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PREFACE

In this edition, volume 22 no. 1 March 2017, we proudly present articles from animal and veterinary sciences including feed and nutritive technology, microbiology, physiology, and genetic study on tripanosoma evansi. The articles published in this edition are: "Performance of EPMp Broiler Ducks Feed with Various Levels of Dietary Lysine up to 10 Weeks of Age"; "Path Analysis of Exogenous Variables against Technology Adoption Levels of Dairy Cattle in West Sumatera"; "Production Performance of HyCole, New Zealand White Rabbits and Its Reciprocal"; "Weight Estimation of Empty Carcass and Carcass Cuts of Female SenSi-1 Agrinak Chicken"; "Effectiveness of DMSO Concentration on Recovery Rate and Viability of Primordial Germ Cell of Gaok Chicken" and "Genetic Variability of ESAG 6/7 Gene Isolat *Trypanosoma evansi".*

We extent high appreciation to all peer reviewers who make this journal accademicaly high value. Hopefully these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from other authors to contribute their papers to Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor;

Bogor, March 2017

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LIST OF CONTENT

Performance of EPMp Broiler Ducks Feed with Various Levels of Dietary Lysine up to 10 Weeks of Age

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ABSTRAK

Purba M, Sinurat AP. 2017. Performa itik pedaging EPMp yang diberi pakan dengan berbagai kadar asam amino lisin sampai umur 10 minggu. JITV 22(1): 1-8. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1606

Penentuan kadar asam amino lisin yang optimum dalam ransum, merupakan upaya penting untuk menghindari pertumbuhan yang buruk pada ternak itik. Tujuan penelitian ini adalah untuk mengetahui kebutuhan asam amino lisin optimal dalam ransum untuk itik pedaging EPMp yang dipelihara sampai dengan umur 10 minggu. Penelitian dilakukan dengan menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan ransum dan 4 ulangan. Setiap ulangan terdiri dari 10 ekor itik EPMp unsexed. Perlakuan ransum terdiri dari kadar total lisin: 0,60% (T1); 0,70% (T2); 0,80% (T3) dan 0,90% (T4). Peubah yang diamati mencakup: konsumsi pakan, pertambahan bobot badan, feed conversion ratio (FCR), persentase bobot karkas dan potongan karkas. Hasil penelitian menunjukkan bahwa rataan pertambahan bobot badan dan FCR itik nyata (P<0,05) dipengaruhi oleh kadar total lisin dalam ransum, akan tetapi konsumsi pakan dan persentase bobot karkas maupun potongan karkas tidak nyata (P>0,05) dipengaruhi kadar asam amino lisin dalam ransum. Rataan pertambahan bobot badan itik dengan pemberian ransum T4 (0,90% lisin) nyata (P<0,05) lebih tinggi dibandingkan dengan T1 (0,60% lisin) akan tetapi tidak nyata (P>0,05) untuk ransum T2 dan T3. Rataan FCR itik dengan pemberian ransum T3 dan T4 nyata (P<0,05) lebih baik dibandingkan dengan perlakuan T1. Disimpulkan bahwa total lisin yang optimal untuk menghasilkan pertambahan bobot badan itik EPMp tertinggi sampai dengan umur 10 minggu adalah 0,70%, sedangkan untuk FCR ditunjukkan oleh itik pada ransum berkadar asam amino lisin 0,80% dan 0,90%.

Kata Kunci: Itik Pedaging EPMp, Asam Amino Lisin

ABSTRACT

Purba M, Sinurat AP. 2017. Performance of EPMp broiler ducks feed with various levels of dietary lysine up to 10 weeks of age. JITV 22(1): 1-8. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1606

Determining the optimum level of lysine in the ration will be a significant effort to avoid poor growth in duck husbandry. The purpose of this study was to determine the optimum dietary lysine requirement for EPMp broiler ducks, raised up to 10 weeks of age. The study was designed in a completely randomized design (CRD) with 4 dietary treatments, and 4 replicates. Each replicate consisted of 10 ducks. The 4 treatments were diets, containing lysine: 0.60% (T1); 0.70% (T2); 0.80% (T3) and 0.90% (T4). Variables measured included: feed consumption, body weight gain, feed conversion ratio (FCR), carcass and carcass cuts percentages. The results showed that the performance of 10 weeks of age EPMp broiler duck was significantly affected by the level of dietary lysine (P<0.05). The average body weight gain and the FCR of the duck were significantly affected (P<0.05) by dietary lysine level, but not for feed consumption and percentage of carcass (P>0.05). The average body weight gain of EPMp duck with T4 treatment (0.90% lysine) was significantly higher than that T1 (0.60% lysine) but did not significantly difference (P>0.05) with T2 and T3. The average FCR of EPMp duck under T3 and T4 were significantly (P<0.05) higher than that of EPMp duck under T1. It was concluded that the optimum dietary lysine to produce maximum body weight gain of EPMp duck raised up to 10 weeks of age was 0.70%, while for minimum FCR were at the level of 0.80% and 0.90%.

