opinion of Risnajati (2014), which states that differences in body weight, in environmental conditions and maintenance management.

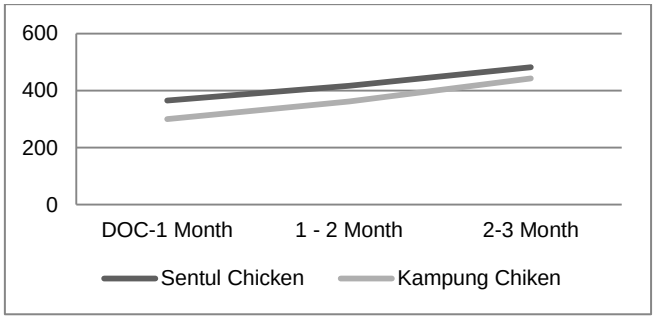
The results of the average difference test (t-test) also showed that the average body weight gain of Sentul chickens from the age of DOC-1 month, 1-2 months, and 2-3 months were significantly different (P<0.05) higher than the Kampung chicken. The difference is thought to be due to genetic influences. Rahayu *et al.* (2021) state that one of the factors that can affect chicken body weight gain is the genetic factor. This means that the body weight gain of Sentul Sentul chickens was better than that of Kampung chickens.

The results of the average difference test showed that the average body weight gain of Sentul chickens and Kampung chickens aged 2-3 months was significantly different (P<0.05) higher than the average body weight gain of 1-2 months old and the DOC-1 month old. The age of 1-2 months was significantly different (P<0.05) higher

Tabel 2. Body weight gain of Sentul chicken and Kampung chicken

Strain	Doc-1 month	1-2 month	2-3 month
Sentul chicken	364.65 ± 23.94^{cA}	381.00 ± 54.00^{bA}	499.86 ± 45.03^{aA}
Kampung chicken	335.43 ± 23.60^{cB}	359.26 ± 14.05^{bB}	387.39 ± 54.40^{aB}

$^{a, b, c}$ Different letter superscripts on the same row are significantly different (P < 0.05).
$^{A, B}$ Different letter superscripts on the same column are significantly different (P < 0.05).



Graph 2. Body weight gain of Sentul and Kampung chickens.

Table 4. Morphometric characteristics of Sentul chickens and Kampung chickens at 3 months

Body measurements	Sentul chickens	Kampung chickens
BeLe (mm)	38.34±1.62[a]	29.43±0.75[b]
BeW (mm)	40.89±1.93[a]	36.26±1.79[b]
LeH (mm)	7.88±0.60[a]	7.18±0.36[b]
HeHe (mm)	31.99±1.58[a]	29.99±1.31[b]
HeCc (mm)	114.91±1.20[a]	112.98±0.43[b]
NeLe (mm)	140.56±1.42[a]	133.12±0.69[b]
NeCc (mm)	93.63±1.59[a]	91.63±0.38[b]
WLe (mm)	220.12±1.79[a]	218.90±1.11[b]
UBLe (mm)	289.83±1.50[a]	267.83±0.78[b]
LBLe (mm)	301.35±1.51[a]	276.13±1.02[b]
BHe (mm)	311.93±2.92[a]	285.93±1.96[b]
ChLe (mm)	134.31±7.10[a]	130.31±5.45[b]
CW (mm)	67.73±7.21[a]	63.73±2.41[b]
CCc (mm)	330.93±1.98[a]	277.93±0.83[b]
ShLe (mm)	86.66±5.49[a]	78.66±3.31[b]
ShCc (mm)	46.21±0.54[a]	43.82±0.26[b]
TLe (mm)	133.01±5.42[a]	123.42±6.98[b]
TCc (mm)	107.34±1.50[a]	100.41±0.59[b]
TFLe (mm)	60.77±5.07[a]	55.47±3.80[b]
PuBD (mm)	14.24±0.40[a]	13.13±0.37[b]

[a, b] Different letter superscripts on the same row are significantly different (P < 0.05).

Beak length (BeLe). beak width (BeW). length head (LeH). head height (HeHe). head circumference (HeCc). neck length (NeLe). neck circumference (NeCc). wing length (WLe). Upper body length (UBLe). lower body lengte (LBLe). back height (BHe). chest length (ChLe). chest width (CW). chest circumference (CCc). shank length (ShLe). shank circumference (ShCc). tibia length (TLe). tibia circumference (TCc). third finger length (TFLe). pubic bone distance (PuBD).

than the average body weight gain of the doc-1 month of age. This means that the highest body weight gain of Sentul and Kampung chickens was achieved at the age of 2-3 months than 1-2 months of age and 1 month of doc. This is consistent with the opinion of Agustina *et al.* (2013), which states that a period of growth acceleration occurs before the cattle experience puberty (genital maturity) and then experiences a slowdown when they become sex adults. At the age of 12 to 20 weeks, there will be a decrease in the rate of growth until it reaches sexual maturity (Trisiwi, 2017).

Morphometric characteristics of Sentul chickens and Kampung chickens at 3 months

The T2-hotelling test is a test conducted on body sizes between strains simultaneously on Sentul and Kampung chickens. The results of the T2-Hotelling test analysis of Sentul chickens and native chickens have a statistical value of 0.160326, an F value of 0.007233, and a P-value of 0.01. The results of the T2-hotelling test above showed that the body sizes of Sentul chickens were significantly (P<0.01) higher than those of Kampung chickens. This means that Sentul chickens have a larger body size than Kampung chickens. This is presumably due to genetic influences so that the sizes of the two chicken strains are different, according to the opinion of Milas *et al.* (2020), which states that differences in body size are caused by a trait found in livestock genes. Based on the T2-Hotelling test, it can be concluded that the body sizes of Sentul chickens are better than those of Kampung chickens.

The results of the average difference test on body Measurements can be seen in Table 4 below. Based on Table 4, Sentul chickens have higher average body sizes than Kampung chickens. This means that the body size of the Sentul chicken is larger than that of the Kampung chicken. This happens because Sentul chickens have a higher weight than Kampung chickens. According to Sitanggang *et al.* (2016), The greater the size of an individual's body frame, the larger the body size will be. A large body size has a large carcass. According to Wahyudi *et al.* (2017), the greater the body size has a large carcass, the greater the bodyweight of the livestock.

Based on Table 4 above, the results of the average difference in body sizes of Sentul chickens were significantly higher (P<0.05) compared to native chickens. This difference in body size is thought to be due to genetic influences, so according to Putri *et al.* (2020), if the diversity of environmental conditions does not exist, then the difference in body size is caused by genetic diversity.

Principal component analysis

The equations, determining the body size and body shape, total diversity, and eigenvalues of Sentul chickens and Kampung chickens at 3 months, are presented in Table 5. Based on Table 6 above shows that the body size score equation for Sentul chickens has a total diversity of 85.8%, while Kampung chickens have a total diversity of 33.5%. The presentation above is the largest proportion among the main components determining body size. The highest eigenvector in the equation of body size in Sentul and Kampung chickens is chest circumference. The body size equation above is thought to occur due to the similarity of the maintenance environment according to Sartika and Iskandar (2019), which states that the determinants of body size are caused by different environmental conditions.

Sentul chicken body shape score equation has a total diversity of 4.0%, while the total diversity in Kampung chicken has a total diversity of 8.3%. The presentation above is the largest proportion of the main components determining body shape. The highest eigenvector in the equation of body shape in Sentul chickens is

chest width, while in Kampung chickens it is wing length. The difference in determining body shape above is thought to have occurred due to genetic differences between the two strains, according to opinion. According to Mahmudi *et al.* (2019), differences in body shape determinants in livestock strains are caused by differences in genetic factors.

These results can be used as a way of considering livestock breeding and as a consideration for the purification policy of Sentul and Kampung chickens in the future. Body

Table 5. Equation of body size and body shape with total diversity and eigenvalues in Sentul chickens and Kampung chickens

Type		Equations	KT (%)	Λ
Sentul chickens	body size	0.229 BeLe + 0.118 BeW + 0.200 LeH +0.232 HeHe + 0.232 HeCc 0.232 NeLe + 0.236 NeCc + 0.218 WLe +0.237 UBLe + 0.233 LBLe +0.236 BHe +0.236 ChLe + 0.150 CW + **0.238 CCc** + 0.234 ShLe + 0.233 ShCc + 0.234 TLe +0.235 TCc + 0.229 TFLe + -0.235 PuBD	85.8	17.16
	body shape	0.062 BeLe + -0.961 BeW + -0.006 LeH + 0.032 HeHe + 0.078 HeCc + 0.038 NeLe + -0.017 NeCc + -0.024 WLe + -0.011 UBLe + 0.052 LBLe + 0.036 BHe + 0.022 ChLe + **0.232 CW** + -0.027 CCc + -0.002 ShLe + 0.019 ShCc + -0.002 TLe + 0.030 TCc + -0.041 TFLe + 0.017 PuBD	4.0	0.79
Kampung chickens	body size	0.228 BeLe + 0.265 BeW + 0.241 LeH +0.281 HeHe + 0.218 HeCc + 0.204 NeLe + 0.127 NeCc + 0.035 WLe + 0.057 UBLe + 0.132 LBLe + 0.070 BHe + 0.282 ChLe + 0.279 CW + **0.289** CCc + 0.271 ShLe + 0.077 ShCc + 0.284 TLe + 0.224 TCc + 0.282 TFLe + 0.280 PuBD	33.5	0.8
	body shape	-0.371 BeLe + 0.113 BeW + -0.171 LeH + -0.041 HeHe + -0.221 HeCc + 0.083 NeLe + -0.312 NeCc + **0.486 WLe** + 0.138 UBLe + -0.286 LBLe + 0.465 BHe + 0.058 ChLe +0.019 CW + 0.056 CCc + 0.055 ShLe + 0.107 ShCc + -0.159 TLe + -0.05 TCc + 0.225 TFLe + 0.022 PuBD	8.3	1.66

Beak length (BeLe). beak width (BeW). length head (LeH). head height (HeHe). head circumference (HeCc). neck length (NeLe). neck circumference (NeCc). wing length (WLe). Upper body length (UBLe). lower body lengte (LBLe). back height (BHe). chest length (ChLe). chest width (CW). chest circumference (CCc). shank length (ShLe). shank circumference (ShCc). tibia length (TLe). tibia circumference (TCc). third finger length (TFLe). pubic bone distance (PuBD).

Table 6. Selection response

Strain	Selection diferential (g)	Selection response (g)	h^2
Sentul chicken	23.8	7.5	0.35
Kampung chicken	26.4	9.3	0.32

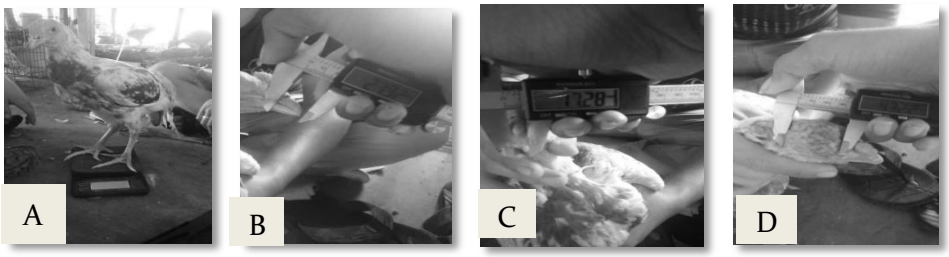


Figure 1. Measurement of body weight (A), beak length Head (B), beak width (C), and head length (D).

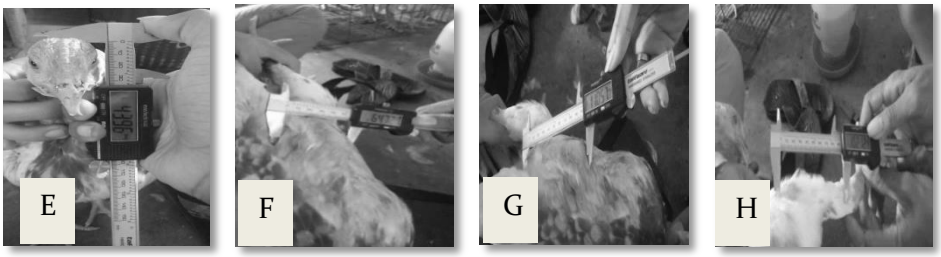


Figure 2. Measurement of head height (E), chest width (F), chest length (G), and tibial length (H).

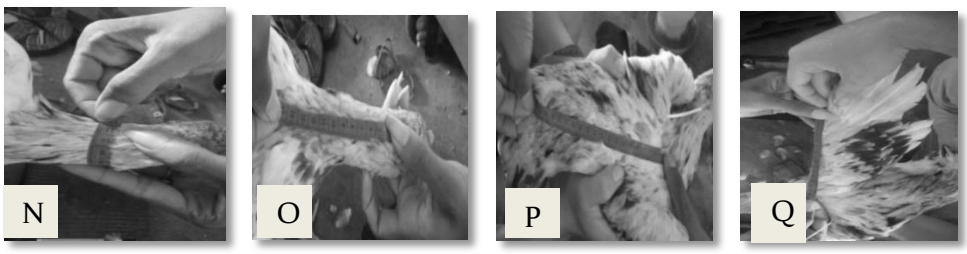


Figure 3. Measurement of neck circumference (N). neck length (O), upper body length (P) and wing length (Q).

Figure 4. Measurement of chest circumference (R), lower body length (S), and tibia circumference (T).

Figure 5. Measurement of shank circumference (U), back height (V).

measurements can be used to predict body shape as a characteristic of a particular nation (Putri *et al.*, 2020). The results of this study indicate that chest circumference (CCc) can be used as a selection parameter in increasing body scores and as a consideration for purification policies for Sentul and Kampung chickens.

Selection response

The response to the generation of Sentul and Kampung chicken lines is presented in Table 6. Based on Table 6 of the selection responses above, it is known that the value of the selection differential, the selection response, and the heritability value of Sentul chickens body weight were 23.8 g, 7.5 g, and 0.32 g, respectively. While the value of the selection differential, the selection response and the heritability value of Kampung chicken body weight were 0.26 g, 9.3 g, and 0.35 g. The heritability value of Sentul chickens and Kampung chickens in this study was higher than that of Pamungkas (2005), which stated that the heritability value of chickens aged 12 weeks was 0.22.

The heritability value of Sentul and Kampung chickens is categorized as high, according to Rotimi *et al.* (2016) stated that heritability values were categorized into low, medium and high. The low value is h^2<0.20, medium h^2 0.20-0.30, and high h^2 >0.3. High heritabilities reflect the genetic variability. On the contrary, low heritabilities were likely to reflect genetic invariability. This means that through selection can increase the productivity of both Sentul and Kampung chickens (G1).

Conclusions

Based on the results and discussion, it can be concluded that: Egg weight, body weight, body weight gain, and body sizes of Sentul chickens are higher than Kampung chickens. The size characteristic of Sentul and Kampung chickens is chest girth. The characteristic of the shape of the Sentul chickens is the width of the chest while the character of the shape of the Kampung chickens is the length of the wings. The selection response and the heritability value of Sentul and Kampung G1 chickens was high. The selection response and the heritability value of the Sentul chickens was higher than Kampung chikens.

References

Agustina, D., N. Iriyanti, and S. Mugiyono. 2013. Pertumbuhan dan konsumsi pakan pada berbagai jenis Itik Lokal betina yang pakannya disuplementasi probiotik. Jurnal Ilmiah Peternakan 2: 691-698.

Amlia, A., M. A. Pagala, and R. Aka. 2016. Studi karakteristik sifat kualitatif dan kuantitatif Ayam Kampung di Kecamatan Lasalimu Kabupaten Buton. Jurnal Ilmu dan Teknologi Peternakan Tropis 3: 31-39.

Ashifudin, M., E. Kurnianto, and Sutopo. 2017. Karakteristik morfometrik ayam Kedu jengger merah dan jengger hitam generasi pertama di Satker Ayam Maron-Temanggung. Jurnal Ilmu Ternak 17: 40-46.

Gaspersz, V. 2006. Teknik Analisis dalam Penelitian Percobaan. Tarsito, Bandung.

Hardjosubroto, W. 1994. Aplikasi Pemuliabiakan Ternak di Lapangan. PT Grasindo, Jakarta.

Irianti, N. and B. Hartoyo. 2019. Kualitas fisik dan kimiawi telur ayam Sentul dengan pemberian Fermeherbafit-encapsulasi sebagai feed aditif alami. Prosiding Seminar Nasional dan Call for Papers ”Pengembangan Sumber Daya Perdesaan dan Kearifan Lokal Berkelanjutan IX”. 123-131.

Irmaya, D., Depison, and Gurhairiyanto. 2021. Quantitative characteristic of indonesian native chickens at the age of 4 months. Anim. Res. 19: 108-119.

Kestaria, H. Nur, and B. Malik. 2016. Pengaruh substitusi pakan komersil dengan tepung ampas kelapa terhadap performa Ayam Kampung. Jurnal Peternakan Nusantara 2: 43-47.

Kurnia, A. D. 2019. Pertambahan bobot badan harian (pbbh) pada ayam layer starter grower yang diberi resveratrol tepung biji anggur. Jurnal Ternak 10: 7-11.

Lestari, E., Ismoyowati, and Sukardi. 2013. Korelasi antara bobot telur dengan bobot tetas dan perbedaan susut bobot pada telur entok (*Cairrina moschata*) dan itik (*Anas plathyrhinchos*). Jurnal Ilmiah Peternakan 1: 163-169.

Mahmudi, M., R. Priyanto, and J. Jakaria. 2019. Karakteristik morfometrik sapi Aceh, sapi PO dan sapi Bali berdasarkan analisis komponen utama (AKU). Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 7: 35–40.

Mariandayani, H. C., D. S. Dedy, S. Sri and S. Cece. 2013. Keragaman fenotipik dan pendugaan jarak genetik pada Ayam Lokal dan Ayam Broiler menggunakan analisis morfologi. Jurnal Veteriner 14: 475-484.

Milas, E. S. S., Josephine, L. P. Saerang, Lucia, J. Lambey, and B. J. Takaendengan. 2020. Karakteristik fenotipe beberapa sifat kuantitatif ayam Kampung di Minahasa. Zootec. 40: 603 – 614.

Muhlishah, S. L., T. Wiwin and Y. A. Indrawati. 2016. Inventarisasi, daya tetas telur, dan bobot tetas doc berdasarkan umur induk Ayam Sentul Barokah Abadi Farm Ciamis. Fakultas Peternakan Universitas Padjadjaran, Bandung.

Nuraini, Hidayat, Z. and K. Yolanda. 2018. Performa bobot badan akhir, bobot karkas serta persentase karkas Ayam Merawang pada keturunan dan jenis kelamin yang berbeda. Sains Peternakan 16: 69–73.

Okatama, M. S., S. Maylinda, and V. M. A. Nurgiartiningsih. 2018. Hubungan bobot telur dan indeks telur dengan bobot tetas Itik Dabung di Kabupaten Bangkalan. J. Tropical Anim. Prod. 19: 1-8.

Pagala, M. A., A. S. Aku, R. Badaruddin, and H. Has. 2018. Karakteristik fenotip dan genotip gen GH (growth hormon) pada Ayam Tolaki. Jurnal Ilmu dan Teknologi Peternakan Tropis 5: 1–4.

Pamungkas, F. A. 2005. Beberapa kriteria analisis penduga bobot tetas dan bobot hidup umur 12 minggu dalam seleksi ayam kampung. JITRO 10: 281-285.

Puteri, N. I., Gushairiyanto, and Depison. 2020. Growth patterns, body weight, and morphometric of Kub Chicken, Sentul Chicken And Arab Chicken. Buletin Peternakan 44: 67-72.

Putri, A. B. S. R. N., Gushairiyanto, and Depison. 2020. Bobot badan dan karakteristik morfometrik beberapa galur Ayam Lokal. Peternakan Tropis 7: 256-263.

Rahayu, F. F. Depison. Gushairiyanto. 2021. Performance of Kampung Super chicken and Bangkok chicken first generation (G1) until the age of 12 weeks. Livest. Anim. Res. 19: 326-336.

Risnajati, D. 2014. Pengaruh jumlah ayam per induk buatan terhadap performan ayam petelur Strain Isa Brown periode starter. Sains Peternakan: Jurnal Penelitian Ilmu Peternakan 12: 10–14.

Rotimi, E. A., J. O. Egahi and O. M. Momoh. 2016. Heritability estimates for growth traits in the Nigerian Local Chicken. J. Appl. Life Sci. Int. 6: 1-4.

Sartika, T. and S. Iskandar. 2019. Produktivitas ayam petelur KUB-2 generasi ke-4. JITV. 24: 151-157.

Sitanggang, E. N., Hasnudi, and Hamdan. 2016. Keragaman sifat kualitatif dan morfometrik antara Ayam Kampung, Ayam Bangkok, Ayam Katai, Ayam Birma, Ayam Bagon dan Magon di Medan. Jurnal Peternakan Integratif 3: 167–189.

Subekti, K. and F. Arlina. 2011. Karakteristik genetik eksternal Ayam Kampung di Kecamatan Sungai Pagu Kabupaten Solok Selatan. Jurnal Ilmiah Ilmu-Ilmu Peternakan 14: 74-86.

Susanti, I. M. N. I. and M. Sri 2015. Pengaruh bangsa pejantan terhadap pertumbuhan pedet hasil ib di wilayah Kecamatan Bantur Kabupaten Malang. J. Ternak Tropika. 16: 41-47.

Tarigan, J. H. 2015. Identifikasi bobot badan dan ukuran ukuran tubuh Itik Bali (kasus di kelompok ternak Itik Manik Sari Dusun Lepang Desa Takmung Kecamatan Banjarangkan Kabupaten Klungkung Provinsi Bali). Studens e-jurnal. 4: 1-7.

Trisiwi, H. F. 2017. Pengaruh level protein pakan pada masa pertumbuhan terhadap penampilan pada awal peneluran ayam betina hasil persilangan ayam kampung jantan dan ayam ras petelur betina. Jurnal Ilmu dan Teknologi Hasil Ternak 12: 61–68.

Wahyudi, E., G. Ciptadi, and A. Budiarto. 2017. Studi kasus tingkat pemotongan kambing berdasarkan jenis kelamin, kelompok umur dan bobot karkas di tempat pemotongan hewan Kota Malang. Jurnal Ternak Tropika 18: 69–76.

Wardono, H. Ponco, C. Sugihono, H. Kusnadi and Suprijono. 2014. Korelasi antara beberapa kriteria peubah produksi pada Ayam Buras. Prosiding Seminar Nasional "Inovasi Teknologi Pertanian Spesifik Lokasi".Pp. 578-582.

Buletin Peternakan 46 (1): 31-35, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.67523

The Effect of Flushing Premating with Spirulina Platensis Supplementation on Ewes Postpartum Estrus

Diahanvika Tri Sarvinda[1], Sigit Bintara[2], I Gede Suparta Budisatria[3], Kustantinah[4] and Endang Baliarti[3]*

[1]Postgraduate Student, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Department of Animal Breeding and Reproduction, Faculty Animal Science Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[3]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[4]Department of Animal Nutrition, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Article history
Submitted: 9 July 2021
Accepted: 7 February 2022

* Corresponding author:
Telp. +62 811 257 207
E-mail: bali_arti@ugm.ac.id

ABSTRACT

Lactating ewes require high nutrients for basic life requirements and milk production. If not fulfilled, it can have an impact on Negative Energy Balance (NEB) that reduces body weight, Body Condition Score (BCS), and extend the appearance of Postpartum Estrus (PPE). Premating flushing feed is an effort to improve ewe nutrients by adding high nutrition for preparation before mating so that after lambing and suckling, the ewe immediately estrus. This research aimed to evaluate the performance of ewes through premating flushing feeding. The research was conducted at Mendo Galak Farm, Sleman, Yogyakarta. Twenty ewes 2-3 years old with BCS 2-3 divided into two groups; the group with flushing treatment consisted of dried water spinach (Ipomoea reptans poir), concentrate feed with Spirulina sp. (14,92% crude protein, 60,28% total digestible nutrients), and the control group (PS) without Spirulina sp. (crude protein 11,82%, total digestible nutrients 53,20%). Flushing feed was given after a month postpartum as much as 3% dry matter of body weight. The recorded parameter was daily feed consumption, monthly body weight, BCS, and postpartum estrus. The data obtained were tested by an independent T-test with Statistical Product and Service Solution (SPPS ver. 22). The results showed the consumption and digestibility of CP, TDN, and ewe's ADG had a significant difference ($P<0.05$). Postpartum estrus (PPE) of flushed ewes had no significant difference ($P>0.05$), 73.90±11.55 vs. 77.60±14.65 days, respectively. The conclusion was that flushing premating treatment with the addition of Spirulina platensis increased the nutrient intake and digestibility of CP, TDN, and ADG but had not shortened on postpartum estrus of lactating ewes.

Keywords: Flushing premating, Spirulina sp., Body weight, BCS, Postpartum estrus

Introduction

Sheep is one of the meat-producing commodities that is widely bred. It has the prolific potential of having more than one lamb on a period, and the lambing interval is relatively shorter than cows. Farmers have carried out most breeding efforts in rural areas; the feed given to the ewe at various physiological phases did not differ, whereas the requirement was different. Theoretically, the nutrient for lactating ewes is higher to fulfill the essential metabolic nutrient and complete the nutrient needed for milk production. If the feed given is the same as that of maintenance ewe, it does not fulfill the requirement, and a Negative Energy Balance can occur. Nutritional adequacy in the lactation phase is crucial to maintain the Body Condition Score (BCS); thin ewes increase the chance of silent heat so that it directly affects the appearance of Post Partum Estrus (PPE) (Ashari *et al.* , 2018). If the nutritional requirement of the ewe is insufficient for the early lactation period caused by feed intake of postpartum ewes are slower than the energy production needed to produce milk, the body fat and muscle tissue are mobilized as the body's adaptation to physiological conditions prioritized for milk production (Gross *et al.* , 2011). The mobilization of energy and body fat for milk production reduces ewes body weight and disrupts the hormonal cycle for the occurrence of estrus. Negative Energy Balance during the early 30 days of lactation will be prolonging the PPE cycle (Butler, 2001). The solution for the problem of premating ewes due to NEB is flushing.Flushing in ewe is a technique to increase nutrient intake for increas ovulation rate. Flushing that improves the condition of the ewe's body through feed improvement in the reproductive process

optimizes the production and reproductive performance. According to (Shad *et al.* , 2011) the bodyweight of ewes with the flushing method increased at 3-4 weeks before mating, had more potential for pregnancy than thin ewes without flushing.