Key Words: EPMp Broiler Duck, Dietary Lysine

INTRODUCTION

EPMp duck is one of the local broiler ducks that has been being developed at the Indonesian Research Institute of Animal Production at Ciawi, Bogor. The nutrient requirements of the local broiler duck is very important to understand in order to formulate optimum diets that can effectively and efficiently support high production. The nutritional needs of birds can be affected by several factors including: the type, age, anatomical structures and physiological status of the animal (Helmbrecht 2012). Lysine is the second limiting amino acid in poultry diets and the level of this amino acid is usually critically considered in diet formulation for ducks, but there is little research on the lysine requirements of early starting and growing Pekin ducklings. Lysine is an essential amino acid as it cannot be synthesized in the body of the poultry. Insufficient or

excessive dietary lysine can result in poor performance of the animal, thus increasing the cost of feed for livestock production (Dozier et al. 2010).

Nutritional requirements, especially lysine for both laying and broiler meat type of chicken has been reported quite a lot (Bouvarel et al. 2004; Kamran et al. 2008; Dozier et al. 2010), but relatively little for ducks (Adeola 2006; Cho et al. 2014). The nutritional requirements of the duck generally refer to the NRC recommendations (NRC 1994; Chen 1996) including the nutritional requirements of local ducks as well as layer and broiler. Lysine requirement for Pekin duck at growing-finishing period was 0.65% and a total energy was 3000 kcal ME/kg (NRC 1994). The nutritional value of protein and energy in the feed serves as a building block and regulators in animal body to achieve maximum production (Kamran et al. 2008; Pesti 2009). Adeola (2006) reported that lysine, methionine and threonine considered as the essential amino acids to help increase growth in commercial ducks. Lysine deficiency in the body could lead to duck slow growth, whereas when the energy content of the diet was not enough, the feed consumption was then high. In addition to protein level, appropriate energy level also played an important role in determining the cost of feed (Adeola 2006). Metabolizable energy requirements for Pekin duck of growing and finishing period were 3050 and 3075 kcal/kg (Leeson et al. 1996).

Level of lysine in the feed was varied for local broiler ducks and FCR generated was also relatively high. Ketaren et al. (2011) reported the need of lysine to PMp duck (crossed of Pekin duck male with white Mojosari female) finisher period was 0.90%. The average FCR generated during the period finisher was 4.33; while Purba et al. (2015) showed that the need lysine to produce optimal performance during the starting period for PMp duck was 0.70 to 0.85%. FCR local broiler ducks achieved in previous studies was still relatively high around 4.68 to 5:34 (Purba et al. 2014b), so that the optimal nutritional requirements for broiler ducks still needs to be researched and studied to obtain a better FCR values and the resulting in the best performance.

The requirement of lysine for poultry not only needs for growing and production but also for basic needs (maintenance). Lysine requirement in Pekin ducks of 0-2 weeks of age was 0.90%, while at the age of 2-7 weeks was 0.65% (NRC 1994). Adeola (2006) stated that the requirement of lysine and methionine of Pekin ducks aged one week after hatching was not more than 1.20% (lysine) and 0.60% (methionine). Information needs of the amino acid lysine, especially for the local ducks are still varied. Choo et al. (2014) reported that the optimum level of lysine, SAA (total sulfur amino acids) and threonine to produce maximum growth in local ducks in Korea were 1.20%, 0.98% and 0.93% respectively. Lysine requirement for the most optimal and efficient in EPMP broiler ducks aged 10 weeks were conducted as described in this paper.

MATERIALS AND METHODS

The material used were EPMp broiler ducks obtained from crossing of male Muscovy duck by artificial insemination (AI) with female PMp duck (one of new strain from the IRIAP). The whole process of raising of parent ducks was conducted according to standard operational research of IRIAP. EPMp ducklings were then reared in 16 cages, equipped with feed and drinking water. The ducklings were allocated at random from age of 0 to 10 weeks. The study was satistically analyed using completely randomized design (CRD) with 4 dietary treatments and 4 replications. Each replication consisted of 10 ducks. Dietary treatments consisted of four difference lysine with the same metabolizable energy content. The dietary treatments followed:

T1: Diet containing total lysine of 0.60 %, crude protein 16.26%, energy of 2768 kcal EM/ kg;
T2: Diet containing total lysine of 0.70%, crude protein 16.44%, and energy of 2765 kcal ME/kg.
T3: Diet containing total lysine content of 0.80%, crude protein of 16.68%, and energy of 2758 kcal ME/kg.
T4: Diet containing total lysine of 0.90%, crude protein of 16.16%, and energy of 2769 kcal ME/kg.

The nutrient content of rations was formulated based on nutritional requirement for duck under the recommendations of NRC (1994), and Ketaren (2002). Feed was given twice a day in the morning and afternoon, while drinking water was provided *ad libitum*. Ingredients and the composition of the dietry treatment are presented in Table 1.

Ducks were weighed once a week to collect body weight data as well as consumption and feed efficiency data. At the age of 10 weeks, ducks were weighed and slaughtered for carcass analysis. Slaughtering was practiced following Islamic way by cutting the carotid artery and jugular vein. The dead ducks were dipped into a bucket of hot water with a temperature 60-70°C for 40-50 seconds and then immersed in cold water to keep the carcass skin quality. After soaking the body of the ducks was cleanly plucked. The whole carcass was cut to separate the head, neck and legs. The body cavity was opened by applying incision from the sternum to the cloaca.

Cloaca and viscera or internal organs of were then removed. Liver, gall bladder, gizzard, heart, and intestine were separated. The content of gizzard was removed as well as the bile of ducks was separated from the liver then weighed. Variables measured were: feed intake, body weight gain, feed conversion ratio (FCR), carcass and viscera weight.

Table 1. The composition and nutrient content of the dietary treatments

Ingredients	T1	T2	T3	T4
Rice bran (%)	36.31	36.33	36.40	36.70
Corn (%)	34.35	34.10	33.94	34.00
Soybean meal (%)	7.95	8.20	8.50	7.00
Fish meal (%)	6.30	6.25	6.25	5.50
Commercial Broiler Ration (%)	14.00	13.85	13.25	15.00
Methionine (%)	0.00	0.02	0.19	0.08
Lysine (%)	0.00	0.13	0.25	0.42
Vitamin and mineral premix (%)	0.09	0.07	0.07	0.07
Crude palm oil (%)	0.35	0.40	0.50	0.58
Dicalcium phosphate (%)	0.50	0.50	0.50	0.50
Salt (%)	0.15	0.15	0.15	0.15
Total (%)	100	100	100	100
Calculated nutrient content				
Crude protein, (%)	16.26	16.44	16.68	16.16
Lysine (%)	0.60	0.70	0.80	0.90
Energy (kcal ME[1)]/kg)	2768	2765	2758	2769
Crude fiber (%)	6.15	6.15	6.15	6.18
Calcium (%)	0.93	0.93	0.92	0.88
Phospor (%)	0.78	0.78	0.78	0.77

[1)] ME = Metaboliz able Energy

Data were analyzed by procedure of General Linear Model (GLM) applying Statistical Analysis System (SAS, ver. 6.12, 1997).

RESULTS AND DISCUSSION

Feed consumption

The average feed, protein, and lysine consumption are presented in Table 2. Feeding with various lysine levels did not significantly (P>0.05) affect feed consumption of EPMp broiler ducks during the first 10 weeks of age. The average feed consumption of EPMp broiler duck feed under T3 and T4 treatments seemed to be lower than of them under T1 and T2 treatments, but based on statistical analysis, it was not significantly different (P>0.05). The lowest average protein intake in this study was found in the T4 treatment (1499 g/bird), although it showed the insignificant (P>0.05) highest lysine consumption (83.50 g/bird). The study showed that the increasing total lysine content in the ration up to 0.90%, which caused the slight increase in the amount of lysine consumed and decrease in total feed consumption, indicated the phenomena of decreasing feed consumption by increasing dietary lysine.

It was thought that feed consumption to be related to the response of each individual to take advantage of the nutrients, especially protein content in diet. Alleman et al. (2000) and Pesti (2009) stated that each type of animal had different response to the nutrition, especially protein content and amino acid obtained through diet consumed. The results also showed that the amino acid lysine appeared to have a role to reduce the feed consumption of the duck. The results were consistent with the opinion of Hernandez et al. (2004), Fan et al. (2008), Hidalgo et al. (2004), and Kamran et al. (2008).