Flushing in ewe is a technique to increase nutrient intake to improve ovulation rate. Flushing that improves the condition of the ewe's body through feed improvement in the reproductive process optimizes the production and reproductive performance. According to Shad *et al.* (2011), the bodyweight of ewes with the flushing increased at 3-4 weeks before mating, had more potential for pregnancy than thin ewes without flushing. Various studies have shown that reproductive performance increases with body weight gain during the flushing period. The addition of protein and energy sources of feed affects the mechanism of the ovulation rate (Daghigh *et al.*, 2016). Increasing protein levels in concentrate with Spirulina sp. supplementation, which has a protein content of 65%, accelerates the increase in body weight of the ewes during the lactation period. Spirulina sp. can be a source of feed protein because it has a mucoprotein cell wall that is easily digested (Henrikson, 2010). Spirulina sp. as flushing feed for ewes to increase body weight and performance of sheep in intensive rearing in conditions of smallholder farmers has never been studied. The purpose of this study was to evaluate the performance of ewes through premating flushing feeding with the addition of Spirulina platensis. The ewes performance includes changes in body weight, BCS, and reproductive performance.

Materials and Methods

Twenty ewes of DET-Garut breed 2-3 years in a condition after one month of lambing. Ewes were kept in cages that were individually insulated with 1x1.1 m2, equipped with feeding and drinking places. The ewes were divided into two groups; 10 ewes with lower body weight were put into the treatment group (PF) and the rest into the control group (PS).

The ewes were fed 3% of body weight (dry matter basis) with a proportion of 50% dry water spinach (*Ipomoea reptans poir*) and 50% concentrate KJUB Puspetasari F31. The ewes in the treatment group were given the same feed as the control group. The concentrate was added by additive Spirulina platensis 10 g/head/day. The nutrient content of the feed is shown in Table 1. Feeding was twice a day at 07.00 AM and 03.00 PM and drinking water ad-libitum.

The data observed included feed consumption and digestibility, ADG, BCS, and PPE of ewes. Feed consumption was calculated from the total of feed given minus the remaining feed. Digestibility was determined based on the total feed intake (in DM) minus feces (in DM) and digested nutrients. The nutrient consumption and digestibility measured were dry matter, crude protein, and total digestible nutrients (TDN). ADG was obtained from weighing carried out before and after the feed treatment, in the morning before the ewes were fed, then the final weight was reduced by the initial weight divided by the time of maintenance. Assessment of BCS with a scale of 1 (thin) – 5 (obesity) using visual and palpability methods, before and after the feed treatment that refers to Thompson and Meyer (2006).

Observation of estrus was carried out with the help of a ram given an animal coloring marker on the lower chest to mark the occurrence of mating. The marker imprinted a color mark at the tail head and the outer vulva of ewes. In addition, the onset of estrus symptoms can be seen visually with the behavior of letting the back of the ewe be sniffed, not refusing to be climbed, followed by copulation.

The research data obtained were analyzed descriptively with an independent T-test with Statistical Product and Service Solution (SPSS ver. 22).

Results and Discussion

Nutrient intake

The feed consumption data of ewes are shown in Table 2. The average consumption of DM, CP, and TDN of ewes between treatments was significantly different ($P<0.05$). The supplementation with Spirulina sp. effect on ewe consumption. According to Khotijah *et al.* (2020), DM consumption between the same groups showed that the addition of raw materials did not influence the ration palatability.

Crude protein consumption is affected by Spirulina sp. as a protein source. Protein utilization was also associated with low body weight and in the NEB state to recover ewes after lambing and lactation. The nutritional requirements of the ewes are higher at the end of pregnancy and the early onto the third week of lactation (Joy *et al.*, 2014), but at the same time,

Table 1. Nutrient content of treatment feed

Nutrient content (%)	Group	
	Control	Flushing
*Water content	86.94	95.35
**Crude protein	11.82	14.92
*Crude fiber	19.72	18.90
*Crude fat	3.62	3.24
*Ash	11.31	11.04
***Total digestible nutrient	53.20	60.28

*Result of forage and pasture science laboratory, Animal Science UGM; **Result of BPMSP, Agriculture Ministry; ***Result based on Hartadi *et al.* (1980) formulation.

Table 2. Nutrient consumption

Variables	Treatments	
	Control	Flushing
Dry matter (g/bb^0.75/d)	113.66±25.23	134.34±17.66
Crude protein (g/bb^0.75/d)	13.50±3.22[a]	20.00±2.52[b]
Total digestible nutrient (g/bb^0.75/d)	60.47±13.42[a]	80.98±10.65[b]

[a,b] Different superscript at the row indicates siginifcance difference (P<0.05).

Table 3. Nutrient digestibility

Variables	Treatments	
	Control	Flushing
Dry matter (%)	59.73±6.94	61.40±4.16
Crude Protein (%)	54.88±7.48[a]	65.76±4.89[b]
Total digestible nutrient (%)	31.78±3.69[a]	37.01±2.50[b]

[a,b] Different superscript at the row indicates siginifcance difference (P<0.05).

the ability to consume feed decreases that limited nutrient availability. TDN was an illustration of the total energy derived from feed consumption. The averages of TDN consumption in the control and flushing treatments were 649.83±109.69 and 789.71±96.01 g/head/day, respectively. Consumption of TDN treatment was in beneath with the ewes basal requirement in the flushing phase of 940 g or 55% (NRC, 2007).

The digestibility of the ration was measured to determine the number of nutrients absorbed by the ewes. Suardin *et al.* (2014) stated that digestibility is an indicator of ration quality; a high DM digestibility indicates a high digestibility value of feed ingredients. The results showed that the DM between treatments showed no significant difference (P>0.05) (Table 3). It showed that there were the same nutrient supplies for rumen microbial growth. The DM digestibility was in line with the dry matter consumption that also showed no differences (Ratu *et al.* , 2020).

Table 3 shows that CP and TDN digestibility of flushing treatment were significantly different (P<0.05). Supplementation of protein feed sources and the increase of TDN levels increased the digestibility in the feed. According to Mathius *et al.* (2003), the different digestibility coefficients in the availability can be more absorbed. The higher the protein content of the ration, the higher the digested CP level. The role of Spirulina in CP digestibility by ewe was due to bypassing degradation in the rumen. Gouveia *et al.* (2008) reported that Spirulina increased the rumen microbial CP production and reduced retention time in rumen. Approximately 20% of Spirulina feed had bypassed rumen degradation. Spirulina sp. has a high Protein Efficiency Ratio (PER) (Borowitzka and Borowitzka, 1988). The cell walls were made of mucoproteins, polysaccharides, and the enzyme superoxide dismutase (SOD), which was an intercellular antioxidant with an activity of 10,000 - 37,500 u/10g Spirulina sp. and it does not have cellulose, so it was easier to digest and absorb by the body (Henrikson, 2010).

Significant differences in TDN digestibility were followed by an increase in TDN consumption in the ratio to balance protein levels as a stimulant for rumen microbial growth. Supplementation of protein sources cannot stimulate rumen microbial growth without supplementation of dissolved carbohydrates (Parakkasi, 1999). Klein (2020) added that synchronizing glucose availability from carbohydrates as energy and peptides in the form of nitrogen protein in the rumen increases microbial activity and rumen microbial protein synthesis. Freer and Dove (2002) mention a central sensor-integrator that processes complex metabolic information about nutritional status and translates into reproductive responses. Added by Sargison *et al.* (2018), follicular development was controlled by hormones, leptin which was influenced by body fat levels, increasing glucose and insulin during the late luteal phase to increase the number of follicles ovulated.

Ewes performance

The ewes performance, such as body weight and ADG during treatment, was presented in Table 4. Flushing treatment significantly affected ewes ADG within a month of treatment (P<0.05). Bodyweight and ADG have a positive correlation indicating the fulfillment of the basal requirement of the ewe during lactation. Bodyweight gain in a month of treatment of flushing group was increased by 2.2 kg, heavier than the control group 1.36 kg, with ADG of the flushing ewe 96.174±23.72 and control 44.80±9.17 g/head/day. This result was similar to Karikari and Blasu (2009), the bodyweight of the flushing ewe increased by 2 kg, and ADG was 40.80±4.97 g/head/day, after six weeks of flushing. Adding 10% of Spirulina increased bodyweight with ADG 200 g/head/day compared

Table 4. Bodyweight and average daily gain of ewes

Variables	Treatments	
	Control	Flushing
Bodyweight 1 month (kg)	23.98±5.81	19.88±2.61
Bodyweight 2 months (kg)	25.34±5.62[b]	22.08±2.31[a]
ADG (g/d)	44.80±9.17[a]	96.17±23.72[b]

[a,b] Different superscript at the row indicates significance difference (P<0.05).

Table 5. Body condition score of ewes

Variables	Treatments	
	Control	Flushing
BCS 1 month	2.90±0.21[a]	2.55±0.28[b]
BCS 2 months	2.90±0.21	2.70±0.26

[a,b] Different superscript at the row indicates significance difference (P<0.05).

Table 6. Postpartum estrus of ewes

Variables	Treatments	
	Control	Flushing
PPE	77.60±14.65[ns]	75.90±11.55[ns]

[Ns] Non Significan.

to the control and 20% Spirulina treatment in the ratio (Holman *et al.*, 2013). The increase in body weight indicates that the nutrients consumed by ewes have fulfilled their basal nutrient requirement and were also partially used for reproductive organ growth (Freer and Dove, 2002).

The difference in BCS of ewes before treatment showed significant results (P<0.05). It indicates that ewes during the first month of lactation after lambing decreased BCS because the ewe's nutrient consumption had not sufficient the growth of muscle mass and fat reserves. In addition to fulfilling the basal nutrient requirement, the ewe needed more energy for milk production. Barbato *et al.* (2021) stated that feed supplements did not affect the increase in BCS and hormones, and at least the supplementation had protected the ewe from the decrease in BCS commonly associated with lactation. On the other hand, higher milk production encourages more feed consumption. Holman *et al.* (2013) have another opinion that Spirulina sp. contains the essential amino acid ɣ-linolenic which is stored subcutaneously in the form of triacylglycerol in adipose tissue. With the addition of Spirulina sp., BCS of the control group was 2.90±0.21 and 2.70±0.26 at weaning, still below the recommended range from Thompson and Meyer (2006) that BCS was 3.0-3.5 for ewes suckling single and 3.5-4.0 suckling twins, it for milk production and composition, as well as for optimal reproductive performance.

According to Sutiyono *et al.* (2010), the average time interval for the emergence of PPE was 2.84-3.44 months. The results of this study were slightly shorter PPE, 2.58±0.48 months for the control and 2.46±0.38 months for the flushing ewes. Fuquay *et al.* (2011) reported that the onset of PPE was strongly influenced by uterine involution. The ewes enter the postpartum anestrus period takes 6-8 weeks before the uterus recovers after lambing, the ovaries resume their normal activities, and the ewe can be mating again. Several factors influencing the PPE period include the breeding season, nutritional status, and lactation.

The flushing ewes had a faster estrus period than the control, but statistically no significant difference. The faster PPE possibly be affected by the provision of good nutrients improved the nutritional status of ewes to reproduce optimally. El-Shahat and El Maaty (2010) reported a significant interaction between nutrient intake and BCS. Supplementation in ewe increase BCS making normal reproductive cycle. The research conducted showed the impact of 3 weeks of feed supplementation on the ovarian activity of ewes. The number of medium-sized (>3-5 mm) and large (>5 mm) follicles by ultrasonography showed an increase compared to control (Scaramuzzi *et al.*, 2006).

Supplementation with Spirulina sp. affected omega-6 as a precursor to steroid hormones in the blood, such as estrogen, impacting the reproductive cycle. Kabinawa (2014) reported that Spirulina sp. contains 24.9% GLA (Gamma Linoleic Acid) or omega-6 as a precursor stimulating the hormone prostaglandin. The ratio of omega-3 and omega-6 fatty acids increases blood cholesterol levels, which are precursors for the formation of reproductive hormones for steroid hormones such as estrogen have an essential role in the occurrence onset of estrus (Pujiawati *et al.* , 2018).

Conclusions

Flushing premating treatment with Spirulina platensis increased the CP and TDN intake and average daily gain but did not shorten postpartum estrus of lactating ewes with intensive systems.

References

Ashari, M., Rr. A. Suhardiani and R. Andriati. 2018. Reproductive efficiency analysis of sheep fat tail in East Lombok. Indonesian Anim. Sci. Tech. J. 4: 207-213.

Barbato, O., E. D. Felice, L. Todini, L. Menchetti, A. Malfatti and P. Scocco. 2021. Effects of feed supplementation on Nesfatin-1, insulin, glucagon, leptin, T3, cortisol and BCS in milking ewes grazing on semi-natural pastures. Animals. 11: 1-13.

Borowitzka, M. A. and L. J. Borowitzka. 1988. Micro-alga biotechnology. Cambridge University Press, New York.

Butler, W. R. 2001. Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. Anim. Sci. Occas. Publ. 26: 133-145.

Daghigh, K. H., A. Fazel and A. H. Khani. 2016. Different sources of protein effect in the flushing rations on some blood parameters and the reproductive performance of

Ghezel sheep. Irian J. App. Anim. Sci. 6: 629-638.

El-Shahat, K. H. and A. M. A. El-Maaty. 2010. The effect of dietary supplementation with calcium salts of long chain fatty acids and/or L-cartnitine on ovarian activity of Rahmani ewes. Anim. Repro. Sci. 117: 78-82.

Freer, M. and H. Dove. 2002. Sheep Nutrition. CABI Publishing, Australia.

Fuquay, J. W., P. L. H. McSweeney and P. F. Fox. 2011. Encyclopedia of dairy sciences. Academic Press. Elsevier Inc.

Gouveia, L., A. P. Batista, I. Sousa, A. Raymundo and N. M. Bandarra. 2008. Microalgae in novel food products, In: K. N. Papadopoulos ed. Food Chemistry Research Developments. Nova Sci. Pub., New York: 1–37.

Gross, J., H. A. Van Dorland, F. J. Schwan and R. M. Bruckmaier. 2011. Endrocine changes and liver mRNA abudance of somatotropic axis and insulin syste constituents during negative energy balance at different stages of lactation in dairy cows. J. Dairy Sci. 94: 3484-3494.

Henrikson, R. 2010. Spirulina world food; how this micro algae can transform your health and our planet. Ronore Enterprises, Inc., Hawaii.

Holman, B. W. B., A. Kashani and A. E. O Malau-Aduli. 2013. Growth and body conformation responses of genetically divergent Australian sheep to Spirulina (*Arthrospira platensis*) supplementation. American J. Experimental Agriculture. 2: 160–173.

Joy, M., R. Ripoll-Bosch, A. Sanz, F. Molino, I. Blasco and J. A. Rodriguez. 2014. Effects of concentrate supplementation on forage intake, metabolic profile and milk fatty acid composition ofunselected ewes raising lambs. Anim. Feed Sci. Technol. 187: 19-29.

Kabinawa, I. N. K. 2014. Healthy food and biological herbs from Spirulina microalgae. Indonesian Food Tech. 3: 103-109.

Karikari, P. K. and E. Y. Blasu. 2009. Influence of nutrition flushing prior to mating on the performance of West African Dwarf goats mated in the rainy season. Pakistan J. Nutr. 8: 1068-1073.

Khotijah, l., Nurmiasih and D. Diapari. 2020. Nutritional intake, blood profile and metabolites in ewes fed a fat-rich diet of vegetable oils. JINTIP. 18: 38-42.

Klein, B. G. 2020. Cunningham's textbook of veterinary physiology. 6th edn. Elsevier. St. Louis-Missouri: 364-380.

Mathius, I. W., D. Sastradipradja, T. Sutardi, A. Natasasmita, L. A. Sofyan and D. T. H. Sihombing. 2003. Strategic study on energy-protein requirements for local sheep: 5 ewes during lactation phase. JITV. 8: 26-39.

NRC. 2007. Nutrient requirements of small ruminants. The National Academies Press, Washington.

Parakkasi, A. 1999. Ilmu nutrisi dan makanan ruminansia. Universitas Indonesia Press, Jakarta.

Pujiawati, Y., L. Khotijah, A. Sudarman and I. Wijayanti. 2018. Effect of different ratio omega-3 and omega-6 in total mix ration on productive performance, blood metabolites and estrus characteristic of ewes. Buletin Peternakan 42: 295-300.

Ratu, L. H. S., G. A. Y. Lestari and M. Nenobais. 2020. The effect of lemongrass powder as a natural antibiotics on intake and digestibility nutrient of "Kacang" goat. Anim. Nucleus J. 7: 95-102.

Sargison, N., J. O. Crilly and A. Hopker. 2018. Practical lambing and lamb care: a veterinary guide 4th edn. Willey Blackwell, UK.

Scaramuzzi, R. J., B. K. Campbell, J. A. Downing, N. R. Kendal, M. Khalid, M. M. Guttirrez and A. Somchit. 2006. A review of the effect of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reprod. Nutr. Dev. 46: 339-354.

Shad, F. I., N. A. Taufanil, A. M. Ganie and H. A. Ahmed. 2011. Flushing in ewes for higher fecundity and fertility. Livestock Int. 15: 10-14.

Suardin, S., N. Sandiah, and R. Aka. 2014. Digestibility of dry matter and organic matter of Mulato grass mixed (*Brachiaria hybrid. cv. mulato*) with different types of legume using cow rumen fluid. JITRO. 1: 16-22.

Sutiyono, Y. S. Ondho, S. Johari and Sutopo. 2010. The relationship between the appearance of ewes in various litter size. JIP. 20: 24-30.

Thompson, J. M. and H. Meyer. 2006. Body Condition Scoring of Sheep. Oregon State University.

Buletin Peternakan 46 (1): 36-40, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.69012

The Quality of Buffalo Sperm Following Preservation Using Different Diluents and Sperm Concentrations

Yendraliza[12]*, Muhammad Ridho[1], Muhamad Rodiallah[12], and Zumarni[1]

[1]Departemen of Animal Science, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru, 28293, Indonesia
[2]Local Animal Research Center in Riau Province, Universitas Islam Negeri Sultan Syarif Kasim Riau, Pekanbaru, 28293, Indonesia

Article history
Submitted: 8 September 2021
Accepted: 21 January 2022

* Corresponding author:
Telp. +62 812 689 370 4
Email: yendraliza@uin-suska.ac.id

ABSTRACT

Artificial Insemination (AI) success depends on the quality of the frozen semen. The quality of the frozen semen of swamp buffalo in Indonesia is still low. The study was conducted to determine the quality of buffalo sperm following freezing using three different diluents and three different doses. The study used buffalo semen from the Tuah Sakato Artificial Insemination Center, Payakumbuh (n = 3). The semen collecting was carried out once a week for 10 weeks (replication). The research method used was 3x3 factorial randomized block design. The first factor was diluent (Triladyl®, Andromed® and Tris egg- yolk) and the second factor was the dose of spermatozoa (10 and 15 and 20 x 10^6 sperm/ml). Data were analyzed using variant analysis, while the differences between treatments were tested by Duncan Multiple Range Test. The results showed that the plasma membrane integrity of buffalo sperm was found in Andromed® diluent, while tris egg-yolk diluent gave better motility, viability, plasma membrane integrity and recovery rate at a sperm concentration of 20 x10^6 sperm/mL compared to triladyl® diluent and a sperm concentration of 10 and 15 x10^6 sperm/mL. It was concluded that andromed® diluent and tris egg-yolk gave better motility, viability, plasma membrane integrity and recovery rate at a sperm concentration of 20 x10^6 sperm/mL compared to triladyl® diluent and a sperm concentration of 10 and 15 x10^6 sperm/mL. of Buffalo of sperm abnormalities not sicnificantly by the type of diluent but are influenced by sperm concentration.

Key words: Abnormality, Buffalo, Motility, Membrane plasma integrity, The recovery rate

Introduction

Artificial insemination (AI) is a reproductive technology that is proven to be effective and can be widely applied in the field (Singh and Balhara, 2016). The quality of semen is one of the factors that can succeed in the AI program. Freezing technique, type of diluent and sperm concentration are those the indicators of semen quality (Ariantie *et al.*, 2013). One of the purposes of the use of semen diluents is to maintain the quality of spermatozoa during preservation. Diluent of Tris egg-yolks, andromed®, and triladyl® have been used in semen cryopreservation of various animal species. However, the contradictory results related to the ability of these three diluents to maintain semen quality during freezing has been reported by some researchers. Dorado *et al.* (2010) and Alamaary *et al.* (2019) stated that tris egg-yolk is better in goat and horses semen preservation. While Optixell's diluent (Naz *et al.*, 2018) and andromed® diluents (Ansari *et al.*, 2017) were both better for Nili-Ravi Buffalo semen preservation. Researchers' reports on sperm concentration doses in diluents are also different. Alvarez *et al.* (2012) stated that the best concentration in Pigs is 800 x 10^6 sperm/mL. In contrast to Stuart *et al.* (2019), the best concentration of sperm on alpacas is 50 x 10^6 sperm/mL and Gaviraghi *et al.* (2013) reported that sperm concentrations of 4, 6 and 8 x 10^6 sperm/mL in 0.25 mL straw were not significantly associated with pregnancy in Mediterranian Italian buffalo.

Buffalo semen has 60% phosphatidylcholine that composes the spermatozoa membrane (Andrabi, 2009). This makes the chances of choline in inducing acrosomes higher, thus causing damage to the sperm membrane of buffalo (Manjunath, 2012). Cold shock due to the decrease in temperature during freezing requires a balanced diluent and sperm concentration so as not to cause structural changes, membrane damage and decreased

metabolic function (Holt, 2000). The purpose of the study was to find the right type of diluent and sperm concentration for the freezing of swamp buffalo.

Materials and Methods

Semen was collected using artificial vagina from three buffalo-bulls raising in Tuah Sakato Artificial Insemination Center. The age of bulls were 7 years old in averages and the weighs ranged between 450-500kg. The semen was collected in the morning (at 08.00 am) once a week for 10 weeks. Semen had motility >60% and >800 x 10^6 sperm/mL of sperm concentration were used for frozen processing.

Preparation of diluents

The Andromed® and *Tryladyl* (Minitub Germany) diluents were each mixed with aquabidest (20/80, v/v), homogenized and stored in the refrigerator. While Tris-egg yolk diluent were obtained by mixing 3,634g tris (hydroxymethyl) aminomethane, 0.5g of glucose and 1.99g of citrate acid, and diluted into 80mL aquabidest. The tris diluent were homogenized for 15 minutes, boiled and cooled to a temperature of 37^0C before mixed with 20mL of egg yolks. This tris-egg yolk diluent were then homogenized for 60 minutes, added by 6% glycerol, 1000IU/mL benzylpenicillin and 1000mg/mL streptomycin sulfate, homogenized and cooled at a temperature of 37^0C (Herbowo *et al.*, 2019). All chemicals purchased from Sigma-Aldrich Co, USA.

Dilution and freezing

Semen samples with different concentrations (10, 15, and 20 x 10^6 sperm/mL) were each diluted with andromed®, triladyl® and tris-egg yolk diluents. The diluted semen was loaded into 0.5mL straw (Minitub, Tiefenbach, Germany) and equilibrated at 4^0C for 5h. After equilibration, the straws were placed on 8cm above liquid nitrogen (LN_2) for 10 minutes for freezing process, and finally plunged into LN_2 (-196^oC) for storage.

Post thawed evaluation of frozen semen

After 24h of storage, the straw were thawed in the water at 37^0C for 5 seconds. Sperm motility evaluation were conducted by mixing one drop of semen with four drops of saline solution and put on the clean object-glass then covered with cover glass and evaluated under a light microscope at 400x magnification. While sperm viability and abnormality evaluation were observed by mixing one drop semen and 4 drops eosin-nigrosin on clean object-glass, homogenized, smeared, and dried above the heating plate. The smear was then evaluated using light microscope at 400x magnification. Sperm plasma membrane integrity (PMI) was evaluated by using the HOST (Jeyendran *et al.*, 1984 cit. Yendraliza *et al.*, 2019). The value of PMI was shown as a percentage of intact sperm (total 200 sperms counted). The recovery rate (RR) is comparison sperm motility after thawing to fresh semen was determined.

Statistical analysis

All data were shown as mean value ± SEM and analyzed using variant analysis. While the differences between treatments were analyzed using Duncan's multiple range tests. Differences were considered significant at $p<0.05$. All statistical analyses were performed with SPSS 16 package program for windows.

Results and Discussion

The quality of fresh semen

The quality of fresh semen buffalo was presented in Table 1. The average volume was 1.20±0.30 mL with pH 7±0.0 and the color of semen buffalo in this study was a cream color and watery consistency.

The frozen semen quality

The frozen semen quality of buffalo was presented in Table 2. The post thawing quality of buffalo sperm showed highest motility in andromed® diluents at sperm concentration of 20 x 10^6 sperm/mL ($P<0.05$). The sperm abnormalities not sicnificantly by the type of diluent but are influenced by sperm concentration ($P<0.05$). The observed viability of buffalo sperm had the highest values on tris egg-yolk diluents and sperm concentrations of 20 x10^6 sperm/mL compared to andromed® and triladyl® diluents ($P<0.05$). However, were the highest value of sperm PMI was obtained in the tris egg-yolk diluent and andromed® at a sperm concentration of 20 x10^6 sperm/mL compared to triladyl® diluents ($P<0.05$). The best recovery rates of buffalo sperm were obtained on andromed® diluents followed by tris egg-yolk compared to triladyl® diluents at sperm concentrations of 20 x 10^6 sperm/mL.