Table 2. The average feed, protein and lysine consumption of EPMp broiler ducks fed various levels of dietary lysine up to 10 weeks of age

Treatments	Feed consumption (g/bird)	Consumption of	
		Protein (g/bird)	Lysine (g/bird)
T1 (total dietary lysine content 0.60%)	9472±23.3	1540	56.83
T2 (total dietary lysine content 0.70%)	9498±21.5	1561	66.49
T3 (total dietary lysine content 0.80%)	9237±21.5	1541	73.90
T4 (total dietary lysine content 0.90%)	9278±16.1	1499	83.50

The effect of nutrient content in diets to support good performance depends on the capacity of the duck to change dietary intake to meet changing of calories needs. Feed intake during the starter period will differ from grower and finisher period. In the starter period, duckling is likely to have physical limitations in consuming huge amount of feed. During growing and finishing period, eventually feed consumption will increase according to the increase in physical capacity and stability of the digestive organs.

The results presented in Table 2. were similar to the previous study of Purba et al. (2015). The study provided information that the average lowest of feed consumption was achieved under T4 treatment (0.90% of lysine), with average feed intake of 8037 g/bird. Therefore in terms of efficiency, the feed containing 0.90% of lysine level was most advisable to obtain a lower feed consumption of EPMp broiler ducks at the age of 10 weeks. Increasing dietary lysine content of 0.3% which reduced feed up to 200 g/bird, seemed to be economically advantage in broiler duck husbandry.

Body weight gain

The average body weight gain of EPMp broiler ducks by administration with various total lysine content in the treatment rations are presented in Table 3. Overing of various total lysine levels in the ration had significant ($P<0.05$) effect on the body weight gain of EPMp broiler duck up to 10 weeks of age. The average body weight of EPMP broiler duck under T4 treatment (0.90% total lysine) was significantly higher ($P<0.05$) when compared to the one under T1 treatment, but not significantly different ($P>0.05$) from the one under T2 and T3 treatments.

The highest body weight gain in this study was shown under T4 treatment (2351±22.93 g/bird) while the lowest was found under T1 treatment (2161±20.10 g/bird). The higher body weight gain of EPMp broiler duck under T4 treatment was influenced by the amount of lysine amino acid consumption. When seen in Table 2, the amount of lysine consumption under T4 treatment was higher when compared with other treatments that affected the increase in body weight gain of the ducks up to 10 weeks of age. The results of the study are in line with the results of the other studies. Bons et al. (2002) and Xie et al. (2006) reported that the growth and performance of male Pekin duck was significantly increased in line with the increase of the amino acid lysine in the diet. The administration of lysine of 1.20% in feed weight gain produced increased body weight of local duck in Korea (Choo et al. 2014).

Normal growth in ducks was highly dependent on nutritional content, especially essential amino acids (Kamran et al. 2008). Other researchers also stated that besides proper nutritional diet, genetics, and management had great influence on the performance and carcass quality of Pekin ducks (Adeola 2006; Xie et al. 2014). Normal growth in poultry is not enough only with limited availability of source materials or energy substrate as a result of the synthesis of proteins (amino acids), but also very influential in the groove in the regulation of growth, protein synthesis by their interaction with growth hormone (Dorup 2004). During the period of growth, Zeng et al. (2015) suggested that the level of energy and digestible lysine in Pekin ducks each of 13.75 MJ ME/kg (approximately 3280 kcal ME/kg) and 1.21% digestible lysine. Their lysine and energy level were much higher than the level used in this study. Lysine requirement is also influenced by the type of poultry. Nutritional requirement (lysine) of Muscovy duck or mule duck in Taiwan according to Chen (1996) for finisher period was 0.90% supporting the average weight gain of about 2.77 kg/bird and the feed consumption was 8.86 kg/bird at 10 weeks age. Dozier et al. (2008) reported that lysine requirement to produce weight gain and FCR optimal for broiler finisher period was 0.79 to 0.83%. This study gave that the impression that diet contained 0.70% of lysine with 16.26% of crude protein and 2768 kcal ME/kg was considered to be sufficient to produce maximum weight gain of EPMp broiler duck up to10 weeks of age.

Table 3. The average of weight gain of EPMp duck fed various levels of dietary lysine up to 10 weeks of age

Treatments	Weight gain (g/bird)
T1 (total dietary lysine content of 0.60%)	$2161^{a1)}$±20.10
T2 (total dietary lysine content of 0.70%)	2244^{ab}±19.90
T3 (total dietary lysine content of 0.80%)	2261^{ab}±23.64
T4 (total dietary lysine content of 0.90%)	2351^{b}±22.93

[1)] Values in the same column with the different superscript are significantly difference (P<0.05)

Table 4. The average of FCR of EPMp duck fed various levels of dietary lysine up to 10 weeks of age

Treatments	FCR
T1 (total dietary lysine content of 0.60%)	$4.39^{a1)}$±0.08
T2 (total dietary lysine content of 0.70%)	4.23^{ab}±0.03
T3 (total dietary lysine content of 0.80%)	4.09^{bc}±0.05
T4 (total dietary lysine content of 0.90%)	3.95^{c}±0.07