Table 1. Quality of fresh buffalo sperm

Variable	Mean ±SEM
Volume (mL)	1.20 ± 0.30
pH	7 ± 0.0.
The color of semen	cream
consistency	watery
Concentration (sperm/mL)	1.200 x 10^6 ± 4.75
mass activity	++
Motility (%)	75.5 ± 5.5
Viability (%)	90
Abnormality (%)	10 ± 1.0
Plasma membrane integrity (%)	70 ± 5.7

Table 2. Quality of buffalo sperm after thawing with difference diluents and sperm concentration (mean±SEM)

Variable	Diluents	Sperm concentration		
		10 x 10^6	15 x 10^6	20 x 10^6
Motility	Tris-eggyolk	48.00±9.64^{aB}	55.00±7.26^{aB}	62.83±3.69^{bB}
	Andromed®	50.50±0.39^{aB}	64.83±0.46^{bC}	68.50±0.31^{cC}
	Triladyl	33.67±4.48^{aA}	47.33±4.75^{bA}	53.00±4.77^{bA}
Abnormality	Tris egg-yolk	10.00±1.00^{A}	12.67±1.53^{A}	11.33±1.15^{A}
	Andromed®	11.00±0.39^{A}	10.33±0.46^{A}	9.67±0.31^{A}
	Triladyl	21.00±1.00^{B}	20.33±2.52^{B}	18.33±2.52^{B}
PMI	Tris egg-yolk	54.67±6.11^{aB}	50,33±2.52^{aB}	60.00±4.00^{bB}
	Andromed	49.33±0.39^{aB}	55.00±0.46^{bB}	60.33±0.31^{bcB}
	Triladyl	28.67±3.21^{aA}	30.67±3.06^{aA}	32.33±3.21^{aA}
Viability	Tris egg-yolk	57.33±2.52^{cB}	64.33±3.79^{cC}	66.67±3.21^{cC}
	Andromed®	58.00±0.39^{cB}	50,67±0.46^{bB}	57.00±0.31^{cB}
	Triladyl	35.33±5.03^{aA}	41.67±1.53^{bcA}	43.67±3.21^{bcA}
Recovery rate	Tris egg-yolk	63.58±7.70^{aB}	72.85±9.60^{bB}	83.22±4.80^{cB}
	Andromed®	66.89±1.39^{aB}	85.87±1.40^{bC}	90.51±1.31^{bcC}
	Triladyl	39.74±7.80^{aA}	62.69±6.29^{bA}	70.20±6,32^{cA}

Means in the same columns (uppercase) and rows (lowercase) with different superscripts differ significantly (P<0.05), PMI: plasma membrane integrity, mean ± stdev

The characteristics of fresh buffalo semen in this study have normal buffalo semen characteristics following the Indonesian Standardzation National (INS) year 2008 (Standardisasi Nasional Indonesia (SNI), 2008). The results of this study also support the findings of previous research Bhakat *et al.* (2011), Ghodasara *et al.* (2016) and Kaka *et al.* (2016).

The results of this study indicated that there was a significant interaction effect of diluent and sperm concentration on motility, viability, PMI and RR. But, the type of diluent did not have a significant effect on the concentration and abnormality of buffalo sperm. The results of this study differ from of Akhter *et al.* (2010), Ansari *et al.* (2017), Rakha *et al.* (2016) who stated that the motility and intact plasma membrane in Nili-Ravi buffalo were not significantly different in the diluents of bioexcel®, tris citrate, and andromed®.

The best post-thawing buffalo sperm quality in this study at a sperm concentration of 20 x10^6 sperm/mL was different from reports Morton *et al.* (2007) and Stuart *et al.* (2019) that the best sperm concentration is 50 x 10^6 sperm/mL. The effectiveness of cryopreservation depends on the number of spermatozoa viable to process (Naz *et al.*, 2018). Spermatozoa's resistance to cold shock is related to the permeability of hydraulic membranes, tolerance levels and domain order to membrane phase changes (Sieme *et al.*, 2016). Changes in membrane function due to decreased temperature will lead to dysfunction of ion canal regulation which will eventually result in decreased motility and viability function (Oldenhof *et al.*, 2013). This is seen in the decrease in the value of intact plasma membranes in each type of diluent and in each sperm concentration used in this study. Buffalo sperm on andromed® diluents has a higher motility than buffalo sperm in tris egg-yolk and triladyl® diluents. However, buffalo sperm on andromed diluents and tris egg-yolk at sperm concentrations of 20 x 10^6 sperm/mL have the same PMI but have different viability of sperm. Repairing the cell plasma membrane will have a positive impact on the motility and vitality of spermatozoa. This is because the motility of spermatozoa is highly dependent on the energy supply in the form of Adenosine Triphosphate (ATP) which is the result of metabolism (Manjunath, 2012).

The sperm motility of buffalo in this study (33.67%-68.50%) was different from that of the Nili-Ravi buffalo sperm in Pakistan (26.51-35.11%) (Naz *et al.*, 2018) and buffalo in Egypt (33.00-41.50%) (El-Sisy *et al.*, 2016). The death of spermatozoa is caused by the process of freezing and re-thawing. Khalil *et al.* (2018) states that cell damage due to freezing can occur due to dehydration, increased electrolyte concentrations, and the formation of intracellular ice crystals which can affect cell wall permeability and ultimately spermatozoa lose their motility. The loss of spermatozoa motility during the frozen process will affect the rate of recovery of sperm after thawing (Singh *et al.*, 2018). This can be seen from the low abnormality and high motility resulting in high recovery rate in this study.

The percentage of abnormalities in buffalo sperm in this study (9.67 - 21.00%) was lower than the abnormalities sperm in bulls in Egypt (20.33 - 33.00%) (El-Sheshtawy *et al.*, 2018). However, the abnormality value in this study was almost the same as the abnormality of buffalo sperm in tris-egg yolk diluent at different glycerol concentrations (7.80-16.30%) (El-Sisy *et al.*, 2016). The percentage of PMI of buffalo sperm in this study was higher than PMI of buffalo sperm in Pakistan (44.4-46.8%) that used andromed® and tris egg-yolk diluent (Ansari *et al.*, 2017) but not different from PMI of buffalo sperm in Ciawi that used tris egg-yolk diluent (51.38-62.41%) (Herbowo *et al.*, 2019).

The viability of buffalo sperm in this study (35.33 – 66.67%) was different from reports Herbowo *et al.* (2019) which used tris egg-yolk diluent (49.08- 61.52%) and reports Ansari *et al.* (2017) which used andromed® and tris egg-yolk diluents (61.5-67.5%). The difference in viability was caused by different types of buffalo, type of diluent and feed (Manjunath, 2012). In this study it was seen that egg yolk has the potential to overcome ROS-mediated loss of sperm integrity during freeze thawing process. The recovery rate of buffalo sperm after thawing in this study was

different from the recovery rate of FH bull sperm using tris egg-yolk diluents, andromed® and soyben lecithin (Arifiantini and Yusuf, 2010) and RR on Alpaca sperm in tris egg-yolk diluent with a sperm concentration of 50 $x10^6$ sperm/mL. The differences in abnormalities, plasma membrane, viability and RR values were due to different types of treatments, sperm and diluents (Holt, 2000).

Conclusions

Andromed® diluent and tris-egg yolk gave better motility, viability, plasma membrane integrity and recovery rate at a sperm concentration of 20 $x10^6$ sperm/mL. Buffalo sperm abnormalities were not different in all types of diluents, but the best sperm abnormality value was at a sperm concentration of 20 x 10^6 sperm/mL.

Acknowledgement

Thanks to Rector UIN Suska Riau for financial support in this research and BIB Tuah Sakato manager and team for use of the place.

References

Akhter, S., M. S. Ansari, B. A. Rakha, S.M.H. Andrabi, S. Iqbal, and N. Ullah. 2010. Cryopreservation of buffalo (*Bubalus bubalis*) semen in Bioxcell® extender. Theriogenology. 74: 951–955.

Alamaary, M. S., A. W. Haron, M. Ali, M. W. H. Hiew, L. Adamu, and I. D. Peter. 2019. Effects of four extenders on the quality of frozen semen in Arabian stallions. Veterinary World. 12: 34–40.

Alvarez, M., J. Tamayo-Canul, E. Anel, J. C. Boixo, M. Mata-Campuzano, F. Martinez-Pastor, and P. de Paz. 2012. Sperm concentration at freezing affects post-thaw quality and fertility of ram semen. Theriogenology. 77: 1111–1118.

Andrabi, S. M. H. 2009. Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. Reproduction in Domestic Animals. 44: 552–569.

Ansari, M. S., B. A. Rakha, and S. Akhter. 2017. Cryopreservation of Nili-Ravi buffalo (*Bubalus bubalis*) semen in AndroMed ® extender; in vitro and in vivo evaluation. Reproduction in Domestic Animals 52: 992–997.

Ariantie, O., T. Yusuf. D. Sajuthi, and R. Arifiantini. 2013. Pengaruh krioprotektan gliserol dan dimethilformamida dalam pembekuan semen kambing Peranakan Etawah menggunakan pengencer tris modifikasi. JITV 18: 239–250.

Arifiantini, R. I. and T. L. Yusuf. 2010. Developing of tris soy milk diluent for Frisian Holstein Bull Frozen Semen. HAYATI Journal of Biosciences 17: 91–94.

Bhakat, M., T. K. Mohanty, V. S. Raina, A. K. Gupta, and H. M. Khan. 2011. Frozen Semen Production Performance of Murrah Buffalo Bulls. Buffalo Bulletin 30: 157–162.

Dorado, J., A. Muñoz-Serrano, and M. Hidalgo. 2010. The effect of cryopreservation on goat semen characteristics related to sperm freezability. Animal Reproduction Science. 121: 115–123.

El-Sisy, G. A., M. I. Shahba, and R. I. El-Sheshtawy. 2016. Freezability of buffalo semen with TRIS extender enriched with disaccharides (trehalose or sucrose) and different glycerol concentrations. Asian Pac. J. Rep. 5: 416–418.

El-Sheshtawy, R. I. and W. S. El-Nattat. 2018. Effect of tris-extender supplemented with various concentrations of strawberry *(Fragaria* spp.) on bull semen preservability. Asian Pac. J. Reprod. 18: 93-6.

Gaviraghi, A., R. Puglisi, D. Balduzzi, A. Severgnini, V. Bornaghi, G. Bongioni, A. Frana, L. M. Gandini, A. Lukaj, C. Bonacina, and A. Galli. 2013. Minimum number of Spermatozoa per dose in Mediterranean Italian buffalo (*Bubalus bubalis*) using sexed frozen semen and conventional artificial insemination. J. Theriogenology. 79: 1171-1176.

Ghodasara, S. N., P. U. Gajbhiye, A. R. Ahlawat and K. S. Murthy. 2016. Evaluation of fresh semen quality and predicting the number of frozen semen doses in Jaffrabadi buffalo bull. Buffalo Buletin. 35:65-72

Herbowo, M. T., R. I. Arifiantini, N. W. K. Karja, and R. G. Sianturi. 2019. Cryopreservation of swamp buffalo semen in skim milk yolk-based diluent with two different cryoprotectants. Tropical Anim. Sci. J. 42: 13–18.

Holt, W. V. 2000. Basic aspects of frozen storage of semen. Anim. Rep. Sci. 62: 3–22.

Kaka, A., A. A. Memon, P. Khatri, H. K. Kumbhar, D. H. Kalhoro, M. Tariq, S. A. Pirzado and A. N. Tunio. 2016. Determination of quality characteristic of Kundhi semen. J. of Basic and Applied Sciences. 12: 394-397

Khalil, W. A., M. A. El-Harairy, A. E. B. Zeidan, M. A. E. Hassan, and O. Mohey-Elsaeed. 2018. Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. Int. J. Vet. Sci. Med. 6: S49–S56.

Manjunath, P. 2012. New insights into the understanding of the mechanism of sperm protection by extender components. Anim. Reprod. 9: 809–815.

Morton, K. M., R. Bathgate, G. Evans, and W. M. C. Maxwell. 2007. Cryopreservation of epididymal alpaca (Vicugna pacos) sperm: A comparison of citrate-, Tris- and lactose-based diluents and pellets and straws. Reproduction, Fertility and Development. 19: 792–796.

Naz, S., M. Umair, and S. Iqbal. 2018. Comparison of Tris egg yolk- based, Triladyl® and Optixell® extender on post-thaw quality, Kinematics and in vivo fertility of Nili Ravi Buffalo (*Bubalus bubalis*) bull spermatozoa. Andrologia. 50: 1–6.

Oldenhof, H., M. Gojowsky, S. Wang, S. Henke. C. Yu, K. Rohn, and H. Sieme. 2013. Osmotic stress and membrane phase changes during freezing of stallion sperm: Mode of action of cryoprotective agents. Biology of Reproduction. 88: 1–11.

Rakha, B. A., M. S. Ansari, I. Hussain, M. Anwar, S. Akhter, and E. Blesbois. 2016. Comparison of extenders for liquid storage of Indian Red Jungle Fowl (*Gallus gallus murghi*) spermatozoa. Avian Biology Res. 9: 3-9.

Sieme, H., H. Oldenhof, and W. F. Wolkers. 2016. Mode of action of cryoprotectants for sperm preservation. Anim. Reprod. Sci. 169: 2–5.

Singh, I. and A. K. Balhara. 2016. New approaches in buffalo artificial insemination programs with special reference to India. Theriogenology. 86: 194–199.

Singh, M., J. S. Rajoriya, A. Kumar, S. K. Ghosh, and J. K. Prasad. 2018. Cryopreservation of Buffalo (*Bubalus bubalis*) Semen : Current Status and Future Prospective. Buffalo Bulletin 37: 109–128.

Standardisasi Nasional Indonesia (SNI). 2008. SNI 4869.2-2008 Semen Beku Kerbau. Available at: https://id.scribd.com/document/345026647/SNI-4869-2-2008-Semen-Beku-Kerbau. Date accessed: February, 10, 2019.

Stuart, C. C., J. L. Vaughan, C. M. Kershaw, S. P de Graaf, and R. Bathgate. 2019. Effect of diluent type, cryoprotectant concentration, storage method and freeze/thaw rates on the post-thaw quality and fertility of cryopreserved alpaca spermatozoa. Scientific Reports 9: 1–8.

Yendraliza, Y., E. Yuliana, M. Rodiallah, and Z. Zumarni. 2019. Kualitas Semen Kerbau pada Waktu Ekuilibrasi dan Inkubasi yang Berbeda dalam Larutan Hipoosmotic Swelling TEST. Agripet. 19: 22–30.

Buletin Peternakan 46 (1): 41-45, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.67188

Single Nucleotide Polymorphism of Partial GDF9 Gene in Three Local Goat of Indonesia Compare with Several Goat in Asia

Fatimah Az Zahra Chairunissa, Sigit Bintara and Tety Hartatik*

Department of Animal Breeding and Reproduction, Faculty of Animal Science Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Article history
Submitted: 30 June 2021
Accepted: 21 January 2022

* Corresponding author:
E-mail: tety@ugm.ac.id

ABSTRACT

The GDF9 gene is a gene that affects the maturation of oocytes. GDF9 is expressed in oocytes and granulosa cell, it can stimulate granulose cell proliferation and regulate cumulus cell function from pre-ovulation to ovulation. The GDF9 gene is associated with an increase in the ovulation rate and litter size in animals. This study aims to determine the kinship relationship of local goats compared to goats in Asia on prolific traits and to determine the restriction mapping of the GDF9 gene in goats based on the different SNP locations. The local goat comes from the Bligon goat, Kacang goat and Kejobong goats which is compared to the GenBank data (EF446168, EU883989 and KY780296). GDF9 sequences were analyzed using BioEdit and sequencing results to identify Single Nucleotide Polymorphism (SNP) and using NEBCutter V2 to determine the restriction enzyme which recognized the sequence around SNP. The result shows that three variations of SNP were found in exon 2 (g.3615T>C, g.3760T>C and g.3855A>C). Identification of SNP position found 1 SNP position identified by restriction enzyme at g.3855A> C. The identified restriction enzyme is HpaII and MspI. The results of this study are expected to provide genetic information that will be used for further research on the relationship between GDF9 gene polymorphisms to animal prolific.

Keywords: GDF9, Goat, Phylogenetic, Restriction mapping, SNP

Introduction

Bligon is the one of goat breeds which has good fertility and productivity. In Yogyakarta, this goat population accounts for 60% from the whole goats population and takes up to 12.000 heads per year of their productivity. Kejobong goats are one of local breeds in Purbalingga, Central Java which has similar productivity with Bligon goats. Their population is 15.317 head in Kejobong distric, Purbalingga, Central Java. Kejobong and Bligon goats are known as local Indonesian goat breeds that have good productivity (Hanim *et al.,* 2020). The Bligon goat is a crossbreed between the Kacang goat and the Etawah cross-breed goat. The Bligon goat is the maximum several and great goat breed in Indonesia. Goat farming is one of the most important livelihood and food approaches for most Indonesian farmers (Kurniawati *et al.,* 2021). Kacang goat is an indigenous small goat breed of goat that is used as a meat source and is widely reared as a side business on farms in Indonesia due to the ability of these goats to reproduce and survive with simple rearing and feeding practices. These goats are commonly raised in small flocks that are confined in a simple colony house at night and tethered to graze, typically from noon through the late afternoon (Khalil *et al.,* 2019). Bligon goat has good reproductive performance. The average length of pregnancy for the Bligon goat was 5.5 months with a range of 5 to 6 months. The number of litter sizes produced by the Bligon goat is 1.74 with a range of 1 to 2. The level of litter size is influenced by genetic factors, age of the parent, body weight of the parent, and nutritional level (Murdjito *et al.,* 2011).

Prolific trait is a characteristic of animals that can give birth to one or more children per birth. Types of birth can be divided into single and twin. The birth of twins (more than one) is one thing that is highly expected because it can provide economic benefits (Tiesnamurti, 2002). To exploit the potential of increasing the prolific potency in goats, and these properties must be studied molecularly using genes relate to the nature of litter size or the nature of prolification. Several genes including GDF9. Growth Differentiation Factor (GDF9) is located on chromosome number 7 and consists of 2 exons and 1 intron with a length of 5644 bp. GDF9 could influences prolific traits of goats. It was a gene group of TGF beta superfamily which plays a role in the process of folliculogenesis and prolification.

GDF9 triggers the secretion of progesterone in luteal cells. The process of folliculogenesis was essential in follicular development. The GDF9 gene strongly influences increased ovulation rate. Polymorphism in the GDF9 gene was associated with litter size. Prolification trait was essential in determining the amount of litter size in goats (Mudawamah *et al.,* 2019). One point mutation of the GDF9 gene in Chinese goats that is associated with ovulation rate (Du *et al.,* 2008). Many significant associations have been reported between GDF9 polymorphism and ovulation rate, prolificacy, and fertility in sheep (Yuliana *et al.,* 2019). The study of polymorphism can be done by aligning the deoxyribonucleic acid (DNA) sequences. The program that usually uses DNA alignment and compares two or more sequences is the BioEdit program and Mega 10 (Anugratama and Hartatik, 2020). Research on the GDF9 polymorphism of local goats has not yet been carried out clearly. Therefore this study aims to identification the SNPs and restriction enzymes of partial GDF9 in local goat of indonesia. This information hopefully give beneficial for the further research on molecular genetic analysis as a marker for economical trait in livestock.

Materials and Methods

DNA Sample resources

The DNA materials come from sample extracts that have been isolated in previous studies sample, we used in study were 10 samples from extract DNA of Bligon goat, two samples of Kacang goat, and two samples Kejobong goat. Reference Genbank uses from NCBI with Genbank accession number EF446168, EU883989 and KY780296.

DNA amplification and sequencing

Ten genomic DNA samples from Bligon goat, two samples from Kacang goat, and two samples of Kejobong goat were amplified used PCR method to get targeted sequence. The primer design uses bioinfo.ut.ee/primer3-0.4.0. At positions 3549 to 4004 along 456 bp, the primers used were forward primer CTCCTCTTGAGCCTCTGGTG and reverse primer TCCAGTTGTCCCACTTCAGC.. Polymorphism Chain Reaction performed in a total reaction of 23 µl, containing 12,5 µL PCR Kit (KAPA BIOSYSTEMS, USA), 9,5 µL DDW, 2 µL of DNA, 0,5 µL of both forward and reverse primers. The reactions were performed using a thermal cycle (PEQLAB Primus 25 advanced, Germany) with a pre-denaturation temperature at 94°C for 1 minute, followed by 35 cycles of reaction; denaturation at 94°C for 1 minute, annealing at a temperature of 57°C for 1 minute and extension at 72°C for 1 minute, then the last step was a final extension at 72°C for 5 minutes. The quality of the PCR product was determined using gel electrophoresis (1%), the thick appearance of DNA bands were the preferred result (Albakri and Hartatik, 2021). The sequencing was done by LPPT UGM.

Sequence comparison analysis

Single nucleotide polymorphism identification, comparison sequences, and amino acid change were performed using BioEdit software. A total of 14 sequences of GDF9 gene (ten sample Bligon goat, two sample Kacang goat, two sample Kejobong goat) were aligned using ClustalW on BioEdit ver. 7.2.5 to reveal the SNPs and to perform the amino acid change. Identification of SNP position based on GenBank accession number EF446168.2 as sequence reference. Identification of restriction enzymes was recognized by using Nebcutter V2 which available online in http://nc2.neb. com/NEBcutter2/. Individual target sequences of genes are entered in NEBCutter V2 with all other parameters in their default settings. The program will calculate the positions of all restriction enzymes and then display all restriction enzymes that recognize the target sequence. Specific restriction enzymes that can recognize targets are determined by the appearance of a red line under a sequence (Albakri and Hartatik, 2021).

Results and Discussion

Study reference

The target DNA in this study was GDF9 gene fragment based on genbank accession number EF446168.2. Based on the alignment results, 3 SNP positions were found in positions of GDF9 from 3549 to 4004 along 456 bp found three SNPs g.3615T> C, g.3760T> C and g.3855A>C. Single Nucleotide Polymorphism can cause change in amino acids. These changes can be either silent or missense. The silent mutation occurs if the SNP only changes the DNA but does not change the amino acid. Missense mutation will occur if the SNP not only change the DNA but also an amino acid. Previous research by Xue-qin *et al.* (2009) stated that it was found in the polymorphism analysis of the GDF-9 gene in exon 2 of white goats Guizhou Province. The goat has a heterozygous genotype that mutates at 791 bp (G / A) which results in a substitution of valine to isoleucine in the residue of 79 mature peptide GDF-9 gene. Due to the mutation of valine into isoleucine, it will increase the methyl group in the amino acid chain.

In SNP g.3615T>C there is 1 genotype, namely CC is indicated by a single peak on base C. SNP g.3760T>C has 1 genotype, namely TT is indicated by single peak on base T. SNP g.3855A>C has 2 genotypes namely AA and AC, with AA genotype marked single peak on base A, and AC genotype marked double peak on base A and C.

Based on the results of the analysis of changes in the amino acid SNP g.3615T>C, there change the amino acid from valine to alanine. The position of SNP g.3760T>C did not changes the

amino acid, namely proline. The position of SNP g.3855A>C did not change the amino acid, namely proline, the X sign indicates heterozygous. Research by Ghoreishi et al. (2019) detected the GDF9 SNP in Markhoz goats with mutations at position g.183C>A which did not change the amino acid Leucine. Previous research, Du et al. (2008) detected a heterozygous genotype (g.1189G>A mutation) of the GDF9 gene in Guizhou white goats, as many as 8 samples from 33 high-productivity broods, of which 8 mothers were found to produce 3 offspring per birth rather than 112 low-productive goats with homozygous genotype. Aboelhassan *et al.* (2021) stated that the genetic polymorphism of the GDF9 gene was revealed to affect the nature of fecundity in animals, where heterozygous genotypes were found to cause an increase in ovulation rates and consequently lead to an increase in litter size in livestock brooders, compared to homozygous broods in wild animals. Changes in amino acids can be seen in Table 1.

The restriction enzymes found in GDF9 are HpaII and MspI with SNP g.3855A> C. There was one fragment AA with a size of 456 bp and three fragments AC with sizes 456 bp, 306 bp and 149 bp. Restriction mapping can be seen in Table 2.

3541 tgtgacgg**ct cctcttgagc ctctggtg**gc ctcccacaag aggaatattc acatgtctgt

3601 aaattttaca tgtgtgaaag accagctgca gcatccttca gcgcgggaca gcctgtttaa

3661 catgactctt ctcgtagcgc cctcactgct tttgtatctg aacgacacaa gtgctcaggc

3721 ttttcacagg tggcattccc tccaccctaa aaggaagcct tcacagggtc ctgaccagag

3781 gagagagcta tctgcctacc ctgtgggaga agaagctgct gagggtgtaa gatcgtcccg

3841 tcaccgcaga gaccaggaga gtgtcagctc tgaattgaag aagcctctgg ttccagcttc

3901 agtcaatctg agtgaatact tcaaacagtt tctttttccc cagaatgaat gtgagctcca

3961 tgactttaga cttagcttta gtca**gctgaa gtgggacaac tgga**ttgtgg ccccacacaa

Figure 1. Sequence target of GDF9 in goat GenBank acc no EF446168.2 exon 2 (underline: primer).

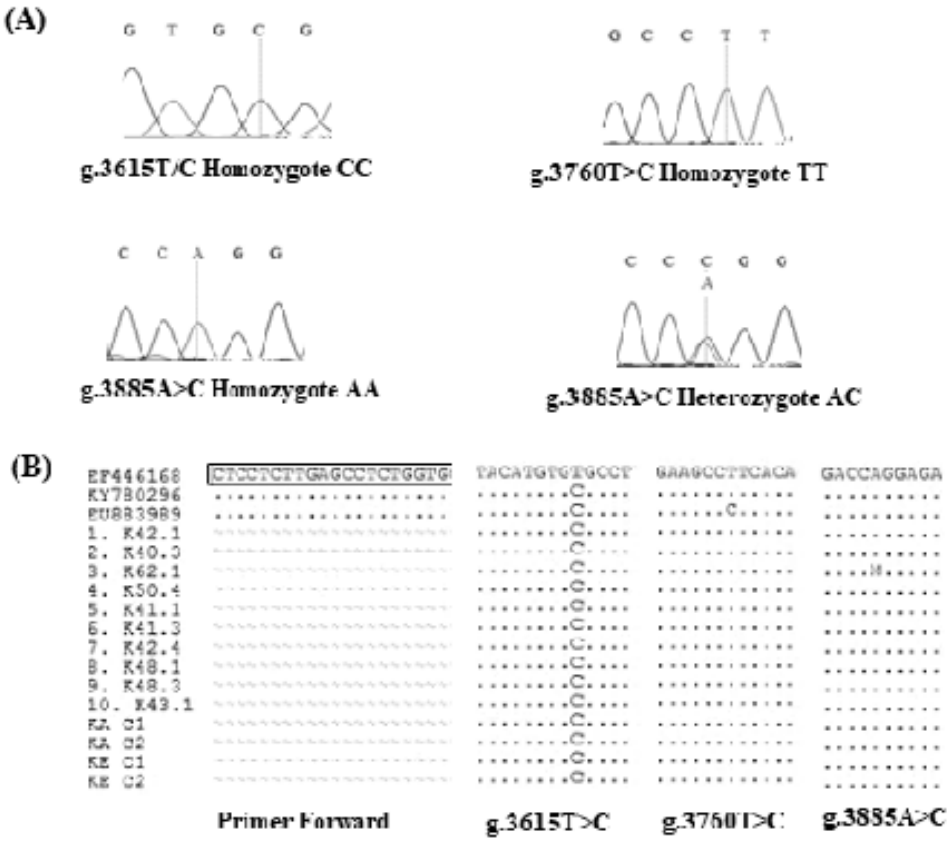


Figure 2. SNPs variants of Local goat compare to the GenBank data. (A) Chromatogram at SNP position. (B) Alignment three GenBank of Capra hircus and local goat (No.1-10=Bligon Goat, KA=Kacang Goat, KE=Kejobong Goat).

Table 1. Amino acids analysis of GDF9 gene from representative local goat and GenBank data

SNP	Genbank	Codon	Amino Acid	Mutation type	Genotype
g.3615T>C	EF446168.2	GUG	Valine	Non	TT
	KY780296	GCG	Alanine	synonimous	CC
	EU883989	GCG	Alanine		CC
	SampleK42.1	GCG	Alanine		CC
	Sample K62.1	GCG	Alanine		CC
	KA C1 (Kacang)	GCG	Alanine		CC
	KE C1 (Kejobong)	GCG	Alanine		CC
g.3760T>C	EF446168.2	CCU	Proline	synonimous	CC
	KY780296	CCU	Proline		CC
	EU883989	CCC	Proline		CC
	Sample K42.1	CCU	Proline		CC
	Sample K62.1	CCU	Proline		CC
	KA C1 (Kacang)	CCU	Proline		CC
	KE C1 (Kejobong)	CCU	Proline		CC
g.3855A>C	EF446168.2	CCA	Proline	synonimous	CC
	KY780296	CCA	Proline		AA
	EU883989	CCA	Proline		AA
	Sample K42.1	CCA	Proline		AA
	Sample K62.1	CCC	Proline		AC
	KA C1 (Kacang)	CCA	Proline		AA
	KE C1 (Kejobong)	CCA	Proline		AA

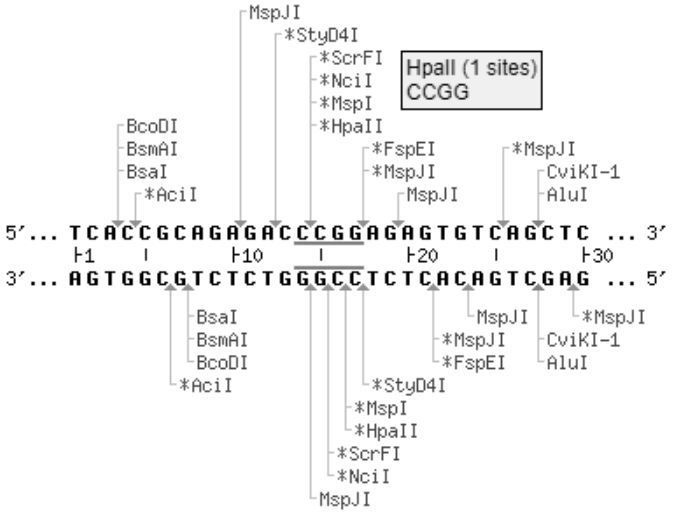


Figure 3. Restriction enzymes g.3855A>C.

Table 2. Restriction enzyme mapping

Sampel	MspI (C^CG_G)		HpaII (C^CG_G)		Genotip
	Site	Fragment	Site	Fragment	
K42.1	0	456	0	456	AA
K40.3	0	456	0	456	AA
K62.1	1	456, 306, 149	1	456, 306, 149	AC
K50.4	0	456	0	456	AA
K41.1	0	456	0	456	AA
K41.3	0	456	0	456	AA
K42.4	0	456	0	456	AA
K48.1	0	456	0	456	AA
K48.3	0	456	0	456	AA
K43.1	0	456	0	456	AA
KA C1	0	456	0	456	AA
KA C2	0	456	0	456	AA
KE C1	0	456	0	456	AA
KE C2	0	456	0	456	AA

The restriction enzyme price list shows that the price of the HpaII enzyme is $ 67 for 2000 units and the MspI for 5000 units is $ 67 (New England Biolabs, 2021). The criteria for selecting restriction enzymes is ease of use: only 1 µl is required for each reaction, and standard enzyme concentrations are sold at 2000-20,000 units / ml (2-20 units / µl). A specific intersection. Problems can occur if the intersection is not identified and you are better aware of other intersections. The number of pieces is not too large and the band size is not too short, exceeding 100 bp (Gerstein, 2001). Based on the restriction enzyme selection criteria and considering price factors, the MspI enzymes met these criteria.

Conclusions

Based on the research that has been done, it can be concluded that there are 3 SNPs, namely g.3615T>C, g.3760T>C, and g.3855A>C. The restriction enzymes identified were found in target sequence SNP g.3855A>C, namely HpaII and MspI. The recommended restriction enzymes for further use are MspI.

Acknowledgements

This research was supported by research grant Rekognisi Tugas Akhir (RTA) Universitas Gadjah Mada year 2021 with Letter of assignment No.3143/UN1.P.III/DIT-LIT/PT/2021. The authors thank to Retno Setyawati for the technical assistance in Laboratory of Animal Breeding and Genetics. We also would like to thanks to Aldi, Jalu, Fuad, Nabil and Yoga for helping when amplification of DNA.

References

Aboelhassan, D. M., A. M. Darwish, N. I. Ali, I. S. Ghaly, and I. M. Farag. 2021. A study on mutation points of GDF9 gene and their association with prolificacy in Egyptian small ruminants. J. Gen. Engin. Biotechnol. 19: 1-11.

Albakri, M. W. and T. Hartatik. 2021. Restriction mapping of melanocortin 4 receptor in *Bos taurus* and *Bos indicus* based on genbank data. Res. J. Biotechnol. 16: 52-55.

Anugratama, L. E. and T. Hartatik. 2020 Short Communication:Identification of Leptin gene in crossbred beef cattle. Biodiversitas. 21: 226-230.

Du, Z.Y., J. B. Lin, C. Tan, J. F. Wang, and X. Q. Ran. 2008. Study on the polymorphisms of exon 2 of GDF9 gene in Guizhou White goat. Anim. Husb. Vet. Med. 40: 46–48.

Gerstein, A. S. 2001. Molecular Biology Problem Solver: A Laboratory Guide. John Wiley & Sons Inc., New York.

Ghoreishi, H., S. F. Yosefabad, J. Sayegh, and A. Barzegari. 2019. Identification of mutations in BMP15 and GDF9 genes associated with prolificacy of Markhoz goats. Archives Animal Breeding. 62: 565-570.

Hanim, C., L. M. Yusiati, I G. S. Budisatria, and F. W. Rachman. 2020. Comparison of nutrient digestibility of bligon and kejobong goats fed by king grass and peanut straw. Buletin Peternakan 44: 6-9.

Khalil, A. Bachtiar, and Evitayani. 2019. Reproductive performance of female Kacang goats supplemented by mineral under a tethering feeding system. Tropical Anim. Sci. J. 42: 215-223.

Kurniawati, N., Latifah, Kustantinah, M. D. E. Yulianto, and T. Hartatik. 2021. Identification of MC4R gene markers in Bligon goats with single and twin birth type. IOP Conf. Series: Earth Environ. Sci. 667: 012-074.

Mudawamah, M., I. D. Ratnaningtyas, M. Z. Fadli, and G. Ciptadi. 2019. Individual mutations in Indonesian local ettawah goats based on the GDF9 gene. IOP Conf. Series: J. Physics Conf. Series. 1146: 012-023.

Murdjito, G., I G. S. Budisatria, Panjono, N. Ngadiono, and E. Baliarti. 2011. Kinerja kambing Bligon yang dipelihara peternak di Desa Giri Sekar, Panggang, Gunungkidul. Buletin Peternakan 35: 86-95.

New England Biolabs. 2021. 2021-22 Price List. https://www.neb.com/-/media/nebus/files/misc/neb_pricelist_2019-20.pdf. Accessed 11 January 2022.

Tiesnamurti, E. Martyniuk, E. Eythorsdottir, P. Mulsant, F. Lecerf, J. P. Hanrahan, Z. G. E. Bradford, and T. Wilson. 2002. DNA test in prolific sheep from eight countries provide new evidence on origin of the booroola (feeB) Mutation. Biol. Reprod. 66: 1869-1874.

Xue-qin, R., L. Jian-bin, D. Zhiyoung, Q. Cheng, and W. Jia-fu. 2009. Diversity of BMP15 and GDF9 genes in white goat of Guizhou Province and evolution of encoded proteins. Zoological Res. 30: 593-602.

Yuliana, R., Sumadi, and T. Hartatik. 2019. Identification of single nucleotide polymorphisms in GDF9 gene associated with litter size in Garut sheep. Indonesian J. Biotechnol. 24: 51-56.

Buletin Peternakan 46 (1): 46-53, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.69784

Expression of Myostatin Gene in Belgian Blue and Ongole Grade Crossbred Cattle

Winni Liani Daulay[1], Putri Indahningtyas[3], Cece Sumantri[2], and Jakaria[2]*

[1]Graduate School of Animal Science Faculty, IPB University, Bogor, 16680, Indonesia
[2]Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia
[3]Livestock Embryos Centre (LEC) Cipelang, Bogor, 16740, Indonesia

Article history
Submitted: 14 October 2021
Accepted: 29 December 2021

* Corresponding author:
Telp. +62 816 4831 050
E-mail : jakaria@apps.ipb.ac.id

ABSTRACT

Investigating Myostatin (MSTN) as a potent inhibitor of skeletal muscle growth and development to produce excessive muscles is extremely essential for livestock breeding. This study aimed to analyze the expression of the MSTN gene and its relationships with genotype and phenotype (normal-muscled vs double-muscled) of Belgian Blue (BB) x Ongole Grade (PO) crossbred cattle. For that purpose, 12 animals from BB, PO, BB x PO F1, and BB x PO F2 cattle (3 animals each) raised at Balai Embrio Ternak (BET) Cipelang Bogor, West Java were used for blood sample collection. Genotyping analysis was performed using the PCR-RFLP method withprimer F: 5'-CTC TTC TTT CCT TTC CAT ACA GAC-3' and R: 5'-AGG GGA AGA CCT TCC ATG TT-3', while the MSTN gene expression was analyzed using the qPCR technique. As results, three genotypes: del.11/del.11, +/del.11, and +/+ were detected. The del.11/del.11 genotype, which showed a double-muscled phenotype was found in BB cattle and BB x PO F2 cattle. The +/del.11 genotype was found in BB x PO F1 cattle and BB x PO F2 cattle. The +/+ genotype, which showed a normal phenotype was only detected in PO cattle. There was a significant difference of the MSTN gene expression in the sampled animals among genotypes and between phenotypes (normal-muscled vs double muscled). The MSTN expression in animals with del.11/del.11 genotype was higher than that in animals with +/del11 and +/+ genotypes ($P<0.05$). Animals with +/+ genotype showed the lowest MSTN expression. It was concluded that double-muscled animals showed higher MSTN expression than normal-muscled animals.

Keywords: Cattle, Crossbreeding, MSTN gene, PCR-RFLP, q-PCR

Introduction

Myostatin (*MSTN*) is a member of the growth and differentiation factor superfamily (GDF-8), which is the sole inhibitor of skeletal muscle growth (Patel and Amthor, 2005). The inhibition of the *MSTN* activity can cause an excessive muscle growth, such as an increase in the number and diameter of muscle fibers (Zhang *et al.,* 2012). The bovine *MSTN* gene consists of three exons and two introns, of which the coding region encodes a protein with 375 amino acids (Jeanplong *et al.,* 2001).

Natural mutation in *MSTN* gene can produce double muscles in animals, consequent to loss of functional *myostatin* by disrupting several physiological processes involved in the creation and determination of the functional characteristics of muscle fibers (Cassar-Malek *et al.,* 2007). Many studies have been carried out by modifying the *MSTN* gene to obtain superior livestock that has a high percentage of carcass and meat quality. Naturally occurring mutations in the *MSTN* gene leading to excessive muscle build-up in mammals have been documented in sheep (Clop *et al.,* 2006; Boman *et al.,* 2009), dog (Osman *et al.,* 2021), and cattle (Kambadur *et al.,* 1997; McPherron and Lee, 1997).

The most renowned double muscle phenomenon is in Belgian Blue cattle, which were obtained from crosses between Holstein Friesian (FH) cattle and Shorthorn cattle, which have been developed in Belgium since 1850 (Purchas *et al.,* 1992). McPherron and Lee (1997) found the cause of double muscle in Belgian Blue cattle was due to a deletion of 11 nucleotide bases in exon 3 of the *MSTN* gene, while in Piedmontese cattle it was caused by a G–A transiton mutation at position 941 of the coding region in the *MSTN* gene that converts cysteine residues into tyrosine (Kambadur *et al.,* 1997).

The polimorphysm of the *MSTN* gene has been linked to increased growth traits and carcass in several cattle populations in various countries, including Bali cattle in Indonesia (Prihandini *et al.,* 2021). Khasanah *et al.* (2016) reported that the *myostatin* promoter gene was polymorphic in Bali cattle and there were 2 SNPs (g.-7799T>C and g.-7941C>T) associated with carcass quality. Other previous studies have also found the MSTN gene in cattle, including Qinchuan cattle (Zhang *et al.,* 2007), Angus cattle (Gill *et al.,* 2009), Nellore cattle (Grisolia *et al.,* 2009), Hanwoo cattle (Han *et al.*, 2012), and Marchigiana cattle (Sarti *et al.,* 2014).

The Belgian Blue cross-program with other cattle breeds as an attempt to increase cattle productivity has been carried out with Swiss Brown, Simmental, and Rendena cattle breeds (Tagliapietra *et al.,* 2018), Jersey dairy cow (Goni *et al.,* 2016), Hereford and Angus (Freetly *et al.,* 2011). Cross-breeding of beef cattle and dairy cows has a positive impact and produce several benefits (Weaber, 2015). Fundamentally, the goal of the cross-program is to obtain the effect of heterosis or hybrid vigor and to get the best combination of the two cross elders or races (Weaber, 2015). Belgian Blue (BB) cattle, which are double-muscled cattle from Belgium, were introduced to Indonesia in 2013 (in the form of embryos and semen) and began to be developed in 2015 at the Livestock Embryo Center (BET) Cipelang Bogor (Jakaria *et al.,* 2019). While the Ongole Grade cattle are one of the local Indonesian cattle breeds that have good adaptability in tropical environmental conditions with low feed quality (Romjali, 2018). The BB x PO cross was carried out to produce a generation of cattle that had a combination of superior traits from both parents. Agung *et al.* (2016) reported the F1 generation (Belgian Blue x FH and Belgian Blue x Sumba Ongole (SO)) had the MSTN gene in a heterozygous condition, thus providing scientific evidence that deletion of 11 bases in exon 3 of the MSTN gene is also exists or can be inherited. The evaluation of cross-breeding Belgian Blue cattle with Ongole Grade (PO) in the first generation (F1) that was conducted at LEC Cipelang Bogor showed a significant effect on increasing weaning weight and weight per year (Jakaria *et al.,* 2019). SNPs and indel 11-bp of *MSTN* genes associated with double-muscled phenotype in Belgian Blue crossbred with PO cattle were also found (Jakaria *et al.,* 2021). The analysis of MSTN gene expression at the mRNA transcript level to identify the role of the MSTN gene in producing the double-muscled trait has been reported (Kambadur *et al.*, 1997; Oldham *et al.,* 2001) that MSTN mRNA was higher in double-muscled than normal cattle. The aim of this study was to analyze the expression of the *MSTN* gene in the first and the second generation of Belgian Blue x Ongole Grade crossbreed to determine the involvement of *MSTN* gene in producing the double-muscled cattle breed.

Materials and Methods

D blood collection

All procedures involving animals were approved by the Livestock Embryos Center (LEC) in Cipelang, Bogor, Indonesia. The procedures for blood collection also followed the principles of animal welfare. Blood samples were collected from a total of 12 individual animals including *Belgian Blue cattle (n=3), Ongole Grade (PO) (n=3), F1 offspring (n=3), and F2 offspring (n=3).* F1 was the first generation of individual crossbred (BB × PO) cattle (*B. Taurus* × *B. Indicus*) with blood composition of 50% BB and 50% PO, while the second generation crossbred (F2) had a blood composition of 75% BB and 25% PO.

Blood samples were taken from coccygeal vein using multi Venoject needle with 5 mL vacutainer tubes contain EDTA. The blood samples were divided into two parts for DNA and RNA analysis. The blood samples used for RNA analysis were put in a tube 2 mL and immediately stored in a liquid nitrogen tube (temperature -81^{o}C) before being used for further analysis.

Amplification and genotyping of *MSTN* gene

Genomic DNA was isolated from whole blood samples using the modified Geneaid^{TM} Kit DNA extraction protocol. A pair of primers were used to amplify part of *MSTN* gene in exon 3. The forward primer: 5'-CTC TTC TTT CCT TTC CAT ACA GAC-3' and the reverse primer: 5'-AGG GGA AGA CCT TCC ATG TT-3' had a product length of 451 bp (Jakaria *et al.*, 2021). Amplification condition of PCR consisted of predenaturation at 95°C for 5 min, followed by denaturation at 95°C for 10 s, annealing at 60°C for 20 s, extension at 72°C for 30s and final extension at 72°C for 5 min. PCR Premix in tube 0.2 mL were made of a mixture consisting of 0.6 µL of primer, 12.5 µL of MyTaq HS RedMix, 9.9 µL of nuclease free water (NFW) and 2 µL of DNA samples. The *MSTN* gene was genotyped using enzymes *NmuCI* (*Tsp45I*) (Jakaria *et al.*, 2021) and R buffer by PCR-RFLP for 4 h at 37^{o}C. The digested product were separated using 2% agarose gel with current strength of 100 volt for 35 min and documented using a UV transilluminator (AlphaImager; Alpha Innotech, CA, USA).

Analysis of *MSTN* gene expression

Primer. The primers used in this experiment were picked from National Center for Biotechnology Information (NCBI) referring to accession number of each gene listed in Table 1. The primer length was determined using the Primer 3 program. The primers used for *MSTN* gene expression were determined and analyzed using the Multiple Primer Analyzer and Primer Stat programs. The β-actin gene was used as a housekeeping gene.

RNA isolation. Blood samples which were preserved at -81°C for five days before were isolated using the Qiagen^{TM} RNeasy fibrous tissue

mini kit with a modified procedure. The thawed blood sample was then added with 1:1 PBS into a tube 2 mL. Samples were centrifuged for 10 min at 10,000 rpm. The supernatant was discarded, and the washing process was repeated three times using PBS. RLT buffer in the amount of 800 l was added, homogenized, and incubated at room temperature for seven minutes. The solution was homogenized using a 1 cc syringe with a needle bent in a zigzag form, then incubated at room temperature for 5 minutes. The solution was homogenized with 800 l of 70% ethanol until it was transparent. The solution was placed in the RNeasy Spin Column and centrifuged at 9,000 rpm for 1 minute. The filtrate was discarded after centrifugation for 1 minute at 8,000 rpm with 350 l RW1 buffer. The DNAse incubation mix was added to the spin column tube and the solution was incubated for 15 minutes. RW1 Buffer was added and centrifuged again. Then, 500 l of RPE buffer was added and centrifuged. This process is repeated twice. Furthermore, 30 l of RNAse-free water was added to a 1.5 mL tube that had been packed with a RNAsy spin column, and the sample was incubated for 1 minute at room temperature before centrifugation at 8,000 rpm for 1 minute. The isolated RNA was stored in the freezer at -81°C. RNA quantification was carried out using a Nanodrop Spectrophotometer.

Reverse Transcriptase cDNA. Reverse Transcriptase cDNA was carried out using cDNA synthesis Kit (Toyobo). The total RNA was diluted to 50 ng. 2 µL of total RNA was distributed into 0.2 mL tubes followed by the addition of 2 µL of 4x DNMM and 5 µL of NFW (nuclease free water) then homogenized using vortex and incubated at 37°C for 5 min. Furthermore, 2 µL of 5x RTMM was added and incubated using thermocycler machine Applied Biosystems GeneAmp PCR System 9700 (Thermo Fisher Scientific, Inc., USA) at 37°C for 15 min, 50°C for 5 min and at 98°C for 5 min. Finally cDNA can be stored at -20°C.

Real time q-PCR. Complementary DNA (cDNA) was used for *MSTN* gene expression quantification using real time PCR machine (AG qTower 4 channel Analytic Jena engine, Germany). qRT-PCR was performed using the SYBR green select master kit (Applied Biosystem, USA). The total reaction volume was 10 µL including 5 µL of SYBR green select master kit, 0.5 µL of each forward primer and reverse, 1 µL of cDNA and 3 µL of NFW (nuclease free water). Amplification condition of PCR consisted of predenaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 55 °C for 20 s, extension at 72°C for 30 s and final extension at 72°C for 5 min. *β-Actin* gene was used as a housekeeping gene to normalize the RT-PCR efficiency.

Statistical analysis

All data were analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Scmittgen, 2001). The following formula was used to measure the relative change of gene expression of MSTN gene from tested group to control group compared to the housekeeping gene: ΔΔCT = (average Ct_{MSTN} in the tested group - average $Ct_{\beta\text{-}actin}$ in the tested group) - (average Ct_{MSTN} in the control group - average $Ct_{\beta\text{-}actin}$ in the control group). The +/+ genotypes and normal-muscled phenotypes were appointed as a control group. Statistical comparisons of MSTN gene expression among different genotypes and phenotypes of cattle breeds were determined by the Student t test and $p<0.05$ was regarded as statistically significant (Minitab® 18 Software). The mathematics model was (Kim, 2015):

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s\sqrt{\left(\frac{1}{n_1}\right)+\left(\frac{1}{n_2}\right)}}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(\bar{x}_i - \bar{x}_1)^2 + \sum_{i=1}^{n}(\bar{x}_i - \bar{x}_2)^2}{n_1 + n_2 - 2}}$$

Where:

$\bar{x}_1$ = the average of *MSTN* gene expression of genotype 1 or double-muscled phenotype
$\bar{x}_2$ = the average of *MSTN* gene expression of genotype 2 or normal-muscled phenotype
n_1 = Number of individuals of genotype 1 or double-muscled phenotype
n_2 = Number of individuals of genotype 2 or normal-muscled phenotype
s = the combined of standard deviation

Results and Discussion

Genotyping of *MSTN* gene

The MSTN gene in Belgian Blue, PO and BB x PO crossbred was successfully amplified with product length 451 bp (Figure 1). Agarose gels exhibited bright single bands without smear at the expected size. The results showed that the amplified fragment had a high level of specificity, indicating that RFLP analysis could be carried out directly.

The PCR-RFLP technique using MSTN|*Nmu*CI (Tsp45I) was successfully identified the difference between double-muscled and normal appearance in Belgian Blue, Ongole Grade, and BB x PO crossbred cattle (Figure 2). The del.11/del.11 genotype (350bp and 90bp) showed the double-muscled phenotype was found in Belgian Blue and BB x PO F2 cattle. The +/+ genotype (451bp) was found in all of the PO cattle. The heterozygous genotype (+/del.11) (451 bp, 350 bp and 90 bp) was found in F1 and two F2 crossbred with normal phenotype (Figure 2).

The inheritance pattern of allele + and allele del.11 or +/+ genotype, +/del.11 and del.11/del.11 genotype has been illustrated in Figure 3. The heterozygous F1 offspring was backcrossed with double-muscled Belgian Blue, resulting in a double-muscled and normal F2 offspring.

Based on the results of the genotyping, it was determined that the inheritance pattern of the double-muscled trait was expressed in a recessive homozygous state. Where the double-muscled

Table 1. The primers were used for *MSTN* gene expression using qPCR method

Gene	Primer sequences[1]	Product size (bp)	Annealing temperature (°C)
*MSTN**	F: 5'-GAGAGATGCCAGCAGTGACG-'3 R: 5'-CCTGTCAAGACTCCTGCGAC-'3	213	55
β-actin**	F: 5'-GGACTTCGAGCAGGAGATGG-'3 R: 5'-GCGGCATTCACGAAACTACC-'3	172	55

[1)]Hamny (2020); *) AB076403; **) NM_173979.3 ; F: *forward* and R: *reverse.*

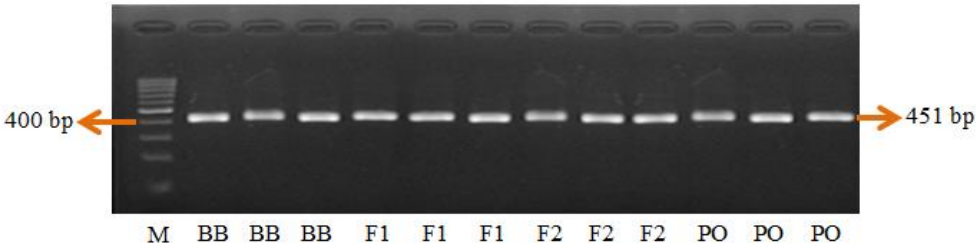


Figure 1. The electrophoresis of PCR product of *MSTN* gene in 1,5% of agarose gel (M: Marker; BB: Belgian Blue; PO: Peranakan ongole; F1: 50% BB x 50% PO; F2: 75% BB x 25% PO).