[1)] Values in the same column with the different superscript are significantly difference (P<0.05)

Feed conversion ratio (FCR)

The average feed conversion ratio (FCR) of EPMp broiler duck up to 10 weeks with feeding treatments containing various levels of dietary lysine was described in Table 4. Provision of various lysine total levels in the treatment ration was significantly (P<0.05) affected the FCR. The average FCR of EPMp ducks under T4 treatment (0.90% total lysine) was significantly different (P<0.05) when compared to T1 and T2 treatments, but not to T3 treatment (P>0.05). There were decline pattern of FCR with increasing dietary lysine (Table 4). The lowest FCR of EPMp broiler duck was found under T4 treatment (3.95±0.07) while the highest was found in T1 treatment (4.39±0.08).

The increase in of feed efficiency seemed to be the effect of reducing feed consumption due to the increase in dietary lysine (Table 2), as it was expected. The results are consistent with the research results of Kamran et al. (2008) who reported that in addition to the role of lysine in the ration not only served to sustain growth, but also reduced feed consumption and lower FCR. Bons et al. (2002) had also noted that the increasing content of digestible lysine decreased FCR of growing Pekin duck. The decrease of FCR due to increasing lysine content in this study also confirmed by other researchers who claimed that application of amino acids in the grower-balanced diet causing low FCR due to the more efficient in forming muscle fibers in the form of meat (Dorup 2004; Fan et al. 2008).

Ketaren (2006) reported that FCR of muscovy duck fed diets contained pollard at level of 30, 40 and 50% respectively were 3.42; 3.39 and 3.47, while feed intake respectively were 6059, 6190 and 6111 g/bird for ducks raised up to 8 weeks of age. The study indicated that the diet contained lysine of 0.80% and 0.90% with 16.16% crude protein and metabolizable energy of 2769 kcal/kg was optimum to support minimum FCR of EPMp duck raised up to 10 weeks of age.

Carcass and carcass cuts

The mean percentage carcass and carcass cuts of EPMp duck under various dietary lysine levels for 10 weeks were presented in Table 5. The average percentage of carcass weight, breast, thigh, back and wings of EPMp ducks were not significantly effected by dietary lysine levels for 10 weeks.

The average carcass weight of EPMp duck ranged from 61.43 to 62.21%. Administration of various levels of dietary lysine did not affect significantly (P>0.05) empty carcass and carcass cuts. Table 5 shows that there was a pattern of increasing percentage of empty carcass of duck in line with the increasing of lysine content in ration. Pesti (2009) reported that the nutritional content of amino acids that were sufficient in accordance with the needs was very useful to encourage the growth of body tissue including the muscle tissue (meat) in poultry. Table 5 shows also that the percentage of the breast, thighs and wings was also not statistically significant (P>0.05) influenced by the administration of various dietry lysine levels. The average weight of duck breast meat ranged from 16.48 to 18.83%. The highest mean weight of breast meat was found under T4 treatment (0.90% lysine). The average thigh meat weight ranged from 14.41 to 14.94%. Feeding various dietary levels of lysine did not significantly affected (P>0.05) percentage of thigh meat.

Table 5. Average percentage of carcass and carcass cuts of EPMp duck fed various levels of dietary lysine up to 10 weeks of age

Treatment	Empty Carcass (%)	Breast (%)	Thigh (%)	Back (%)	Wings (%)
T1[1)]	61.73±0.27	17.48±0.46	14.94±0.18	18.98±0.24	9.97±0.07
T2	61.43±0.32	17.55±0.74	14.41±0.43	18.58±0.11	10.43±0.14
T3	61.85±0.20	16,49±0.90	14.80±0.52	18.91±0.67	10.39±0.22
T4	62.21±0.48	18.83±0.96	14.63±0.30	18.00±0.39	9.86±0.11

1) T1 = total dietary lysine of 0.60%; T2 = total dietary lysine of 0.70%; T3 = total dietary lysine of 0.80%; T4 = total dietary lysine of 0.90%

Table 6. Average percentage of visceras of EPMp duck fed various levels of dietary lysine up to 10 weeks of age

Treatment	Abdominal fat (%)	Liver (%)	Gizzard (%)	Intestine (%)	Intestine length (cm)
T1[1)]	0.99±0.04	2.07±0.05	3.39±0.06	3.86±0.05	170±1.04
T2	0.84±0.12	2.19±0.14	3.55±0.12	3.80±0.14	169±1.54
T3	0.74±0.19	2.19±0.11	3.54±0.07	3.50±0.35	174±1.70
T4	0.81±0.13	2.04±0.10	3.40±0.19	3.53±0.08	164±1.57

1) T1 = total dietary lysine content 0.60%; T2 = total dietary lysine content 0.70%; T3 = total dietary lysine content 0.80%; T4 = total dietary lysine content 0.90%

Carcass cuts

The average percentage of visceral of EPMp broiler ducks under various levels of dietary lysine up to 10 weeks of age are presented in Table 6. The lowest abdominal fat weight (0.74%) was found under T2 treatment while the highest (0.99%) was found under T1 treatment. Based on statistical analysis, abdominal fat was not significantly affected by administration of various levels of dietary lysine. The results are in line with Hyun et al. (2014), who reported that abdominal content and organ weight in broiler chickens were not significantly influenced by amino acid content in diet given during period finisher.