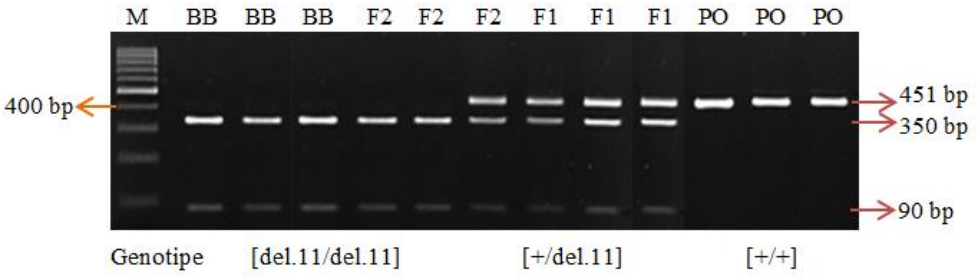


Figure 2. The electrophoresis of PCR-RFLP of *MSTN* │*NmuCI* (*Tsp451*) gene in 2% of agarose gel (M: Marker; BB: Belgian Blue; PO: Peranakan Ongole; F1: 50%BB x 50%PO; F2: 75% BB x 25% PO).

trait did not appear in a heterozygous condition in Belgian Blue and PO crossbred which were grouped into the normal phenotype. It is similar to the double-muscled phenomenon in Belgian Blue and Piedmontese cattle were inherited as recessive (Kambadur *et al.,* 1997; McPherron and Lee, 1997). The double-muscled Marchiagiana cattle were also found in homozygous condition (Marchitelli *et al.,* 2003). In other animals, Boman *et al.* (2009) found a deletion of 1 bp at position c.960delG of the *MSTN* gene in Norwegian white sheep with homozygous condition where the phenotype was characterized by a high carcass conformation class and low fat class. Osman *et al.* (2021) found SNP c.18 G>T and SNP c.241 T>C in the *MSTN* gene in Egyptian sheep were associated with growth traits where the GG genotype showed a higher birth weight and the TT genotype was associated with the average daily gain of sheep.

The phenomenon of double muscles in cattle, especially in Belgian Blue crosses with Indonesian local cattle breeds is important for better breeding strategies in the future. The appearance of the double-muscled phenotype in the second generation of the BB x PO crossbreed was demonstrated to be an effective approach to boosting muscle growth in livestock production.

In addition, there are several problems found in double-muscled cattle, including decreased female fertility, lower offsprings viability, and deferred in sexual maturation (Bellinge *et al.,* 2005; Arthur, 1995). Kolkman *et al.* (2010) also reported high cases of dystocia in the population of double-muscled Belgian Blue cattle, reaching 81.63% or 120 out of 147 calves born by caesarean section due to a greater shoulder width and heart girth. Short *et al.* (2002) reported a decreased pelvic area in double-muscled Piedmontese cattle. Pelvic opening of double-muscled dams was 10 and 6% lower than in normal-muscled Charolais (Arthur *et al.,* 1988) so that the occurrence of dystocia and perinatal mortality was higher in double-muscled cattle. Interestingly, Heterozygous animals did not show any increase in calving difficulty compared to normal animals (Arthur *et al.,* 1988; Blasi *et al.,* 1991; Kišacová *et al.,* 2009). Hopefully, the discovery of genetic markers for the *MSTN* gene (Jakaria *et al.*, 2021) in the crossbreeding program of Belgian Blue cattle with PO or other Indonesian local cattle can reduce the risk of dystocia cases (calving difficulty).

Identification of *MSTN* gene polymorphism and its association with growth traits will provide convenience for breeders to select individual livestock that are considered superior so that they can assist livestock producers in developing breeding strategies to optimize livestock potential. Through various approaches, the exploitation of *MSTN* gene mutations can provide significant benefits for several livestock industries (Ahad *et al.*, 2017).

MSTN gene expression

The mRNA transcription level of *the MSTN* gene was measured via the qRT-PCR technique and the results were performed in Table 2. The *MSTN* mRNA level in del.11/del.11 genotype was lower than +/del.11 (P<0.05). However, The *MSTN* mRNA levels between the del.11/del.11 genotype and the +/+ genotype were not significantly different. Likewise, in the +/del.11 genotype and the +/+ genotype, there was no difference in the levels of *MSTN* mRNA in either (P>0.05) (Table 3). The statistical test results were not significantly different due to the very limited number of samples. The total of samples analyzed was 12 individuals, but only 9 samples were successfully isolated for RNA for qRT-PCR analysis. The *MSTN* gene expression between phenotypes showed a significant difference, where the double-muscled phenotype had a lower *MSTN* mRNA level than in normal-muscled phenotype (P<0.05). The *MSTN* gene expression in BB, PO and their crossbred with different genotypes and phenotypes are presented in Figure 4. The qPCR results indicated that the *MSTN* mRNA transcript level in homozygous double-muscled cattle was substantially decreased compared to heterozygous and homozygous normal-muscled cattle. The heterozygous individuals also encountered a decreased *MSTN* mRNA level compared to normal cattle.

Myostatin (*MSTN*) is the sole inhibitor of skeletal muscle growth and development (Patel and Amthor, 2005). Loss of myostatin fuction increased the diameter and number muscle mass (Zhang *et al.,* 2012). *MSTN*-knockout mice have an incredible increase in skeletal muscle mass and a significantly decreased fat percentage

Table 2. The analysis of qRT-PCR of *MSTN* gene in cattle

Breed	Phenotype	Genotype	ΔCT	Mean ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$
Belgian Blue	Double muscle	del.11/del.11	9.05	9.47 ± 0.59	5,79 ± 0.01	0,02 ± 0.01
F2 (75%BB x 25%PO)	Double muscle		9.89			
F2 (75%BB x 25%PO)	Normal muscle	+/del.11	7.14	5.67 ± 1.01	1,99 ± 0.15	0,3 ± 0.15
F1 (50%BB x 50%PO)	Normal muscle		5.46			
F1 (50%BB x 50%PO)	Normal muscle		4.84			
F1 (50%BB x 50%PO)	Normal muscle		5.25			
PO	Normal muscle	+/+	4.18	3.68 ± 0.78	0.00 ± 0.65	1.00 ± 0.65
PO	Normal muscle		2.79			
PO	Normal muscle		4.08			

Table 3. The differences of *MSTN* gene based on genotype and phenotype

Genotype	*t*-test (P-value)
del.11/del.11 vs +/del	0.035*
del.11/del.11 vs +/+	0.101
+/del vs +/+	0.166
Phenotype	*t*-test (P-value)
Double muscle vs Normal muscle	0.030*

(*) significant at α=5%.

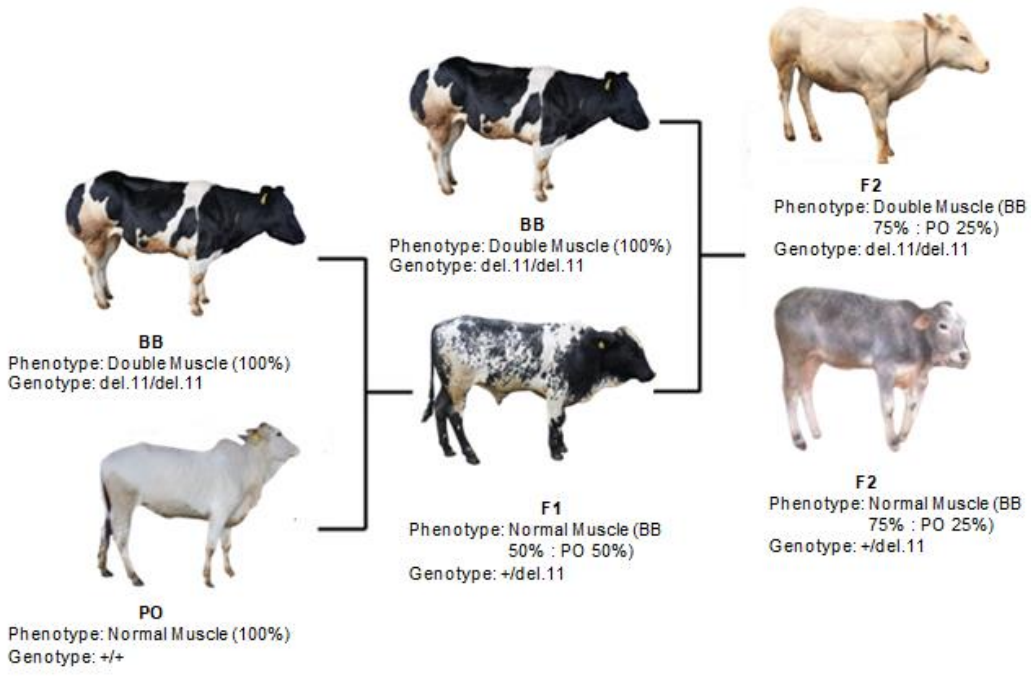


Figure 3. The inheritance of allele + and allele del.11 in Belgian Blue and PO crossbreed.

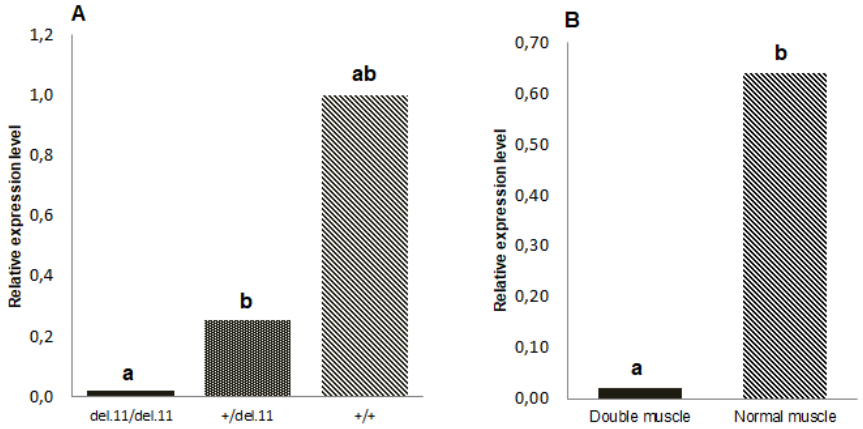


Figure 4. *MSTN* gene expressions (Different A genotype, Different B phenotype). [a,b] indicate significant differences (P<0.05) and [ab] indicates no significant difference (P>0.05).

compared to the wild-type (McPherron *et al.,* 1997). The decreased of mRNA transcription level of mutant *MSTN* in double-muscled cattle suggests that mutant *MSTN* gene can not be successfully transcripted and ultimately produce a disrupted myostatin protein due to the 11-bp deletion in Exon 3. When the structure of the myostatin function is inhibited, resulting in the changes of *CDK2* and *P21* expression levels which are effectively encourage the proliferation of bovine fibroblast cells (Gao *et al.,* 2014). In the double-muscle Javanese cattle, myostatin inhibition can reduce the GLUT4 mRNA to produce the excessive muscle relative to normal-muscled cattle may be due to their greater use of glucose (Takahashi *et al.,* 2014). Hu *et al.* (2013) reported that the *MSTN* gene expression was significantly prevented in transgenic sheep, leading to a faster increase in body weight than in control sheep. Qian *et al.* (2015) showed that *MSTN* gene expression was not detectable in double-muscled Meishan pigs containing a segment with a 193 bp deletion in exon 2 of the *MSTN* gene compared to normal pigs.

On the other hand, Kambadur *et al.* (1997) reported that there was no difference in *MSTN* gene expression between double-muscled Belgian Blue cattle compared to normal muscle using the RT-PCR technique. Evaluation of protein changes in cDNA sequences in double-muscled cattle revealed an 11 bp deletion resulting in the loss of three amino acids (275, 276, and 277) and the presence of a frameshift mutation after amino acid 274.

Frameshift mutations are caused by insertions or deletions that disrupt the DNA sequence. After the insertion or deletion point, each mRNA created from a modified DNA sequence will be read out of the target fragment, resulting in a different protein than usual (Pelley, 2012). The same phenomenon was reported by Boman *et al.* (2009) in Norwegian sheep, which had an increase in muscle mass due to a frameshift mutation in the *MSTN* gene, which caused the formation of a premature stop codon and eventually formed the imperfect protein as a normal, eventually reducing the *MSTN* gene function.

Conclusions

In Belgian blue cattle, PO cattle, and their crosses, *MSTN* gene expression was variable in different genotypes and phenotypes. In the examined cattle, the *MSTN* gene expression in del.11/del.11 genotype (double-muscled) decreased compared with heterozygous (+/del.11) and +/+ genotype. Similarly, the *MSTN* gene expression was lower in the double-muscled phenotype than in the normal-muscled phenotype.

Acknowledgements

This work was financially supported by Doctoral Dissertation Research (DDR), Directorate General of Resources for Science, Technology and Higher Education, Ministry of Research, Technology and Higher Education Fiscal Number: I/EI/KP.PTNBH/2021 date 8st March 2021.

References

Agung, P. P., S. Said, and A. Sudiro, 2016. Myostatin gene analysis in the first generation of the Belgian Blue cattle in Indonesia. J. Indonesian Trop. Anim. Agric. 41: 13–20.

Ahad, W. A., M. Andrabi, S. A. Beigh, R. A. Bhat, and R. A. Shah. 2017. Applications of Myostatin (*MSTN*) gene in the livestock animals and humans: A Review. Int. J. Curr. Microbiol Appl. Sci. 6: 1807–1811.

Arthur, P. 1995. Double muscling in cattle: a review. Aust. J. Agric. Res. 46: 1493-1515.

Arthur, P., M. Makarechian, and M. Price. 1988. Incidence of dystocia and perinatal calf mortality resulting from reciprocal crossing

of double-muscled and normal cattle. Can. Vet. J. 29: 163-167.

Bellinge R. H., D. A. Liberles, S. P. Iaschi, P. A. O'brien, and G. K. Tay. 2005. Myostatin and its implications on animal breeding: a review. Anim. Genet. 36: 1-6.

Blasi, D., W. Lamm, J. Tatum, and J. Brinks. 1991. Growth and fertility traits of calves sired by Piedmontese, Gelbvieh and Red Angus bulls. Livest. Prod. Sci. 31: 259-269.

Boman, I. A., G. Klemetsdal, T. Blichfeldt, O. Nafstad, and D. I. Våge. 2009. A frameshift mutation in the coding region of the myostatin gene (*MSTN*) affects carcass conformation and fatness in Norwegian White sheep (Ovis aries). Anim Genet. 40: 418–422.

Cassar-Malek, I., F. Passelaigue, C. Bernard, J. Léger, and J. F. Hocquette. 2007. Target genes of myostatin loss-of-function in muscles of late bovine fetuses. BMC Genomics. 8: 1–11.

Clop, A., F. Marcq, H. Takeda, D. Pirottin, X. Tordoir, B. Bibé, J. Bouix, F. Caiment, J. M. Elsen, F. Eychenne, C. Larzul, E. Laville, F. Meish, D. Milenkovic, J. Tobin, C. Charlier, and M. Georges. 2006. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet. 38: 813–818.

Freetly, H. C., L. A. Kuehn, and L. V. Cundiff. 2011. Growth curves of crossbred cows sired by Hereford, Angus, Belgian Blue, Brahman, Boran, and Tuli bulls, and the fraction of mature body weight and height at puberty. J. Anim. Sci. 89: 2373–2379.

Gao, F., B. Sun, S. Xing, X. Yu, C. Lu, A. Li, . Zhao, and R. Yang. 2014. The effect of leader peptide mutations on the biological function of bovine myostatin gene. Gene. 540: 171-177.

Gill, J. L., S. C. Bishop, C. Mc Corquodale, J. L. Williams, and P. Wiener. 2008. Associations between the 11-bp deletion in the myostatin gene and carcass quality in Angus-sired cattle. Anim. Genet. 40: 97–100.

Goni, S., C. C. J. Muller, B. Dube, and K. Dzama. 2016. Effect of crossbreeding on beef production of Jersey herd using Fleckvieh sires maintained on a pasture-based feeding system. Open J. Anim. Sci. 06: 163–168.

Grisolia, A. B., G. T. D'Angelo, L. R. P. Neto, F. Siqueira, and J. F. Garcia. 2009. Myostatin (GDF8) single nucleotide poly- morphisms in Nellore cattle. Genet. Mol. Rese. 8: 822-830.

Hamny. 2020. Skrining Pengembangan Sapi Aceh dari Peternakan Rakyat dengan Tinjauan Khusus pada Mikrostruktur Otot dan Ekspresi Gen Myostatin. PhD Thesis. IPB University, Bogor, Indonesia.

Han, S. H., I. C. Cho, M. S. Ko, E. Y. Kim, S. P. Park, S. S. Lee, and H. S. Oh. 2012. A promoter polymorphism of MSTN g.2371T>A and its associations with carcass traits in Korean cattle. Mol. Bil. Rep. 39: 3767-3772.

Hu, S., W. Ni, W. Sai, H. Zi, J. Qiao, P. Wang, J. Sheng, and C. Chen. 2013. Knockdown of myostatin expression by RNAi enhances muscle growth in transgenic sheep. PLoS ONE. 8: e58521.

Jakaria, J., E. Edwar, M. F. Ulum, and R. Priyanto. 2019. Evaluasi kinerja pertumbuhan sapi silangan Belgian blue dan Peranakan Ongole. J. Agripet. 19: 136–141.

Jakaria, J., W. L. N. Aliyya, R. Ismail, S. Y. Siswanti, M. F. Ulum, and R. Priyanto. 2021. Discovery of SNPs and indel 11-bp of the myostatin gene and its association with the double-muscled phenotype in Belgian blue crossbred cattle. Gene. 784: 145-598.

Jeanplong, F., M. Sharma, W. G. Somers, J. J. Bass, and R. Kambadur. 2001. Genomic organization and neonatal expression of the bovine Myostatin gene. Mol Cell Biochem. 220: 31-37.

Kambadur, R., M. Sharma, T. P. L. Smith, and J. J. Bass. 1997. Mutations in myostatin (GDF8) in double-muscled Belgian blue and Piedmontese Cattle.

Khasanah, H., A. Gunawan, R. Priyanto, M. F. Ulum, and Jakaria. 2016. Polymorphism of myostatin (*MSTN*) promoter gene and its association with growth and muscling traits in Bali cattle. Media Peternakan 39: 95-103.

Kim, T. 2015. T test as a parametric statistic. Korean J. Anesthesiol. 68: 540–546.

Kišacová, J., A. Kúbek, V. Meluš, Z. Čanakyová, and V. Řehout. 2009. Genetic polymorphism of myf-5 and *myostatin* in charolais breed. J. Agrobiol. 26: 7-11.

Kolkman, I., G. Opsomer, S. Aerts, G. Hoflack, H. Laevens, and D. Lips. 2010. Analysis of body measurements of newborn purebred Belgian Blue calves. Animal. 4: 661–671.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*. 25: 402–408.

Marchitelli, C., M. C. Savarese, A. Crisà, A. Nardone, P. A. Marsan, and A. Valentini. 2003. Double muscling in Marchigiana beef breed is caused by a stop codon in the third exon of myostatin gene. Mamm Genome. 14: 392–395.

McPherron, A. C. and S. J. Lee. 1997. Double muscling in cattle due to mutations in the myostatin gene. Proceedings, The National Academy of Sciences of the United States of America, Johns Hopkins University School of Medicine, Baltimore, United States of America, August 26, 1997. P. 12457–12461.

McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new *TGF-beta superfamily* member. Nature. 387: 83–90.

Oldham, J. M., J. A. K. Martyn, M. Sharma, F. Jeanplong, R. Kambadur, and J. J. Bass. 2001. Molecular expression of myostatin and MyoD is greater in double-muscled than normal-muscled cattle fetuses. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 280: 1488-1493.

Osman, N. M., H. I. Shafey, M. A. Abdelhafez, A. M. Sallam, and K. F. Mahrous. 2021. Genetic variations in the Myostatin gene affecting growth traits in sheep. Vet. World. 14: 475–482.

Patel, K. and H. Amthor. 2005. The function of myostatin and strategies of myostatin blockade - New hope for therapies aimed at promoting growth of skeletal muscle. Neuromuscular Disord. 15: 117–126.

Pelley, J. W. 2012. Protein Synthesis and Degradation. In Elsevier's Integrated Review Biochemistry. 2nd edn. W.B. Saunders, Lubbock. 149-160.

Prihandini, P. W., D. N. H. Hariyono, and Y. A. Tribudi. 2021. Myostatin gene as a genetic marker for growth and carcass traits in beef cattle. Indones. Bull. Anim. Vet. Sci. 31: 37.

Purchas, R. W., S. T. Morris, and D. A. Grant. 1992. A comparison of characteristics of the carcasses from Friesian, Piedmontese × Friesian, and Belgian blue × Friesian bulls. New Zeal. J. Agric. Res. 35: 401–409.

Qian, L., M. Tang, J. Yang, Q. Wang, C. Cai, S. Jiang, H. Li, K. Jiang, P. Gao, D. Ma, Y. Chen, X. An, K. Li., and Cui W. 2015. Targeted mutations in myostatin by zinc-finger nucleases result in double-muscled phenotype in Meishan pigs. Sci. Rep. 5: 1–13.

Romjali, E. 2018. Program pembibitan sapi potong lokal Indonesia. Wartazoa. 28: 190-210.

Sarti, F. M., E. Lasagna, S. Ceccobelli, P. Di Lorenzo, F. Filip- pini, F. Sbarra, and A. Giontella. 2014. Influence of single nucleotide polymorphism in myostatin and myogenic factor 5 muscle growth-related genes on the performance traits of Marchigiana beef cattle. J. Anim. Sci. 92: 3804-3810.

Short, R. E., M. D. MacNeil, M. D. Grosz, D. E. Gerrard, and E. E. Grings. 2002. Pleiotropic effects in Hereford, Limousin, and Piedmontese F2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele. J. Anim. Sci. 80: 1-11.

Tagliapietra, F., A. Simonetto, and S. Schiavon. 2018. Growth performance, carcase characteristics and meat quality of crossbred bulls and heifers from double-muscled Belgian Blue sires and Brown Swiss, Simmental and Rendena dams. Ital. J. Anim. Sci. 17: 565–573.

Takahashi, H., K. Sato, T. Yamaguchi, M. Miyake, H. Watanabe, Y. Nagasawa, E. Kitagawa, S. Terada, M. Urakawa, M. T. Rose, C. D. McMahon, K. Watanabe, S. Ohwada, T. Gotoh, and H. Aso. 2014. Myostatin alters glucose transporter-4 (GLUT4) expression in bovine skeletal muscles and myoblasts isolated from double-muscled (DM) and normal-muscled (NM) Japanese shorthorn cattle. Domest. Anim. Endocrinol. 48: 62-68.

Weaber, R. L. 2015. Crossbreeding Strategies: Including terminal vs. maternal crosses. Proceedings, Symposium 24, The Range Beef Cow, Kansas State University, Loveland, Colorado, November 17, 18 and 19, 2015. 17: 2–15.

Zhang, C., Y. Liu, D. Xu, Q. Wen, X. Li, W. Zhang, and L. Yang. 2012. Polymorphisms of myostatin gene (*MSTN*) in four goat breeds and their effects on Boer goat growth performance. Mol. Biol. Rep. 39: 3081–3087.

Buletin Peternakan 46 (1): 54-62, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.70195

Developing Strategy to Reduce the Mortality of Native Chicken using Qualitative Modeling

Ismoyowati[1]*, Elly Tugiyanti[1], Diana Indrasanti[2], Novie Andri Setianto[3], and Vony Armelia[4]

[1]Department of Animal Production, Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53123, Indonesia
[2]Department of Animal Science, Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53123, Indonesia
[3]Department of Livestock Socio-economic, Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53123, Indonesia
[4]Postgraduate, Faculty of Animal Science, Jenderal Soedirman University, Purwokerto, 53123, Indonesia

Article history
Submitted: 3 November 2021
Accepted: 26 January 2022

* Corresponding author:
E-mail: ismoyowati@unsoed.ac.id

ABSTRACT

Smallholder native chicken farming continues to face challenges that include simple farming management as well as ND and AI diseases that lead to decreased productivity and increased mortality rate. The aim of the study was to develop a strategy to reduce the mortality rate of native chickens in extensive and semi-intensive rearing systems. This study uses survey method with 78 extensive and 88 semi-intensive native chicken farmers as respondent. This study explores the disease incidence, illness treatment, mortality rate, as well as AI and ND antibody titers which then analyzed descriptively. System dynamic model using Ventana software (VENSIM) was used to identify the contributing factors to the mortality rate of native chicken in smallholder farming. The results showed that the common diseases among native chickens reared in semi-intensive and extensive farming are AI, ND, CRD, and pullorum, with a high rate of disease-specific mortality (>5%). Compared to native chickens in semi-intensive farming, those of in extensive farming showed a higher natural immunity against AI and ND. The qualitative modeling produced seven reinforcing loops and five balancing loops. Some challenges in developing native chicken farming were disease incidence due to lack of proper land and cage, the occurrence of selling unhealthy chickens, farmers opting out for poultry vaccination, high operational cost, lack of business motivation, limited knowledge on poultry management and health, lack of extension programs, and traditional management. We concluded that the rate of disease-specific mortality (ND and AI) remained high in native chickens reared both in extensive and semi-intensive farming. It takes an effort to improve farming management, vaccination, and the government's contribution through extension programs to decrease disease incidence and mortality rate of native chickens.

Key words: Causal loop diagram, Disease**,** Mortality, Native chickens

Introduction

Indonesia has the potential germplasm of native chickens to produce meat and eggs to meet food demand, particularly animal-based protein. Native chickens are commonly reared in rural areas. The national population of native chickens increased by 1.22% to 305.4 million between 2019 and 2020. However, Central Java province saw a declining population by 5.36% from 41,554,574 (2019) to 39,328,326 (2020). Data showed that the 2021's population of native chickens in Central Java is 40,018,923 – a 1.76% increase from the previous year (Director-General of Livestock and Animal Health Services, 2021). Banyumas and Kebumen are two districts with a relatively high population of native chickens, i.e., 1,071,350 and 3,927,820, respectively. The population of native chicken in Banyumas district has increased by 11%, but in the Kebumen district, it has decreased by 19% (Agency of Livestock and Animal Health Service Central Java Province, 2020).

The current challenges to smallholder native chicken farming include simple farming management (extensive and semi-intensive) and incidence of Newcastle Disease (ND) Avian Influenza (AI) diseases that contribute to declining productivity and increased mortality rate among native chickens. Likewise, Newcastle disease (ND) and Avian Influenza (AI) are reported as the most detrimental poultry disease around the world (Alexander, 1995). Newcastle disease is caused by type 1 Avian Paramyxovirus in the family of Paramyxoviridae, while AI comes from influenza

virus type A in the family of Orthomyxoviridae (Alexander, 2000; Swayne and Suarez, 2000; Suarez and Schultz-Cherry, 2000). The most virulent types of ND and AI are listed in the Office International des Epizooties (Swayne and King, 2003). Vaccination to poultry is a successful intervention strategy to control ND and AI (Capua and Marangon, 2004; Veits *et al.*, 2006; Ismoyowati *et al.*, 2013) because it can reduce mortality risk by five times compared to the non-vaccinated poultry reared in household farming or scavenge (Harrison and Alders, 2010).

Native chicken farming belongs to agribusiness that risks contracting disease incidence and mortality. Poultry production is a complex system that is influenced by factors, such as management, disease agents, and environment. on an industrial scale, poultry faces more complicated challenges and high costs. A complex disease-related challenge is attributed to the multifactorial etiology, including variance in pathogenic virulence and pathogenicity, pathogenic interactions, environmental factors, farming management, poultry's immunity status, vaccine efficacy, and feeding. The extensive factors have made it challenging to implement disease control (Galarneau *et al.*, 2020).

A thinking system is a feasible measure to solve these issues. A thinking system is a theory to understand and control any changes that occur in the system. Meanwhile, dynamic modeling attempts to study, understand and analyze a complex system due to perpetual change (Forrester, 1971; Ford, 2010). Modeling has been used for years in poultry production for feed management and growth models, risk factor models for poultry disease recognition and mitigation, and impact of disease on production parameters (Emmans, 1981; Sentíes-Cu e *et al.*, 2010; Volkova *et al.*, 2010) and economic models (Williams, 1999). System dynamics can address the complexity of a dynamic production system and combine inherent feedback loop and system delay (Sterman, 2000; Homer and Hirsch, 2006). System dynamics consist of qualitative modeling with Causal Loop and quantitative modeling with Stocks and Flows diagram (Sterman, 2000).

This study aimed to evaluate the disease incidence and the mortality rate of native chickens reared in extensive farming and semi-intensive farming, investigating the contributing factors to the mortality rate, and develop a Causal Loop Diagram as the basis for investigating the strategy to decrease the mortality rate of native chicken.

Materials and Methods

This study surveyed native chicken farmers and their farms in a structured, questionnaire-based interview. Data collection also included direct observation of poultry conditions and blood sampling for measuring ND and AI antibody titers. The steps of the survey were as follows: 1)We targeted native chicken farmers in Banyumas and Kebumen area. The number of native chicken farmers running extensive farming and semi-intensive farming was 36 vs. 44 in Banyumas and 42 vs. 44 in Kebumen; 2)To collect the samples (farmers), we applied the Purposive Sampling Technique with criteria: smallholder native chicken farming under traditional (extensive) and semi-intensive management. An extensive rearing system is an uncaged rearing of chickens that are grazed around the backyard. Whereas a semi-intensive system refers to chickens farming that are kept in a limited area, the farmer provides cages and feeds with limited quantity and quality. We collected data through interviews with the farmers, questionnaires, and direct observation and measurement in the field. The data included farming system (extensive vs. semi-intensive), disease prevention, total sick chickens, diseases (e.g., ND, AI, Infectious Bronchitis (IB), Infectious Bursal Disease (IBD), Chronic Respiratory Disease (CRD), Aspergillosis, Coccidiosis, Ornithonyssus bursa, and others), disease treatment, medication, vaccination, sales of sick chicken, culling, time of exposure to disease, and the role of extension agent in preventing and medicating disease among native chickens; 3)We drew blood samples from two chickens aged 16-20 weeks from each farm (n=324 birds) to measure the ND and AI antibody titers. Blood sampling was performed according to animal ethic protocols published by The Centre of Research and Community Service No. 1443/UN23.18/PT.01.01/2021. The antibody titers were examined using the Haemagglutination Inhibition (HI) test; 4)Data of disease incidence, treatment to sick chickens, mortality, AI and ND antibody titers were subjected to descriptive analysis. The System Dynamics was modeled to identify the contributing factors to the mortality rate of native chicken in smallholder farming, the inter-element relationship in native chicken farming, and the qualitative and quantitative modeling of native chicken farming. Additionally, strategies were designed to decrease the mortality rate among native chickens. The steps in system dynamics modeling (SDM) include: 1) Identify problems and limitations; 2) Develop a dynamic hypothesis that explains the causes of the problems; 3) Build causal loop diagram (CLD); 4) Develop stock and stream models; and 5) Perform model simulations (Galarneau *et al.*, 2020). The System Dynamic model used a thinking system using Ventana software (VENSIM).

Results and Discussion

Diseases incident and mortality

Disease incidence in Banyumas and Kebumen districts included AI, ND, CRD, and pullorum (Figure 1). In the interview, most farmers mentioned that native chickens often contracted diseases that led to sudden death. Farmers' ignorance of the types of disease contributed to poorly managed poultry farming.

Preventing disease among native chickens can be done by vaccination and cage sanitation.

Our observation showed that only 11.11% of extensive farming-breeders in Banyumas and 5% in Kebumen performed ND or AI vaccination to their native chickens. Native chicken farming is crucial to develop, especially in rural areas to supply meat and eggs and provide additional income for the community (Burhanudin *et al.*, 2019). Banyumas district is one of the centers for developing native chickens in Indonesia (Iswanto *et al.*, 2018), and the Kebumen district has a high population of native chickens (BPS, 2021). Some common diseases contracting poultry in these two districts are AI, ND, CRD, and Pullorum (Figure 1). Avian Influenza (AI) records the highest disease incidence in extensive farming in Banyumas (61,11%) and Kebumen (70%), exceeding the incidence in semi-intensive farming in Banyumas (40,91%) and Kebumen (50%). Avian Influenza is also reported to have the highest incidence among poultry (Sub-Directorate for Observing Animal Diseases, 2014).

Figure 1 shows that AI disease incidence is the highest of other diseases. AI incidence is caused by a seasonal infectious disease that originated from an outbreak in 2012. Extensive or free-range farming would moderate the spread of disease more rapidly and extensively. According to most farmers interviewed in our study, native chickens often contracted diseases that cause sudden death. Farmers' ignorance about the type of diseases that contracted their poultry has led to poor poultry management. The second prevalent disease after AI, Newcastle Disease records a low percentage of incidence probably due to routine ND vaccination among poultry farms that help curb the disease. A survey study of ND vaccination rollout in 11 villages of Chibuto, Mozambique reported that the farms had more chickens reared in the semi-intensive system (15.0) than in extensive farming (8.7). Compared to the non-vaccinated chickens, the vaccinated chickens had a bigger population size (16.9 vs. 10.0), higher average hatchability (80% vs. 70%), and five-time less risking death due to ND. The efficacy of ND 1-2 vaccination is reflected in the average increase of flock size and decreased mortality rate due to ND (Harrison and Alders, 2010). Meanwhile, CRD and Pollurum were of low incidence in Banyumas and Kebumen district, but predators remained prevalent in the semi-intensive farming in Banyumas.

Delabouglise *et al.* (2020) reported the impact of AI outbreak on smallholder chicken farming in south Vietnam. In small broiler flocks (≤16 chicken/flock) the estimated probability of harvest was 56% higher when an outbreak occurred, and 214% higher if an outbreak with sudden deaths occurred in the same month. Vaccination and disinfection combined have a strong and positive correlation with flock size. The majority of poultry producers in the low-income country are smallholder farmers who rely on quick sales of their poultry to compensate for the loss due to disease. AI-infected chickens are distributed to markets or trade networks, thus potentially increasing the spread of AI.

Prevalent diseases to native chickens include ND, gumboro, fowl pox, snot, CRD, AI and paralysis. The common challenges to native chicken farmers include lacking knowledge on the standard practice of commercial farming and biosecurity, and the under optimum sanitation and animal health treatment which may expose the native chickens to many diseases. Diseased livestock would lead to low productivity even death (Libriani *et al.*, 2020). These challenges are also found in Banyumas and Kebumen districts.

Vaccination rollout to chickens reared in different farming systems in Banyumas and Kebumen has reached 40-70%. ND and AI antibody titers are crucial to identify the vaccine potentials to stimulate protective immunity in chickens, especially after the vaccination. This effort is taken to prevent AI or ND outbreaks (Kencana *et al.*, 2016). The percentage of the use of chemical and herbal medicine, the sales of sick chickens, and culling were low, except in semi-intensive farming in Banyumas.

Disease treatment to native chicken may include offering medicine and vitamin or herbal medicine. In Banyumas, 11.11% of the extensive farmers offered herbal medicine for their sick livestock, 9.09% semi-intensive farmers offered vitamins and medicine, and 36.36% used herbal medicine. In Kebumen, 30% of extensive farmers and 15% of semi-intensive farmers preferred medicine and vitamin, 35% of extensive farmers and semi-intensive farmers provided herbal medicine to treat poultry disease (Figure 2).

Native chicken farmers treated their sick chickens in different manners. In Banyumas, 5.56% of extensive farmers sold their sick chickens, while 22.22% opted for culling the sick chickens. In semi-intensive farming, 9.09% of farmers chose to sell sick chickens, while 27.27% preferred culling. In Kebumen, 15% of the extensive farmers sold the sick chickens, and 30% culled them. In semi-intensive farming, 10% of the farmers sold sick chickens, and the rest 30% preferred to cull them for family consumption (Figure 2).

The present study showed that native chicken farms in Banyumas and Kebumen districts tend to use herbal remedies to treat disease in poultry (Figure 2). Herbal remedies are an alternative solution for chemical medicine as a supplement to improve native chicken's body immune against stress and common disease (Mahfuuzhoh *et al.*, 2019) as well as an appetite booster for livestock to improve health less susceptible to disease (Yuliani *et al.*, 2020).

Vaccination refers to an act of injecting antigen (weakened virus or disease agent) into a healthy body to improve antibody or body immune. Vaccination is the most effective measure to give protection to native chickens from disease incidence. The low vaccination rates among native chickens are probably due to farmers' lack of awareness of the importance of

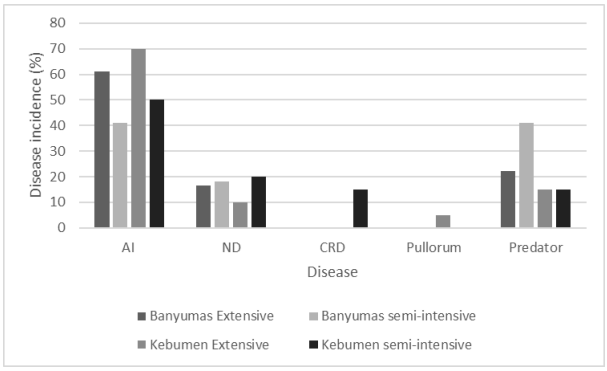


Figure 1. Disease record of Native chicken.

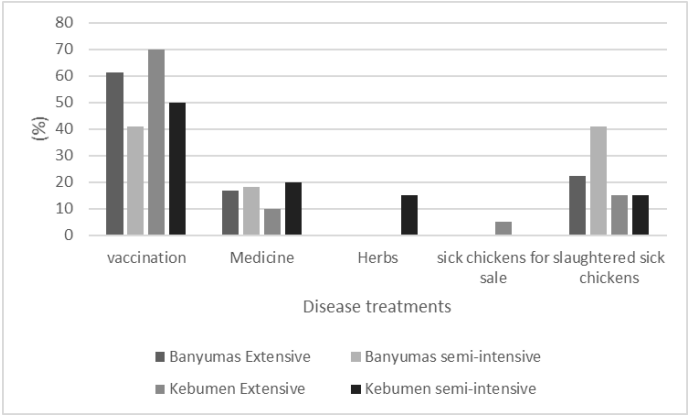


Figure 2. Disease treatment among poultry.

maintaining poultry health through vaccination and performing the vaccination by themselves. To the best of our knowledge, there are no or limited extension programs on poultry health targeting native chicken farmers in Banyumas and Kebumen. Therefore, animal health issue remains an obstacle in the development of native chicken farming.

Rosyidi (2018) stated that many disease incidences and feed poisoning are due to farmers' partial knowledge and consumers' lack of awareness of the safety and halal status of animal-based food remain unreported or unidentified. Sick chickens for sale or culling illustrate such an alarming food safety among Banyumas and Kebumen community; therefore, the government should educate the people regarding the best practice of disease treatment for native chickens.

Some disease incidence and predators are behind the mortality of native chickens. Mortality rate refers to the average death among native chickens in one-year maintenance. The mortality rate of native chicken in Kebumen and Banyumas was relatively high compared to the 5% threshold (Sofjan, 2012). Native chicken mortality in the farms observed in this study ranged from one to three chickens per year (Table 1). Kebumen has a smaller flock size but a higher mortality rate than Banyumas (Table 1), so it is crucial to investigate what has caused the phenomena from a maintenance perspective. Hidayat and Asmarasari (2015) stated that the challenges in native chicken farming are slow growth, high mortality, and low production of eggs. In addition, farmers' low level of knowledge on disease and disease prevention, disease incidence, and environmental factors contribute to the increased mortality rate among native chickens in the location of the study.

The mortality rate of native chicken is generally affected by an extreme environmental condition, disease, antinutritional substances in feed, and competition for feed (Kestaria *et al.*, 2016). Traditional farming systems contribute to 70% of the mortality rate of native chickens (Rajab and Papilaya, 2012). It was in line with Nurmi *et al.* (2018) that the environment contributes 70% to the success of a farming business. The factors influencing mortality rate include body weight, chicken breeds and strains, climate conditions, environmental hygiene, sanitation, equipment, cage, and environmental temperature. Fluctuating weather conditions would cause a decrease in feed intake, which leads to declining body weight, and eventually, death.

Since ND and AI are viral diseases, poultry's health status should be subjected to a serology test to observe the antibody titers in the chickens. Table 2 illustrates the result of the Haemagglutination Inhibition test (HI test) of native chicken.

Table 1. The mortality rate of native chicken in semi-intensive and extensive farming

Area	System	Average number of chickens/farmer (bird)	Mortality/year (%)
Banyumas	Extensive	14.89	6.72
	Semi-intensive	18.59	7.79
Kebumen	Extensive	12.9	14.34
	Semi-intensive	18.15	16.53

Table 2. The Percentage of ND and AI antibody titres in native chicken reared in extensive and semi-intensive system

Antibody titres	Extensive (%)	Semi-intensive (%)	Antibody titres	Extensive (%)	Semi-intensive (%)
	ND			AI	
0	50.00	57.14	0	50.00	54.76
<2^6	39.47	30.95	<2^4	47.37	42.86
≥2^6	10.53	9.52	≥2^4	2.63	2.38

Based on the recommended HI test by the World Organisation for Animal Health (Office International des Epizooties, 2012), AI and ND protective antibody titers are ≥ 2^4 and ≥ 2^6, respectively, reflecting a high level of antibody against the diseases. The protective antibody titers against ND of native chickens reared in extensive and semi-intensive farmings in Banyumas and Kebumen were 2,38% and 10,53%, respectively (Table 2). This result confirmed a study about local chicken raised under a traditional management system in three selected agricultural-climatic zones in Central Ethiopia. The study reported that the hemagglutination inhibition (HI) test for ND antibody titers produced an overall seropositive rate of 32,22%, which varied between farming in highland (28,57%), medium-altitude area (29,69%), and lowland (38,33%). ND is reportedly the main contagious disease that threatens the sustainability and productivity of traditionally-reared native chickens (Tadesse *et al.*, 2005). In Nigeria, the high prevalence of ND antibody among native chickens (17%) which may be due to virulent infections such as mesogenic strain, which is a virus that causes respiratory and neurological symptoms that leads to low mortality, or lentogenic strains (mild respiratory infection without apparent morbidity and mortality) (Oyiguh *et al.*, 2014).

Protective antibody titers of AI in extensive farming and semi-intensive farming are 2,63% and 2,38%, respectively (Table 2). It shows partial efficacy of vaccination rate among native chicken in Banyumas and Kebumen regardless of the farming system. The percentage of protective antibody titers of ND and AI is lower than the non-protective ones, which is probably because the chicken samples in the research location are never naturally infected by AI or ND. Also, it indicates a failed vaccination which may be attributed to different factors, including the chicken conditions, vaccinator, and vaccine equipment.

A previous study reported that AI and ND vaccination records of native chickens reared under an extensive system were non-existent. The Haemagglutination inhibition (HI) test showed that the antibody prevalence against AI and ND was 31,6% and 38,8%, respectively, and the average antibody titers of AI were 0,32 ± 0,1 Log 2 and BD were 0,39±0,2 Log 2. The result showed that 70,4% of serum contained HI antibodies against AI and ND antigens. It was suspected that native chickens reared under an extensive system play a crucial role in spreading ND and AI diseases (Wakawa *et al.*, 2009). Yuliantari *et al.* (2018) reported that AI prevalence is considered relatively high if it is produced from 2.5% of the total blood serum. Sutrisna (2014) stated that antibody titers are not consistently protective; the efficacy will decrease with time, and the declining rate is affected by the disease virulent or animal condition. Therefore, vaccination is the prerequisite for an optimum formation of antibody titers.

Strategy to reduce mortality rate

Factors that were allegedly influencing the mortality rate of native chicken in family poultry farming can be explored and strategies for development can be designed using a model developed in Causal Loop Diagram (CLD). Modeling in the current study found seven reinforcing loops and five balancing loops. Loops of Smallholder native chicken farming (Family poultry production).

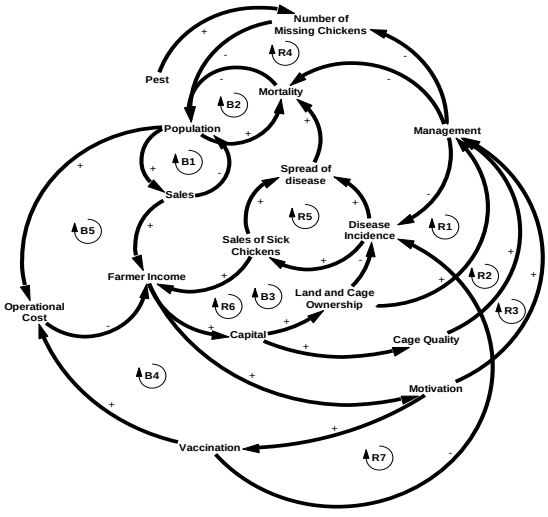


Figure 3. Loops of smallholder native chicken farming.

Figure 4 illustrates three balancing loops (B), namely B1, B2, and B3, and three reinforcing loops (R): R5, R6, and R7. Loop B1 shows that population increases sales, and the higher the sales, the smaller the population. Loop B2 indicates that population increases mortality rate,

which then decreases the population. Based on loops B1 and B2, an effort to maintain the population of native chicken is crucial to curb mortality and sales rate.

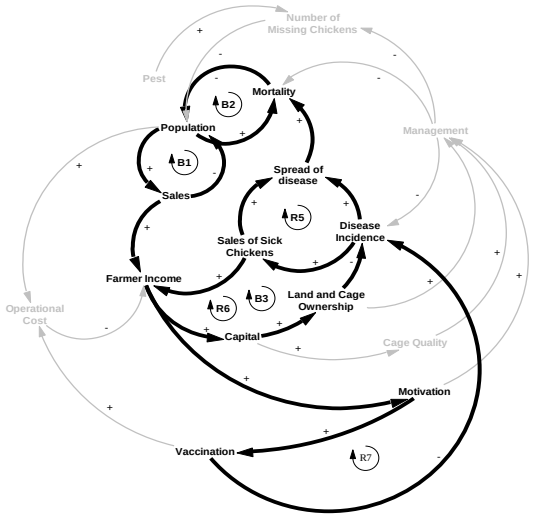


Figure 4. Loop disease incidence.

Figure 4 illustrates three balancing loops (B), namely B1, B2, and B3, and three reinforcing loops (R): R5, R6, and R7. Loop B1 shows that population increases sales, and the higher the sales, the smaller the population. Loop B2 indicates that population increases mortality rate, which then decreases the population. Based on loops B1 and B2, an effort to maintain the population of native chicken is crucial to curb mortality and sales rate.

Loop B3 shows that farmer income increase capital. Capital improves land and cage ownership, reduces disease incidence, thus lowering the total sales of sick chickens. Loop R5 shows that income increases capital, capital improves land and cage ownership, which decreases disease incidence and sales of sick chickens, and eventually, reduces the spread of disease, resulting in an increased population, sales, then income. Loop R6 shows that income increases capital, capital improves land and cage ownership and reduces disease incidence, thus curbing the spread of disease and mortality rate and eventually increasing population, sale, and income. Loop R7 shows that income increases business motivation, motivation improves vaccination rates and reduces disease incidence, the spread of disease, and mortality, and eventually increases poultry population, sales, and farmer income. The sales of sick chicken increase farmers' income and their business capital. The analysis shows that disease prevalence is positively correlated with mortality, so the increased prevalence of the disease will raise the mortality rate. Mortality is negatively correlated with farmer income because a high mortality rate would decrease farmer income. When farmer receives less profit, it is assumed that disease prevention has been improved, thus reducing disease prevalence. These correlations collectively impose a balancing effect on the system and result in declining disease prevalence and mortality (Galarneau *et al.*, 2020).

Based on loops R5, R6, and R7, we suggest farmers allocate their income for vaccination and improve their land and cage ownership so that they can curb the spread of disease and mortality. We found that most farmers in Banyumas and Kebumen districts did not vaccinate their native chickens, which may have caused high susceptibility to disease outbreaks and high mortality rates. Farmer's ignorance of the harmful consequences of consuming sick chickens made them sell the sick chicken for half price. Poultry reared in an extensive farming system does not pose as a serious threat as those in an intensive system that have homogeneity of genetic stock and poor biosecurity. AI pandemic has impacted native poultry, farmers, and traders of native poultry. The impacts include cultural issues, village poultry value chains, approaches to biosecurity, marketing, poultry disease prevention, and control compensation, genetic diversity, poultry as part of livelihood strategies, and effective communication. The first step towards HPAI prevention and control is increasing awareness that poultry health and welfare is vital (Alders *et al.*, 2014). Accordingly, it takes a collaborative measure from multiple stakeholders and the government to increase farmers' level of knowledge on animal health and welfare.

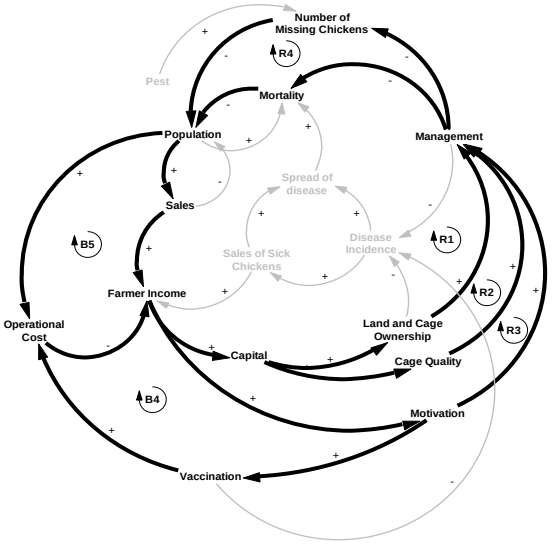


Figure 5. Loop Management.

Figure 5 consists of two balancing loops (B), i.e., loop B4 and B5, and four reinforcing loops, i.e., loop R1, R2, R3, and R4. Loop B4 shows that farmer income increases motivation, improves vaccination rates, increases operational cost, and decreases farmer income. Loop B5 indicates that population increases the operational cost, then decreases farmer income. Income increases management that will increase the

population. Poultry vaccination reduces disease risk among native chickens, but vaccination rollouts will add to operational costs. This monetary factor is crucial in farmers' decision to perform vaccination whereas their knowledge of the importance of vaccination is low. Farmers tend to limit their flock size to curb operational costs because the high maintenance cost is not equal to the low selling price that ranges from Rp20,000,00 to 150,000,00, depending on the chicken's age and sex.

Loop R1 shows that a positive rate is attributed to population – sale – farmer income – capital – land and cage ownership – farming management – mortality – population. The high population would increase farmer income, and then increases capital reinvestment, improve land and cage ownership, and increase management, which then decreases mortality, and eventually increases population. Loop R2 shows that population increases sales, sales increase farmer income, and their capital, then increases cage quality and improves management, reduces mortality rate, and eventually, increases the population. Loop R3 shows that population increases sales, sales increase farmer income, business motivation, and management, then reduces mortality rate, and eventually, increases population. Loop R4 shows that management decreases the number of missing chickens, which means an increased population that gives rise to sales and income, and eventually, increases capital and improves management. The result showed that native chickens reared under extensive farming tend to go missing more often than in a semi-intensive system where farmers invest in more treatment and care for their animals. In extensive farming, chickens roam free in the natural environment, thus more susceptible to going missing. Based on loops R1, R2, R3, and R4, it takes an allocated income for improving land and quality cage to reduce the mortality rate among native chickens.