Based on efficiency aspect to produce low abdominal fat weight administration of dietary lysine of 0.80% was considered sufficient. Abdominal fat content in duck meat was one important factor in the acceptance of duck meat by consumers.

Duck meat that contains high fat is generally less preferred so that it became one of the attention of nutrition researchers preparing proper dietary formula. Another factor that can affect abdominal fat content in ducks was the energy and protein content in the ration. Fan et al. (2008) reported that diet with 18% protein content and 3002 and 3006 kcal EM /kg significantly increased abdominal fat content in Pekin duck at 6 weeks. Purba & Prasetyo (2014b) also reported that a crude fiber content of 9% in feed could lower abdominal fat of EPMp duck for 12 weeks.

The average percentage of liver, gizzard, intestine, and intestine length presented in this study did not significantly ($P>0.05$) influenced by the administration of various dietary lysine levels. The development of liver, gizzard and intestines in poultry were started early because these three organs are very important role for growth process (Leckerck & Carville 1985). The average percentage of liver of EPMp duck ranged from 2.04 to 2.19%. The result was almost equal to the average percentage of Rouen liver ducks. The average percentage of liver of Rouen ducks at 10-week-old according to Omojola (2007) was 2% for male and 2.81% for female, while the average percentage of liver of Pekin ducks at the same age were 2.60% for male and 1.48% for female, respectively.

The average percentage of gizzard under the administration of various dietary levels of lysine ranged from 3.39 to 3.55%. Percentage of gizzard was not affected by the dietary treatment. Percentage of gizzard were considerably normal. The gizzard of the ducks and in poultry mechanically serves as a digestive tool Adeola (2006) reported that the percentage of Pekin duck gizzard at 10 weeks was 2.23% for male and 2.97% of female.

The average percentage of intestine of ducks by giving various dietary levels of lysine ranged from 3.50 to 3.86%, while the average length of intestine ducks ranged from 164 to 174 cm. The intestinal organs are part of the digestive tract until the nutrients derived from the feed are absorbed by the body. Intestines in

poultry can be affected by various factors, including sex. Omojola (2007) reported that the average length of intestinal length of *Chairina moschata* for 10 weeks old was significantly influenced by sex. It was reported that the average intestine length of male C*hairina moschata* was 194.90 cm, while *Chairina moschata* female was 137 cm. The study are also in line with Sutrisna (2010) reported that the length of intestine length and proventrikulus in Tegal ducks were not influenced by the provision of various levels of crude fiber in the ration. The study indicate that the lysine level from 0.60 to 0.90% over for 10 weeks can meet the nutritional requirements of EPMp duck to produce prime percentage carcass cuts.

CONCLUSION

Administration of various dietary lysine significantly affected the weight gain and FCR of EPMp broiler duck up to 10 weeks of age. The optimal total lysine level to produce maximum body weight gain and low FCR of EPMp broiler ducks for 10 weeks was 0.80% and 0.90% and 0.70%, respectively. In terms of efficiency to reduce abdominal fat in EPMp broiler ducks, the use of 0.80% of lysine in the ration was considered optimum.

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Path Analysis of Exogenous Variables against Technology Adoption Levels of Dairy Cattle in West Sumatera

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ABSTRAK

Herawati T, Priyanto D. 2017. Analisis pola variabel eksogen terhadap tingkat adopsi teknologi sapi perah di Sumatera Barat. JITV 22(1): 9-15. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1603