Conclusions

Disease incidence among native chicken reared in semi-intensive and extensive farming includes AI, ND, CRD and pullorum. Disease-causing mortality remains high (>5%). Native chickens reared under extensive farming shows a higher body immune against AI and ND than those in semi-intensve system. CLD qualitative model produces seven reinforcing loops and five balancing loops. The challenges to developing native chicken farming include disease incidence due to lack of appropriate land and cage, sales of sick chicken, zero vaccination rates, high operational cost, low business motivation, low level of knowledge on farming management and animal welfare, non-existent extension programs by the government related to traditional farming management. Strategy to reduce mortality includes preventing the incidence of diseases by providing land and cage for breeding, prohibiting sales of sick chickens, introducing vaccination, improving management through extension program and campaign, and mentoring by medical personnel to native chicken farmers.

Acknowledgement

The authors express their gratitude to the Directorate general of Higher Education, Research, and Technology of the Ministry of Education, Research and Technology for the grant under the Applied Research scheme 2021, contract No: 117/SP2H/LT/DRPM/2021, 18 March 2021.

References

Agency of Livestock and Animal Health Service Central Java Province. 2020. Livestock population by district/municipality and kind of livestock in central java province. https://jateng.bps.go.id/, Accessed 17 October 2021.

Alders, R., J. A. Awuni, B. Bagnol, P. Farrell, and N. de Haan. 2014. Impact of avian influenza on village poultry production globally. EcoHealth, 11: 63–72.

Alexander, D. J. 1995. The epidemiology and control of avian influenza and newcastle disease. J. Comp. Pathol. 112: 105–126.

Alexander, D. J. 2000. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. 19: 443–462.

Burhanudin, D., D. M. Saleh and S. Mugiyono. 2019. Effect of artificial insemination interval and spermatozoa concentration on salable chick and grade out in Sentul chicken. J. Anim. Sci. Technol. 1: 168-177.

Capua, I and S. Marangon. 2004. Vaccination for avian influenza in Asia. Vaccine. 22: 4137–4138.

Central Bureau of Statistics (BPS) Kebumen Regency. 2021. Kebumen Regency in Numbers. https://kebumenkab.bps.go.id. Accessed 2 November 2021.

Delabouglise, A., N. T. L. Thanh, H. T A. Xuyen., B. Nguyen-Van-Yen., P. N. Tuyet., H. M. Lam and M. F. Boni. 2020. Poultry farmer response to disease outbreaks in smallholder farming systems in southern Vietnam. eLife 9. e59212: 1-19.

Director-General of Livestock and Animal Health Services. 2021. Livestock and Animal Health Statistics 2021. http://ditjenpkh.pertanian.go.id/, Accessed 17 October 2021.

Emmans, G. C. 1981. A model of the growth and feed intake of adlibitum fed animals, particularly poultry. Comput. Anim. Prod. 5: 103–110.

Ford, A. 2010. Modeling the Environment. 2nd ed. Island Press, Washington, Covelo, London.

Forrester, J. W. 1971. World Dynamics. 1st ed. Wright-Allen Press, Cambridge, MA.

Galarneau, K. D., R. S. Singer, and R. W. Wills. 2020. A system dynamics model for disease management in poultry production. Poultry Science. 99:5547–5559.

Harrison, J. L. and R. G. Alders. 2010. An assessment ofchicken husbandry including Newcastle disease control in ruralareas of Chibuto, Mozambique. Tropical Animal Health and Production. 42: 729–736.

Hidayat, C. and S. A. Asmarasari. 2015. Native chicken production in indonesia: a review. Indonesian Animal Husbandry Journal. 17: 1-11.

Homer, J. B. and G. Hirsch. 2006. System dynamics modeling for public health: background and opportunities. Am. J. Public Health. 96: 452–458.

Ismoyowati, M. Muft, M. Samsi, and A. Susanto. 2013. Maternal antibody titer against avian influenza transferred from hens to the eggs and ducklings. Animal Production 15: 189-195.

Iswanto, Subiharta, and A. Prabowo. 2018. The dissemination of superior native chicken breeding by Balitbangtan (KUB) supports the Working in Central Java program. Proceedings of the National Seminar on Agricultural Resource Readiness and Location-Specific Innovation Entering the Industrial Era 4.0. BPTP Central Java, Balitbangtan, Ministry of Agriculture.

Kencana, G. A. Y., I. N. Suartha, N. M. A. S. Paramita, and A. N. Handayani. 2016. Newcastle disease combination vaccine with avian influenza triggers protective immunity in laying hens against tetelo disease and bird flu. Veteriner Journal 17: 257-264.

Kestaria, H. Nu, and B. Malik. 2016. Effect of commercial feed substitution with coconut dregs flour on native chicken performance. Archipelago Farming Journal. 2: 43-48.

Libriani, R., L. O. Nafiu, T. Saili, M. Abadi, A. Sulfitrana, W. L. Salido, and P. D. Isnaeni. 2020. Disease prevention in native chicken through technical guidance on sanitation and biosecurity management in Abeli district. Journal of Applied Science Community Service. 2: 111-116.

Mahfuuzhoh, D., N. Prabewi and Susanto. 2019. The response of women farmers groups in banjarsari village towards giving herbs for optimizing the performance of native chicken in the starter period. Jurnal Agricultural Extension Development Program. 16: 47-57.

Nurmi, A., M. A. Santi, N. Harahap, and M. F. Harahap. 2018. Percentage of carcass and mortality of broilers and native chickens given waste of unfermented and fermented palm starch dregs in the ration. Integrated Animal Husbandry Scientific Journal 6: 134-139.

Office International des Epizooties (OIE). 2012. Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.3.14. http://www.oie.int/international-standard-setting/terrestrialmanual/access-online

Oyiguh, J. A., L. K. Sulaiman, C. A. Meseko, S. Ismail, I. Suleiman, S. J. Ahmed, and E. C. Onate. 2014. Prevalence of newcastle disease antibodies in local chicken in federal capital territory, Abuja, Nigeria. International Scholarly Research Notices Volume 2014, Article ID 796148, 3 pages.

Rajab, and B. J. Papilaya. 2012. Quantitative characteristics of local native chicken in traditional maintenance. J. Agrinimal. 2: 61-64.

Rosyidi, D. 2018. Some of the constraints of food ingredients of livestock origin to achieving safe, healthy, whole and halal (ASUH). Proceedings of Nation Seminar on Animal Science Technology and Agribusiness VI: Development of Local Livestock Genetic Resources Towards Self-Sufficiency in Animal Food ASUH, Faculty of Animal Science, Jenderal Soedriman University, 7 July 2018.

Sentíes-Cu e., C. G., R. W. Wills., P. A. Stayer., M. A. Burleson, and D. L. Magee. 2010. Epidemiology and effect on production parameters of an outbreak of inclusion body hepatitis in broilers. Avian Dis. 54: 74–78.

Sofjan, I. 2012. Native chicken superior Balitnak. Agricultural Research and Development Agency. Jakarta.

Sterman, J. D. 2000. Business Dynamics: Systems Thinking and Modeling for a Complex World. McGraw-Hill, Boston, MA.

Suarez, D. L. and S. Schultz-Cherry . 2000. Immunology of avian influenza virus: a review. Dev. Comp. Immunol. 24: 269-83.

Sub-Directorate for Observing Animal Diseases. 2014. Manual of poultry diseases. 2nd printing. Directorate of Animal Health. Directorate General of Livestock and Animal Health. Ministry of Agriculture.

Sutrisna, R. 2014. Isolate of lactic acid bacteria as probiotics with AI and ND vaccination in formation of antibody titres and body weight of medium type roosters. J.Appl. Agric. Res. 14: 124-133.

Swayne, D. E. and D. L. Suarez. 2000. Highly pathogenic avian influenza. Rev. Sci. Tech.19: 463–482.

Swayne, D. E. and D. J. King. 2003. Avian influenza and newcastle disease. J. Am. Vet. Med. Assoc. 222: 1534–1540.

Tadesse, S., H. Ashenafi, and Z. Aschalew. 2005. Seroprevalence study of newcastle disease in local chickens in central Ethiopia. Int. J. Appl. Res. Vet. Med. 3: 25-29.

Veits, J., D. Wiesner, W. Fuchs, B. Hoffmann, H. Granzow, E. Starick, E. Mundt, H. Schirrmeier, T. Mebatsion, T. C. Mettenleiter, and A. Romer-Oberdorfer.

2006. Newcastle disease virus expressing h5 hemagglutinin gene protects chickens against newcastle disease and avian influenza. Proc. Natl. Acad. Sci. USA 103: 8197–8202.

Volkova, V., R. Bailey, M. Rybolt, K. Galarneau, S. Hubbard, D. Magee, J. Byrd, and R. W. Wills. 2010. Inter-relationships of Salmonella status of flock and grow-out environment at sequential segments in broiler production and processing. Zoonoses Public Health 57: 463–475.

Wakawa, A. M., P. A. Abdu, J. U. Umoh, S. Lawal, and R. B. Miko. 2009. Serological evidence of mixed infections with avian influenza and newcastle disease in village chickens in Jigawa State, Nigeria. Veterinarski Arhiv. 79: 151-155.

Williams, R. B. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int. J. Parasitol. 29: 1209–1229.

Yuliani, N. S., G. Y. I. Sakan, and M. D. S. Randu. 2020. Utilization of herbal plants to prevent broiler chicken disease in Noelbaki village farmers group. Scientific Journal of Community Service. 4: 347-355.

Yuliantari, I. A. M., G. A. Y. Kencana, and I. M. Kardena. 2018. Seroprevalence of avian influenza in native chicken in Kerta, Payangan, Gianyar, Bali. Indonesia Medicus Veterinus. 7: 689-698.

Buletin Peternakan 46 (1): 63-72, February 2022

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v46i1.70242

Potential Analysis and Development Strategies Based on Zoning For Beef Cattle Farming in Kepulauan Bangka-Belitung Province

Muhammad Taufiq Alamsyah[1,2], Siti Andarwati[1], Tri Anggraeni Kusumastuti[1], and Panjono[1]*

[1]Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Agency of Agriculture and Food Security, Kepulauan Bangka Belitung Province, 33149, Indonesia

Article history
Submitted: 5 November 2021
Accepted: 10 January 2022

* Corresponding author:
Telp. +62 813 2802 2087
E-mail: panjono@ugm.ac.id

ABSTRACT

This research was aimed in detemining the potency mapping and development strategies based on zoning for beef cattle farming in Kepulauan Bangka Belitung Province. This research was implemented by using two-stages survey method. The first stage was analyzing and making regional potency mapping from secondary data with potential index analyses. The second stage was observing the results of the observation, an in-depth interview, and focus group discussion towards 42 respondents consisted of 14 cattle farmers, 5 cattle sellers, 5 agriculture agency officers, and 18 officials who were in charge of the cattle function in regency/city. Purposive sampling was used to choose respondents. Meanwhile, the data analysis used SWOT analysis. The results of potency mapping showed that Bangka had the highest index (the most potential). Pangkalpinang had the lowest index (potential). The qualitative SWOT analysis resulted in the strategy of SO (Strength-Opportunity), WO (Weakness-Opportunity), ST (Strength-Treat), and WT (Weakness-Threats). Qualitative analysis of SWOT showed the internal factor -0,153 (x) and external factor 0,34 (y). The strategies were in quadrant III; changing the policies by minimizing the weakness to take advantage of opportunities. The analysis for RTRW documents and the result of SWOT analysis generated 6 (six) zones and development priorities. Thus, the development plan of beef cattle consisted of priority zones: 1). I: production center and product processing in Bangka Tengah; 2) II: Cattle farmer integration in Bangka, Bangka Barat, and Belitung Timur; 3) III: Cattle farming in a previously ex-mining land in Bangka, Bangka Barat, and Belitung Timur; 4) IV: production center with local based feed production in Bangka Selatan; 5) V: Modern cattle farm with technology-based in Pangkalpinang; 6) VI: Animal farm with agrotourism based in Pangkalpinang and Belitung.

Keywords: SWOT analysis, Potency mapping, Strategy priority, Beef cattle, Zoning strategy

Introduction

Beef cattle of the the livestock sub-sector commodity as an integral part of livestock sector plays an importance role in supplying meat as a source of animal protein in Indonesia. It shows that beef cattle farms have a bright future since the demand for materials derived from livestock increase as the population, money, and people's awareness of the value of eating healthy food rises. Meat consumption in Indonesia every year also always increases, due to public awareness of the importance of consuming animal protein (Tadete et al., 2016). This condition is caused by important role of meat as a source of high-quality proteins, minerals and vitamins and other compounds, which was difficult to obtain in sufficient amount from other sources (Geiker *et al.,* 2021).

Most of the beef production in Indonesia, 78% from the total of beef cattle production come from traditional livestock, 5% from imports, and 17% from live livestock imports, especially from Australia (Zakiah *et al.,* 2017). The demand also always increases every year, as well as the number of beef cattle import which also continues to grow since domestic cattle farms have not been able to meet market needs. In addition, the quality of imported meat also has several advantages, such as being tenderer than traditional beef cattle, a high degree of marbling, so that it is favored by consumers (Priyanto *et al.*, 2015).

In achieving a national increase in beef cattle population, it is necessary to support the regions in Indonesia which still have potential resources of cattle populations. Kepulauan Bangka Belitung Province (Babel), as the area with the lowest beef cattle population in Sumatra, has the potential to increase its beef cattle population.

Babel Province has natural resources in the form of land as a place for livestock keeping and forage production. Good quality and forage availability can increase production, especially for increasing body weight of cattle (Suhaema *et al.,* 2014). Forage producing areas in Babel Province include gardens (160,327), rice fields (25,093 Ha), palm plantation (48,351.9) and forests (43,661 ha). The beef cattle population in this Province during the 2014-2019 period continued to increase, ranging from 10,136; 10,557; 11,604; 12,664; 13,760; and 14,760 (BPS Provinsi Kepulauan Bangka Belitung, 2020). Until now, the annual increase in population has not been commensurate with the needs of beef cattle in the region, so Babel has to import livestock from outside region, either in the form of frozen meat or feeder cattle.

The development of beef cattle farms has become one of working program priorities of Kepulauan Bangka Belitung Governor, as detailed in the Regional Medium Term Development Plan (*Rencana Pembangunan Jangka Menengah Daerah / RPJMD*) for increasing beef cattle production. The Governor also targets Kepulauan Bangka Belitung Province to become a cattle center in the Sumatera and be able to reach meat self-sufficiency. Therefore, the local government through the Agricultural Agency of Kepulauan Bangka Belitung Province proclaimed the program of *"Babel Lumpat* (*Babel Lumbung Pangan Asal Ternak*)*"* which means the realization of Babel as the livestock center in the Sumatera region.

To achieve the target, an alternative strategy that matches each potential region should be formulated. The alternative strategies must be elaborated in terms of zoning strategy, because the condition of each regency/city is different, especially the Babel contour area which is an archipelago. Very few studies have been carried out on the development of beef cattle farming in the Babel region, because Babel is indeed known as a tin mining area, not an agricultural and livestock area. Research that had been conducted was about the potential of Belitung district as a beef cattle development area (Erbowo, 2013). The results of the research only explained the components, as well as the strategies needed in the development of beef cattle area in Belitung Regency. So far, there is no research and comprehensive analysis that had been conducted to determine the strategy to support the development of beef cattle in Babel. Therefore, this research was aimed at developing an alternative strategy based on zoning for beef cattle according to the potency analysis of each region in Bangka Belitung Province. The findings of this research were expected to provide a guide for local governments in developing the most appropriate program for beef cattle as an important sub-sector for regional development in Kepulauan Bangka Belitung Province.

Materials and Methods

This research used a survey method which was executed by using 2 (two) stages. The first stage was to do an analysis on region potency mapping. The second stage was to create a formula for an alternative strategy.

The region potency analysis and mapping

This research was based on secondary data to analyze and map the potential development of beef cattle farming in each regency/city. The secondary data were gathered from the Ministry of Agriculture Republic of Indonesia, Central Bureau of Statistics (BPS), and the local Agency that was in charge in the cattle farming function in the research area. The data were gathered from the data of physical resources, human resources, social resources, and also from the supporting infrastructures which were taken from the Ministry of Agriculture Republic Indonesia, Central Bureau of Statistics (BPS), and the local Agency in the research area.

Carrying capacity was calculated based on the production of dry matter forage towards the minimum feed requirements of cattle (1 AU) in one year (Sulfiar *et al.,* 2020). The animal unit (AU) was a unit for the ruminant cattle population multiplied by the conversion factor. The conversion factor for beef cattle was 0,7 (Saputra *et al.,* 2016). Dry matter forage production was the amount of natural forage potential, potential agricultural waste, and potential forage from palm plantation, using equations of Yuniar *et al.* (2016), Suhaema *et al.* (2014), and Rizali *et al*. (2018). The minimum cattle feed requirements was estimated by using equation of Rizali *et al*. (2018).

Natural forage potential (ton) = {(Ga x 2.875) + (Fa x 0.6) + (Cpa x 10) + (Cfa x 0.5) + (Cla x 5)} x 0.5 where Ga is garden area, Fa is forest area, Cpa is coconut plant area, Cfa is coffee plant area, Cla is clove plant area. The numbers in the formula are assumed to be natural forage potential produced per hectare of the land area.

Potential of agricultural waste (ton) = {(wr x 0.4) + (fr x 3 x 0.4) + (cn x 3 x 0.5) + (sb x 3 x 0.55) + (pt x 2 x 0.55) + (sp x 0.25/6) + (cs x 0.25/4)} x 0.65 where wr is wetland rice, fr is field rice, cn is corn, sb is soybean, pt is peanuts, sp is sweet potatoes, cs is cassava. The numbers in the formula are the assumptions about the potential waste produced from the production of each type of plant food.

Potential of palm plantation forage = 23 x 0,007 x 312 x 36% = 18 ton dry matter/yearwhere 23 is the amount of midrib; 0,007 is theweight of each midrib; 312 is the amount of working days; 36% is the dry matter of midrib. The numbers in the formula are the estimation about the potential dry matter produced from the palm plantation.

Minimum cattle feed requirements (R) = 3% x 200 kg x 365 = 2,190 ton DDM/year/AU. R is minimum cattle feed requirements (1 AU) in tons of digestible dry matter for 1 year, 3% is the

minimum requirement for the number of forage rations (dry matter) on livestock weight, 200 kg is the live weight of 1 AU of beef cattle, 365 is the number of days in 1 year.

The population pressure value was calculated by using the formula of *Otto Soemarwoto Model III* (Herlindawati *et al.,* 2018) as follows:

$$TKt = (1 - \alpha\, t).zt.\frac{ft.P0(1+r)^t}{Lt}$$

From the formula above, *TK* is the population pressure towards the agricultural land, *t* is the calculation of time, *z* is the land area that supports the life of a farmer at a desired level of living, i.e 0,78 ha/person (Nazam *et al*., 2011), *f* is the farmer percentage of the population. *Po* is the number of population in people reference time (people), *r* is the level average of annual population growth rate, and *L* is the extensive farming land which is available in the related area, α is the income fraction of non-farming (0.35). The classification of population pressure value of < 1 means that it had not experienced the population pressure in this particular area. The value of >1 means that the particular area had experienced population pressure that exceeds the land capacity (critical level).

The potency map of the area was analyzed by using the potential index analysis method by Ahmad and Sugiharto (2018), in which the potential index was determined by weighting each indicator and its variable based on the priority as determined by Expert Judgement towards 5 (five) respondents from the Agricultural Agency of Kepulauan Bangka Belitung Province who was considered well-informed about the condition of beef cattle farming in Babel. Before determining the potential index process was started, the equalization of each variable and indicator was conducted by using measurement scale application between 0 (0%) to 1 (100%).

The indexing measurement was conducted by using the formula:

Xin index variable:

$$\frac{\text{The Variable of Xi District n-Minimum Value Xi All Districts}}{\text{Maximum Value Xi All Districts-Minimum Value of Xi All Districts}}$$

Since the indicator of population pressurehas a negative correlation, the reverse calculation was applied to be: (1-*Xin*). The result of the calculation shows the highest value and lowest value that can be used as the basic standard to determine 4 (four) potential criteria ([most potential, potential, less potential, and low potential) for the index in each variable and indicator.

Alternative strategy formulation

This research used primary data, secondary data, and previous results of the study. This research was aimed at formulating an alternative strategy that includes zoning, priority, and strategy development plan.

Purposive sampling was used to collect data from 14 cattle farmers, 5 cattle sellers, 5 Agriculture agency officers, and 18 officials who are in charge of the animal production in the regency/city. The primary data were taken from the in-depth interview, focus group discussion (FGD), and questionnaire. The primary data were collected from the internal factors, consisted of the strengths and weaknesses, also the external factors from the opportunity and threat towards the development of beef cattle farming. The qualitative and quantitative data were analyzed by using the SWOT method based on Rangkuti (2016).

Results and Discussion

Region potential mapping

The result of Expert Judgement in Table 1 showed the illustration of urgency level indicator and its variable in developing the beef cattle in Babel. The physical resources had the highest urgency level of 32.7%, and then human resources of 29%, social resources of 19.4%, and infrastructure of 19%.

Table 1. Indicator percentage and potential variable

Variable	* Weight (%)	Indicator	**Weight (%)
Physical Resources	32.7	Carrying Capacity	11.8
		Rainfall	6.2
		Population Pressure	7.4
		Temporarily unused land	7.2
Human Resources	29	Total Farmers	3.5
		Total farmer in the productive age	3.6
		Education	3.4
		Internet use	3.4
		Agricultural technology use	3.4
		Ownership of land	2.9
		Number of medic/paramedics veterinary	4.9
		amount of feed and breed supervisor	3.8
Social Resources	19.4	Total farmers group	4.8
		Total farmers group up to beginner class) (group)	4.3
		Total cattle farmers group	6.7
		Total agribusiness cooperative/unit	3.6
Infrastructure	19	Animal health center	6.7
		Artificial insemination center	5.1
		Slaughterhouse	2.5
		Veterinary laboratory	4.7

*The result of *expert judgement* with scale 0-100% from variable total

*The result of *expert judgement* with a scale of 0-100% from the percentage of each variable.

Table 2. Region potency map

Characteristic of potency	Regency / City							
	Bangka	Belitung	Bangka Barat	Bangka Tengah	Bangka Selatan	Belitung Timur	Pangkal pinang	Babel Prov.
Physical resources								
Carrying capacity (AU)	119,567.8	33,281.8	102.445	49.602,7	96.865,5	42.488,3	1.207,2	445.458,2
Rain fall (mm/year)	2,073.3	2,650	2.073,3	2.355,3	2.355,3	2.650	2.073,3	2.588,6
Population Pressure	0,9	0.4	0.5	0.6	0.7	0.5	9.6	0.6
Temporarily unused land (ha)	27,544	2,502	15,820	0	5,590	7,309	410	59,175
Potential criteria	Most Potential	Potential	Most Potential	Less Potential	Most Potential	Most Potential	Low Potential	
Human resources								
Total farmers (%)	19	10.6	13.3	15.2	21.4	18.1	2.8	14.3
Total farmers in productive age (%)	53.4	52	52.5	5.6	58.2	53.6	44	53.9
Total farmers undergraduate of highschool) (%)	22.7	15.4	19.5	17.2	14.9	18.7	26.2	18.9
Internet use by farmers) (%)	21.5	20.5	16.4	16.6	18.5	26	23.3	19.9
Ownership of land up to 0,5 Ha (%)	74.4	54.1	79.7	68.4	79.8	56.8	28	71
Agricultural technology use (%)	39.9	15.7	7.9	17.1	12.7	25	14.5	19
Amount of medic/paramedic veterinary (persons)	4	3	4	5	6	2	4	36
Amount of feed and breed supervisor) (persons)	3	3	1	3	5	1	3	31
Potential criteria	Most Potential	Potential	Potential	Potential	Most Potential	Potential	Potential	
Social resources								
Total farmers group	1,026	351	1,210	1,044	959	445	96	5,131
Total farmers group up to beginner class (group)	18.9	10.8	5.6	27.2	5.3	0.9	20.8	12.8
Total cattle farmers group (group)	6.9	9.1	2.8	7.5	1	4.5	11.5	5
Total agribusiness cooperative (unit)	17	2	3	4	3	0	0	29
Potential criteria	Most Potential	Potential	Potential	Most Potential	Less Potential	Less Potential	Potential	
Infrastructure								
Animal health center (unit)	1	0	0	0	0	0	2	3
Artificial insemination center (unit)	0	1	0	1	0	1	1	4
Slaughter house (unit)	1	1	.0	1	0	1	1	5
Veterinary laboratory (unit)	0	0	0	0	0	0	2	2
Potential criteria	Potential	Potential	Low Potential	Potential	Low Potential	Potential	Most Potential	

The analysis result in Table 2 showed the potential index in each level. The condition of different resources produced the various potential index in each district/city. The highest index of physical resources with the "most potential" criteria is Bangka, followed by Bangka Barat, Belitung Timur, and Bangka Selatan. The best index of human resources with “most potential” criteria is Bangka followed by Bangka Selatan. The best index of social resources with criteria “most potential” ois Bangka followed by Bangka Tengah. The best infrastructure potential index with criteria “most potential” is Pangkalpinang.

The potential region of beef cattle development in Babel can be seen in Picture 1. The analysis result showed that the regency/city in the Babel region had the potential to develop beef cattle farming, with index 4 (most potential) for Bangka, and index 3 (potential) for other regencies/cities. The difference of potential value in each region will be considered in the policy making. By using the region potential map, the comparative superiority of a region can be identified, thus it can be utilized in planning and developing the strategy (Badan Koordinasi Penanaman Modal, 2019).

Alternative strategy

The alternative strategy was taken from the SWOT analysis results qualitatively and quantitatively. It can be seen in Table 3 and Table 4. The qualitative SWOT analysis showed that there were 4 [four] types of strategies that could be applied, i.e the strategy of SO (Strength-Opportunity), WO (Weakness-Opportunity), ST (Strength-Threat), and WT (Weakness-Threats). The quantitative SWOT analysis showed that the average power – the average weakness was 2,890 – 3,043 = -0,153 (x) and the average opportunity – the average threat is 3,244 – 2,845 = 0,34 (y). The strategy matched in the development of beef cattle farming is turning around strategy (quadrant 3), i.e W-O (Weakness-Opportunity) strategy is to minimize the weakness in catching the available opportunities. The strategy of beef cattle development in Bangka Belitung based on SWOT analysis can be seen in Picture 2.