Analisis jalur pada persamaan regresi dapat digunakan untuk melihat pengaruh langsung maupun pengaruh tidak langsung dari beberapa peubah eksogen terhadap peubah endogen. Tingkat adopsi suatu teknologi sebagai peubah endogen dapat dipengaruhi oleh beberapa peubah eksogen, secara langsung maupun tidak langsung. Tujuan penelitian ini adalah untuk menguji beberapa peubah eksogen dari karakteristik usaha sapi perah terhadap tingkat adopsi teknologi pakan sebagai peubah endogen, melalui penelusuran jalur. Pada tahun 2016, dilakukan penelitian di kota Padang Panjang, Sumatera Barat khususnya pada kelompok peternak sapi perah. Peubah endogen dalam hal ini adalah tingkat adopsi teknologi pakan (Z). Peubah eksogen adalah tingkat pendidikan (X1), usia peternak (X3), jumlah sapi laktasi (X2), skala usaha (Y1) dan produksi susu (Y2). Dua peubah terakhir adalah sebagai peubah tengah, yakni yang menjembatani pengaruh tidak langsung. Diperoleh hasil bahwa hanya peubah usia peternak (X3) yang mempunyai pengaruh langsung terhadap Z, dengan nilai ρ_{Zx3}=0,834dan P=0,018. Peubah lainnya yaitu X1 dan X2, berpengaruh signifikan terhadap Y1, dan X2 berpengaruh terhadap Y2 dengan nilai koefisien jalur berturut-turut ρ_{y1x1}=0,133 dan P=0,040; ρ_{y1x2}=0,982 dan P=0,000; ρ_{y2x2}=0,841 dan P=0,008. Oleh karena itu tidak ada kerangka hubungan kausal empiris bersama antara peubah X dan Y terhadap Z, hanya parsial dari X3 dengan struktur model Z= ρ_{Zx3} X3+ $\rho_{Z\varepsilon 2}$= 0,834 X3+0,217ε_2. Disimpulkan bahwa usia peternak sangat mempengaruhi adopsi teknologi pakan sapi perah, semakin tua usia peternak maka semakin sulit untuk adopsi teknologi yang direkomendasikan.

Kata Kunci: Analisa Jalur, Sapi Perah, Adopsi Teknologi Pakan

ABSTRACT

Herawati T, Priyanto D. 2017. Path analysis of exogenous variables against technology adoption levels of dairy cattle in West Sumatera. JITV 22(1): 9-15. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1603

Path analysis of the regression equation can be used to see the direct and also indirect influence of some exogenous variables against endogenous variables. The rate of feed technology adoption as an endogenous variables can be directly or indirectly influenced by some exogenous variables. The purpose of this research was to test multiple exogenous characteristics variables of dairy cows farms against the feed technology adoption rate as endogenous variables, through path analysis. Research conducted in the city of Padang Panjang, West Sumatra in particular farmer group of dairy cattle in 2016. Endogenous variable is the level of adoption of feed technology (Z). Whereas the exogenous variables are the level of education (X_1), the age of farmer (X_3), the amount of cow's lactation (X_2), farm scale (Y_1) and milk production (Y_2). The last two variables are variables which are bridging the influence indirectly. Obtained results showed that only X_3 which directly influenced Z, with a value of ρ_{Zx3} = 0.834 and P = 0.018. Other variables X_1 and X_2 partly significantly influenced Y_1 and X_2 significantly influenced Y_2 with value of path coefficient in successively ρ_{y1x1} = 0.133 and P = 0.040; ρ_{y1x2} = 0.982 and P = 0.000; ρ_{y2x2} = 0.841 and P = 0.008. Therefore, there was no special model of causal relationships between the empirical variables X and Y against Z, except the X_3 which had structure model Z = ρ_{Zx3} X_3 + $\rho_{Z\varepsilon 2}$ = 0.834 X_3 + 0.217 ε_2. It was concluded that the age strongly influenced the feeding technology adoption. The older the age of farmers, the more difficult for adopting recommended technology.

Key Words: Path Analysis, Dairy Cattle, Feed Technology Adoption

INTRODUCTION

Dairy cattle farming, currently still concentrated in the Java Island, even though the dairy consumers spread evenly around Indonesia. Fresh milk production in Indonesia is concentrated in Java Island (95%) with negative total net export-import trading (Hasan 2016; Farid & Sukesi 2017). The efficiency is an obstacle of Indonesian fresh milk production in the outside of Java Island. This as shown by milk production data in 2000, which showed 6,420 dairy cows in North Sumatera producing 4,615 ton fresh milk, meanwhile in West Java with 84,788 dairy cows were able to produce 184,515 ton fresh milk (Yusdja et al. 2016). In 2016 the population of dairy cows in Indonesia, increased up to 533,860 (BPS 2016). Therefore, the improvement

efficiency of rearing dairy cow, in Java Island is crucial in order to fullfil National fresh milk demand.