The W-O strategy covered the improvement of quality and quantity of Human Resources in the agricultural sector. The condition of beef cattle production system in Babel, which is currently dominated by smallholders, requires

Table 3. Matrix of SWOT development of Beef Cattle Farm

	Strength (*Strength*-S)	**Weakness** (*Weakness*-W)
	1. Natural grass potency 2. Cropland potency 3. Palm oil plantation land potency 4. Ownership of land by Farmer 5. Number of farmers (%) 6. Total farmers in the productive age 7. Internet use by farmers	1. Farmer's level education 2. Technology use by farmers 3. Cattle farm as side job and cattle farm as savings 4. Equity and money institutions reach 5. Cattle distribution location 6. Expertise limitation (number and access) 7. Farmer experience 8. Supporting Facility and Infrastructure 9. Product Diversification
Opportunity (*Opportunity*-O) 1. The demand of meat and cattle supply 2. Untapped land availability 3. Supply chain performance efficiency 4. PDRB of agriculture sector always increases 5. No competition with big companies 6. The need for compost in the region is high 7. Climate conditions and geographics which are supported 8. Support from government	**Strategy S-O** 1. The utilization of region potency optimally (S1, S2, S3, S4, S5, S6, O1, O2, O3, O4, O6, O7, O8) 2. Market distribution network development (S5, S6, S7, O1, O3, O5, O8)	**Strategy W-O** 1. The quantitative and qualitative improvement of human resources in the agriculture sector (W1, W2, W3, W4, W7, O1, O2, O3, O5, O6, O7, O8) 2. Increase the number and quality of supporting infrastructure and facilities (W2, W8, W9, O1, O2, O3, O4, O5, O6, O7,O8) 3. Access Facilitation of equity and business relationship through synergy between cattle stakeholders (W4, W6, W8, W9, O1, O2, O3, O4, O5, O6, O7,O8)
Threat (*Threat*-T) 1. Land conversion for tin mining 2. Cattle disease outbreak 3. Female productive slaughter 4. Soil pH condition is too acid 5. The number of regional policies related to beef cattle which is very minimum 6. Cattle theft in some areas 7. Natural disaster (flood)	**S-T Strategy** 1. The development of integrated beef cattle in former mining land (S1, S2, S3, S4, S5, S6, S7, T1, T4, T5, T7) 2. Mentoring and supervising in cattle farmer and *peternak rakyat* (S4, S5, S6, S7 T2, T3, T6)	**W-T Strategy** 1. Improve the agriculture and cattle sector investment (W4, W5, W6, W8, W9, T1, T2, T3, T5, T6) 2. Mining, cattle, and agriculture program evaluation (W1, W2, W3, W7, T1, T2, T3, T6) 3. Formulate the policy of mining land reclamation with the beef cattle land utilization as the basic (W5, W9, T1, T4, T7)

assistance through empowering human resources. Smallholder farmers often have limited access to the inputs, information and services they require to grow a better future. They need to be continuously empowered in terms of input technologies, financial support, information, and markets (Agus and Widi, 2018). Besides that, policies that need to be carried out to attract young people into the agricultural sector are intensive policies for young farmers (Arvianti et al., 2019).

The next strategy was to increase the number and quality of supporting facilities and infrastructure. Livestock technology, as part of supporting facilities and infrastructure, had a huge impact on livestock development. Technologies such as Artificial Insemination (IB) and Embryo Transfer (TE) have a role in support animal husbandry (Rusdiana and Talib, 2019), so the existence of facilities such as animal health center and artificial insemination post is very important.

The next strategy is to facilitate the equity and business relationship. The government must be able to open up and make equity access, as well as commercial relationships and partnerships to be more accessible and simple. Concrete efforts will be needed by government, universities, research centers, extension services, and producers themselves to overcome constraints. Commercialization should be increased in all sectors of the livestock industry and general population (Merkel, 2019). The example of equity access can be implemented through the Program of *Kredit Usaha Rakyat (KUR)* and Corporate Social Responsibility (CSR) which weredirected toward the development of beef cattle for society in Indonesia.

Zoning and strategy priority

The zoning and strategy priority is arranged based on the SWOT analysis result and the documents of spatial lay out and territory planning (*Rencana Tata Ruang Wilayah*/ RTRW). According to Rustiadi *et al.* (2011), the RTRW document has a role in spatial setup, hence it contributes significantly to the regional development program. This analysis identified six zonings and development priorities. These six

Table 4. Internal and External Factors of Beef Cattle Development

	Strength	***Weight**	****Rating**	*****Score**
	Support from government	0.164	3.65	0.598
	Natural grass potency	0.148	3.26	0.482
	Cropland potency	0.114	2.78	0.316
	Palm oil plantation land potency	0.148	2.91	0.430
	Ownership of land by Farmer	0.116	2.74	0.317
	Number of farmer (%)	0.114	2.78	0.316
	Total farmers in the productive age	0.116	2.83	0.328
	Internet use by farmers	0.082	2.78	0.228
	Total	1		3,015
Internal Factors Strategy	**Weakness**	***Weight**	****Rating**	*****Score**
	Farmer's low-level education	0.113	3.04	0.342
	Farming technology use which is low	0.116	3.30	0.384
	Cattle farming becomes a side job and saving	0.122	3.00	0.365
	Equity limitation and money access	0.109	3.04	0.331
	Cattle distribution which has small numbers	0.113	2.78	0.313
	Limitation of experts (number and access)	0.114	3.00	0.343
	Farmer experience	0.103	3.09	0.319
	Supporting Facility and Infrastructure	0.105	3.09	0.325
	Product Diversification	0.105	3.04	0.320
	Total	1		3,043
	average of strength-weakness = 2,890 – 3,043 = -0,153 (x)			
	Opportunity	***Weight**	****Rating**	*****Score**
	Cattle demand and supply are high	0.166	3.52	0.584
	Untapped land availability	0.151	3.30	0.499
	Supply chain performance efficiency	0.136	2.91	0.396
	PDRB of agriculture sector always increases every year	0.129	2.87	0.371
	Low competition with big companies	0.112	2.83	0.317
	The need for compost in the region is high	0.157	3.39	0.534
	Climate conditions and geographics which are supported	0.149	3.26	0.485
External Factor Strategies	Total	1		3,185
	Threat	***Weight**	****Rating**	*****Score**
	Land conversion for tin mining	0.170	3.04	0.517
	Cattle disease outbreak	0.140	2.96	0.414
	Female productive slaughter	0.137	2.87	0.394
	Soil pH is too acid for HPT	0.170	3.13	0.532
	Minim peraturan daerah tentang sapi potong	0.162	2.96	0.478
	Cattle theft	0.111	2.35	0.259
	Natural disaster (flood)	0.111	2.26	0.250
	Total	1		2,845
	average of opportunity - threat = 3,244 – 2,845 = 0,399(y)			

* The weight number of one strategy in one factor divided to total weight number of all strategies in one factor.
** The total rating strategies in one factor is divided to all available ratings.
*** Multiplication result of weight value and rating.

zones can be used comprehensively in the land development instruction to effectively organize the development of the beef cattle farming system.

The first priority zone is in Bangka Tengah regency, i.e. production and processing center of beef cattle, which were focused on increasing cow productivity to meet the demand for livestock products such as feeder cattle, meat, and compost. Because this zone had a high livestock population, it had a better potential of becoming a cattle production center. Other assets of this zone included the availability of farming land, ex-mining land, excellent human resource quality, and a high economic level due to its border to Pangkalpinang city. This zone will be able to solve the issue and problem about sustainable farming system. To achieve the sustainability, extensive production systems should be considered to be shifted to semi-intensive or intensive production systems (Dung *et al*., 2019). Governments, communities (cattleman), and the private sector (investors) must have coordination and cooperation each other so that the development of sustainable beef cattle farms can be achieved (Santoso and Prasetiyono, 2020).

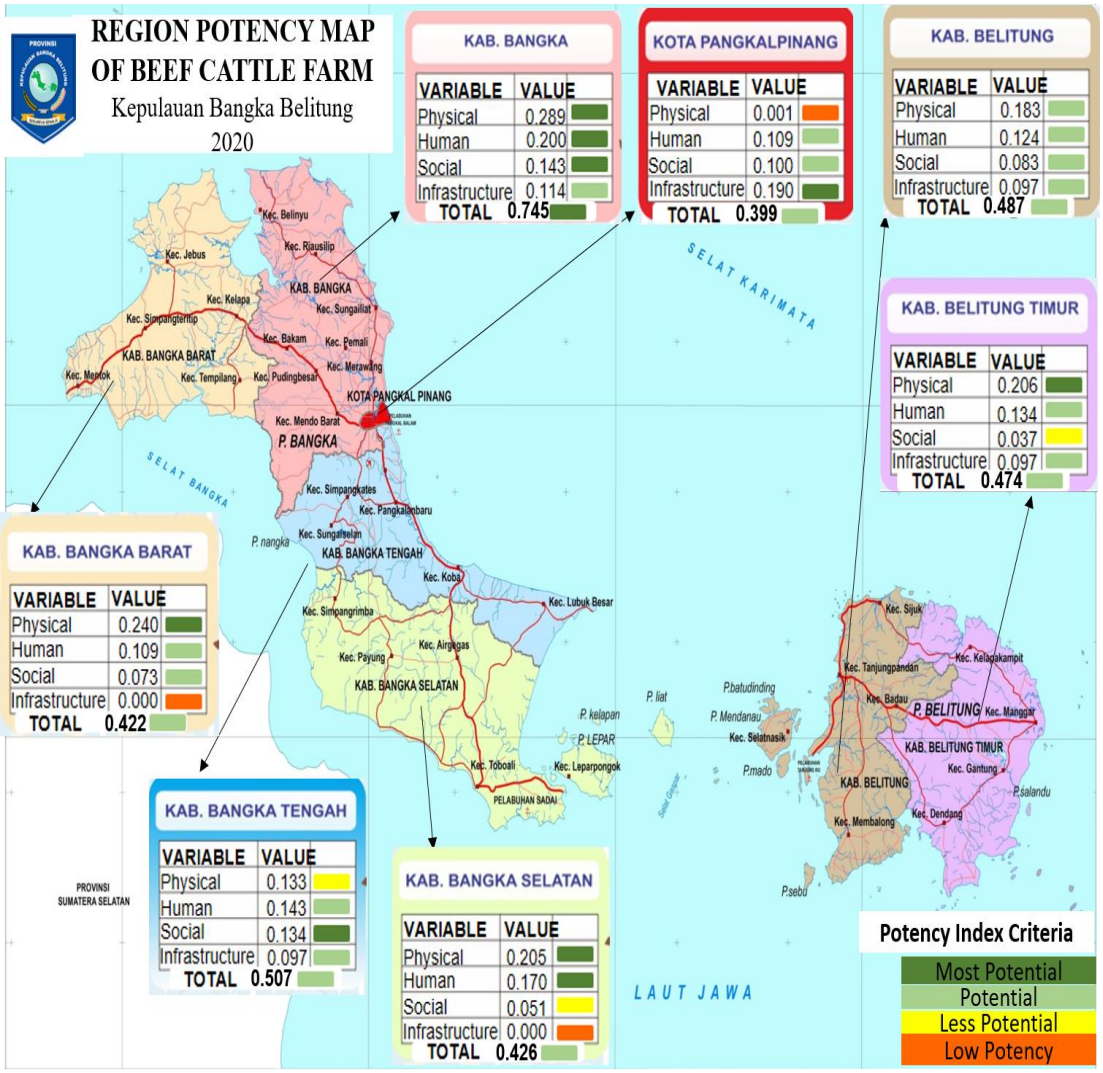
The second priority zone is in the Bangka regency, Bangka Barat, Bangka Tengah, Belitung Timur, and Bangka Selatan, i.e the crop-livestock integration which was focused on the integrated cattle development with the agriculture sector. Integrated farming system is a system that combines two or more fields of agriculture, which was based on the recycling biological concept, and linked of input-output between the mutually commodities which approach of low-external-input utilization, which was done on the land, through the utilization of crop waste, animal manure, fish waste for the purpose of increasing the production and productivity so as to increase farmer income and create farming condition that are environmentally friendly (Mukhlis *et al.,* 2018).

The third priority zone is located in Bangka, Bangka Selatan, Bangka Barat, Bangka Tengah, and Belitung Timur, i.e. cattle farming in ex-tin mining land which was focused on the utilization of the ex-mining land that has gone through the reclamation process and has not seen any mining

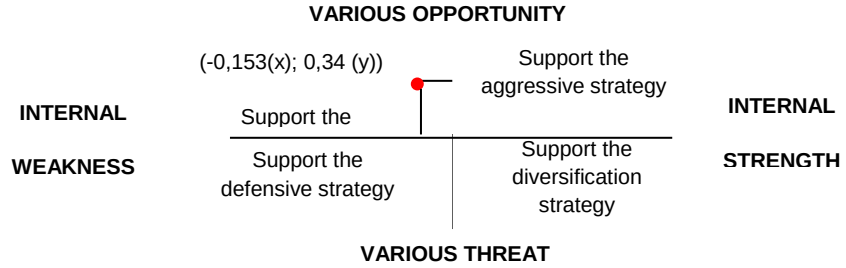
activities in a long time. The implementation of an integrated cattle system in the ex-mining land is a wise choice since it is the majority condition of which the implementation of the beef cattle system took place, related to the close relationship between cattle and plants. The biomass from the main crop commodity can be used as feed and the cattle manure can be used to improve the soil quality, while the underground water is used as the water supply in the agricultural activity (Asmarhansyah, 2017). In addition, this available land will be able to be one of the feed sources since the availability of unused land might be a location where forage grows (Rusdiana et al., 2016).

The fourth priority zone is located in the Bangka Selatan regency, i.e. a feed production facility based on local resources which were focused on producing cattle feed. The size of the farming area in this location was considered as the highest category). Biomass and byproducts of agriculture plant processing and plantation crop processing have a significant potential to be used as feed sources, but the nutritional value in the available raw material must be improved with some treatments including physical, chemical and biological methods (Yanti and Yayota, 2017).

The fifth priority zone was in Pangkalpinang, i.e modern cattle farming with technology-based which was focused on the technology utilization based on the availability of human resources, was supported by the facilities and infrastructure to improve the cattle productivity and the efficiency level of land use. However, the livestock technology was the key to success to the beef production and distribution (Basyar, 2021). The farmer should be able to utilize the technology to increase the efficiency of resources utilization. Applied and simple technologies that were useful and easy to adopt by the farmers are a priority. Feed technologies, by-product technologies including compost management, and parasite control were among the simpler technologies required by farmers to improve cattle performance (Agus and Widi, 2018).



Picture 1. Region Potency Map of Beef Cattle Farm in Bangka Belitung Province.



Picture 2. SWOT analysis of beef cattle development strategy.

The sixth priority zone is located in Pangkalpinang and Belitung i.e the Cattle with agrotourism based, which was focused on the development of agrotourism based on livestock and agriculture sectors. The exploited ground and coastline shore, which can be developed as a beef cattle farming area, have the greatest natural potential in this zone. Pangkalpinang, the most populous city, and the Belitung district which has referred to as the tourism center in Babel have a big potential as the Regional Native Income *(Pendapatan Asli Daerah)* for Babel through the development of beef cattle based on agrotourism. Meanwhile, the development of beef cattle towards agrotourism can give the utilization either for the animal farmer and the tourism. On the other hand, by performing the cow farming activity directly for the tourist, the cattle farmer can improve their income from agrotourism and provide farming experience to the tourist. Raising cattle can also provide an attraction for tourists by using the offered services, and provided a source of income from selling the cattle (Jeczmyk *et al.,* 2021).

The development plans

The development plan of beef cattle farming in Bangka Belitung Province has been formulated in 10 years (2021 – 2030) based on the development priority zone. It is started with the development of a production center and cattle product processing in the first priority zone, because it is matched with the main target of local government to reach meat self-sufficienc*y,* and also make Babel as one of the cattle centers in Sumatera. The first priority zone will be attempted as the cattle barn in the province so it can produce cattle feeder and beef cattle for other regions. To achieve the goal, it is necessary to use field farm potential and available ex-mining land in the second and third priority zones through cattle farmer integration in the area. If farming land and ex-mining land were used optimally, the fourth priority zone, a feed production center based on local resources, can be realized. By having the feed availability quantitatively and qualitatively, the fifth priority zone as the cattle farming with technology-based can be realized. The last is the sixth zone, which can improve the value-added in beef cattle. It is not only as a feed product supplier for the cattle, but it also can boost society's economy through the integration of cattle farming and agrotourism based. It will have a beneficial influence for the cattle farmer and agricultural farmer, al well as for another society.

The participation of all stakeholders in implementing all strategies and solutions determines the level of success of all agriculture development plans in all zones. There must be a synergy between central government, local government, and also all social elements so this development plan can be established. Effective communication and possible outcomes is important, especially for policies with heterogeneous impacts, multiple outcomes, long timescales and large uncertainties. If this complexity does not handled properly, will hinder the potential livestock sustainable development and make it difficult to achieve (Mehrabi *et al*., 2020).

Conclusions

Bangka regency has the highest potential index with the most potential criteria, while the Pangkalpinang has the lowest total index, though it has “potential” criteria. The alternative strategy in the beef cattle development plan consisted of 6 [six] zones and strategic priority. The first priority zone was the production center and cattle product processing in Bangka Tengah. The second priority zone was the cattle farmer integration, in Bangka, Bangka Barat, and Belitung Timur. The third priority zone was the beef cattle farming in ex-mining land in Bangka, Bangka Barat, and Belitung Timur. The fourth priority zone was the feed production center with local based at Bangka Selatan. The fifth priority zone was the modern beef cattle with technology-based in Pangkalpinang. The sixth priority zone was the cattle with agrotourism based in Pangkalpinang and Belitung.

References

Agus, A. and T. S. M. Widi. 2018. Current situation and future prospects for beef cattle production in Indonesia - A Review. Asian-Aust. J. Anim. Sci. 31: 976–83. https://doi.org/10.5713/ajas.18.0233.

Ahmad, A. A., and M. Sugiharto. 2018. Peta pengembangan sapi potong di Kabupaten Banjarnegara. Eko-Regional 9: 106–115. https://doi.org/10.20884/1.erjpe.

2014.9.1.483.

Arvianti, E., Y. Masyhuri, L. R. Waluyati, and D. H. Darwanto. 2019. Gambaran krisis petani muda di Indonesia. Jurnal Sosial Ekonomi dan Kebijakan Pertanian 8: 168–80. https://doi.org/10.21107/agriekonomika.v8i2.5429.g3878.

Asmarhansyah. 2017. Inovasi teknologi untuk peningkatan produktivitas lahan bekas tambang timah. Jurnal Sumberdaya Lahan 11: 91–106. https://doi.org/10.2018/jsdl.v11i2.7305.

Badan Koordinasi Penanaman Modal. 2019. Strategi Pengembangan Potensi Daerah Dalam Peningkatan Iklim Investasi. http://dpmptsp.riau.go.id/media/file/Strategi-Pengembangan-Potensi-Daerah-Dalam-Peningkatan-Iklim-Investasi.pdf (January 15, 2021).

Basyar, B. 2021. Beef cattle farm development policies to overcome beef distribution problem in Indonesia: A Literature Review. American J. Anim. Vet. Sci. 16: 71–76. https://doi.org/10.3844/ajavsp.2021.71.76.

BPS Provinsi Kepulauan Bangka Belitung. 2020. Provinsi Kepulauan Bangka Belitung dalam Angka 2020. Badan Pusat Statistik, Kepulauan Bangka Belitung.

Dung, D. V., H. Roubik, L. D. Ngoan, L. D. Phung, and N. X. Ba. 2019. Characterization of smallholder beef cattle production system in Central Vietnam -Revealing Performance, Trends, Constraints, and Future Development. Trop. Anim. Sci. J. 42: 253–60. https://doi.org/10.5398/tasj.2019.42.3.253.

Erbowo, B., L. Cyrilla, and R. Priyanto. 2013. Potensi Kabupaten Belitung sebagai kawasan pengembangan sapi potong. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 1: 1–7. https://journal.ipb.ac.id/index.php/ipthp/article/view/15472.

Geiker, N. R. W., H. C. Bertram, H. Mejborn, L. O. Dragsted, L. Kristensen, J. R. Carrascal, S. Bügel, and , A. Astrup. 2021. Meat and Human Health-Current Knowledge and Research Gaps. Journal Foods 10: 1–17. https://doi.org/10.3390/foods10071556.

Herlindawati, A., L. Trimo, and T. I. Noor. 2018. Analisis tekanan penduduk terhadap petani padi sawah (Suatu Kasus di Kecamatan Cilamaya Kulon, Kabupaten Karawang, Jawa Barat). Jurnal Pemikiran Masyarakat Ilmiah Berwawasan Agribisnis 4: 12–24. http://dx.doi.org/10.25157/ma.v4i1.715.

Jeczmyk, A., J. Uglis, and R. Steppa, 2021. Can Animals Be the Key to the Development of Tourism: A Case Study of Livestock in Agritourism. Journal Animals 11: 1–15. https://doi.org/10.3390/ani11082357

Mehrabi, Z., M. Gill, M. V. Wijk, M. Herrero, and N. Ramankutty. 2020. Livestock Policy for Sustainable Development. Journal Nature Food 1: 160–65. https://doi.org/10.1038/s43016-020-0042-9

Merkel, R. 2019. Smallholder Livestock Commercialization. Wartazoa 29: 43–50. https://doi.org/10.14334/wartazoa.v29i1.1952

Mukhlis, M. Noer, and M. Nofialdi. 2018. The integrated farming system of crop and livestock : A Review of Rice and International Journal of Sciences. Basic and Applied Research (IJSBAR) 42: 68–82. https: //www.gssrr.org/ index.php/JournalOfBasicAndApplied/article/view/9477

Nazam, M., S. Sabiham B. Pramudya and Widiatmaka. 2011. Penetapan luas lahan optimum usahatani padi sawah mendukung kemandirian pangan berkelanjutan di Nusa Tenggara Barat. Jurnal Agro Ekonomi 29: 113–41. http://dx.doi.org/10.21082/jae.v29n2.2011.113-145.

Priyanto, R., A. M. Fuah, E. L. Aditia, M. Baihaqi, and M. Ismail. 2015. Peningkatan produksi dan kualitas daging sapi lokal melalui penggemukkan berbasis serealia pada taraf energi yang berbeda. Jurnal Ilmu Pertanian Indonesia 20: 108–14. https://doi.org/10.18343/jipi.20.2.108.

Rangkuti, F. 2016. Teknik Membedah Kasus Bisnis Analisis SWOT Cara Perhitungan Bobot, Rating, dan OCAI. PT. Gramedia Pustaka Utama, Jakarta.

Rizali, A., Fachrianto, M. H. Ansari, and A. Wahdi. 2018. Pemanfaatan limbah pelepah dan daun kelapa sawit melalui fermentasi *Trichoderma* Sp. sebagai pakan sapi potong. Enviro Scienteae 14: 1–7. http://dx.doi.org/10.20527/es.v14i1.4886.

Rusdiana, S., U. Adiat, and R. Hutasoit. 2016. Analisis ekonomi usaha ternak sapi potong berbasis agroekosistem di Indonesia. Agriekonomika 5: 137–49. https://doi.org/10.21107/agriekonomika.v5i2.1794.

Rusdiana, S. and C. Talib. 2019. Kebijakan pemerintah mendukung peningkatan usaha sapi potong di peternak. SOCA: Jurnal Sosial Ekonomi Pertanian 13: 380–95. https://doi.org/https://doi.org/10.24843/SOCA.2019.v13.i03.p08.

Rustiadi, E., S. Saefulhakim, and D. R. Panuju. 2011. Perencanaan Dan Pengembangan Wilayah. Cerpen Press, Jakarta.

Santoso, B. and B. W. H. E. Prasetiyono. 2020. The regional analysis of beef cattle farm development in Semarang Regency. Trop. Anim. Sci. J. 43: 86–94. https://doi.org/10.5398/tasj.2020.43.1.86.

Saputra, J., I., Liman, and Y. Widodo. 2016. Analisis potensi pengembangan peternakan sapi potong di Kabupaten Pesawaran. Jurnal Ilmiah Peternakan Terpadu 4: 115–23. http://dx.doi.org/10.23960/jipt.v4i2.p%25p.

Suhaema, E., Widiatmaka, and B. Tjahjono. 2014. The regional development of beef cattle based on physical and forage land

suitability in Cianjur Regency. Tanah Lingkungan 16: 53–60. https://doi.org/ 10.29244/jitl.16.2.53-60

Sulfiar, A., E., T., B. A. Atmoko, B. Guntoro, and I. G. S. Budisatria. 2020. Study of pasture productivity for semi-intensive cattle system during dry season in South Konawe Regency, Southeast Sulawesi. Buletin Peternakan 44: 85-91. https://doi.org/10.21059/buletinpeternak.v44i3.52741

Tadete, M., A., F. H. Elly, L. S. Kalangi, and R. Hadju. 2016. Pengaruh pendapatan masyarakat terhadap konsumsi daging sapi di Desa Kotabunan Kecamatan Kotabunan Kabupaten Bolaang Mongondow Timur. Jurnal Zootek 36: 363–371. https://doi.org/10.35792/zot.36.2.2016.12538.

Yanti, Y. and M. Yayota, 2017. Agricultural by-products as feed for ruminants in tropical area: nutritive value and mitigating methane emission. Agricultural Science 5: 65–76. http://dx.doi.org/ 10.7831/ras.5.65

Yuniar, P., S., A. M. Fuah, and Widiatmaka. 2016. Carrying capacity and priority region for development of beef cattle production in South Tangerang. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 4: 64–268. https://doi. org/10.29244/jipthp.4.1.264-268

Zakiah, A. Saleh, and K. Matindas. 2017. Gaya kepemimpinan dan perilaku komunikasi gppt dengan kapasitas kelembagaan sekolah peternakan rakyat di Kabupaten Muara Enim. Jurnal Penyuluhan 13: 133–42. https://doi. org/10.25015/ penyuluhan.v13i2.14977