The government, through policies package and programs of provincial services, has attempted to improve productivity and income of dairy cattle farmers. The policy of Milk Processing Industry, which obliges to buy domestic milk, is one example policy to protect dairy cattle farmers (Budiyono 2012). Indonesian Agency for Agricultural Research and Development (IAARD), through the technical implementation units has developed feed technology to support the increase of national dairy cattle productivity (Mathius 2014; Ginting & Elisabeth 2014; Adnyana & Mardianto 2016). However, the productivity of dairy cattle in the outside Java Island reminds low (Yusdja 2017; Diwyanto et al. 2017). From the point of view of technology introduction, it is allegedly that technology of dairy cattle production introduced, cannot be adopted well in a location. Not optimal adoption of the technology may be induced by ineffective adoption method (Nugroho et al. 2014; Nuryanti & Swastika 2016).

Factors affecting adoption of dairy cattle technology consist of exogenous and endogenous factors of user. The exogenous factors are farm scale; the amount of cow's lactation and milk production, meanwhile the endogenous factors are level of education and age of the farmers which help determine attitude and level of understanding of the technologies introduced. Study of correlation factors should be conducted to optimize the adoption, since the interaction between factors may be specific both for the commodity or the location (Sudaryanto & Agustian 2017). That is underlying this study conducted.

This study was aimed to test multiple exogenous characteristics variables of dairy cows farms against the feed technology adoption rate as endogenous variables, through path analysis. Research conducted in the city of Padang Panjang, West Sumatra in particular farmer groups of dairy cattle. This location has good potential in dairy cattle development supported by suitable agro-climate, feed source availability and independence level of farmers. The city of Padang Panjang is well known as the biggest agribusiness region of dairy cattle in the West Sumatera (Sartika & Rahmi 2012). Meanwhile, quantification of technology adoption correlation factor r was conducted by assessing that correlation factors through path analysis. This model may be used to find out the direct and also indirect effects on several factors reflected on path coefficient value following structural model mathematically (Trinayani et al. 2013; Azis & Kamal 2017). The understanding of this correlation factors will optimize introduction and adoption process of feed technology of dairy cattle.

MATERIALS AND METHODS

Research conducted in the city of Padang Panjang, West Sumatra in particular farmer group of dairy cattle in 2016. This study used quantitative and qualitative approaching through structured survey. The respondents were four dairy cattle farmer groups and one individual farmer (Table 1).

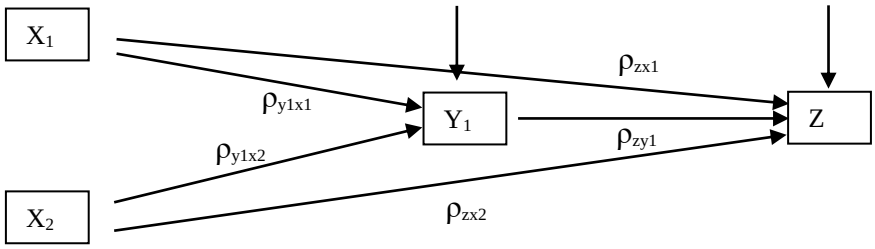
Data analyzed were the age of farmer (year), the level of education of farmer (year), the amount of cow's lactation (head), farm scale (head), milk production (litter/day) and adoption rate (score: 1-4).

The narrative descriptive analysis was used as qualitative analysis model. Quantitative analysis model was performed in the main basis of path analysis model consisting of exogenous (level of education, the age of farmer, the amount of cow's lactation, farm scale and milk production) and endogenous (level of adoption of feed technology) variables with two hypotheses tested:

1. First hypothesis and structural formulation model
   $H_0 : \rho_{Zx1} = \rho_{Zx2} = \rho_{Zy1} = 0$ vs $H_1 : \rho_{Zx1} = \rho_{Zx2} = \rho_{Zy1} \neq 0$
   Hypothesis: Level of education (X_1), Amount of lactating cows (X_2) and farm scale (Y_1) contributed simultaneously to adoption rate of feed technology (Z).
   First structure model and path coefficient shape
2. Second hypothesis and structural formulation model
   $H_0 : \rho_{Zx3} = \rho_{Zx2} = \rho_{Zy2} = 0$ vs $H_1 : \rho_{Zx3} = \rho_{Zx2} = \rho_{Zy2} \neq 0$
   Hypothesis: Age of farmers (X_3), Amount of lactating cows (X_2) and Milk production (Y_2) contributed simultaneously to adoption rate of feed technology (Z).
   Second structure model and path coefficient shape

Table 1. Respondents of the survey in the city of Padang Panjang.

Name	District	Sub-District	The Number of Member (persons)
Serambi Karya Mandiri	Koto Katiak	East Padang Panjang	9
Harapan Baru	Ganting	East Padang Panjang	12
Parmato Mudo Nagari	Silang Bawah	West Padang Panjang	7
Makmur Batu Batire	Kampung Manggis	West Padang Panjang	10
Rafles (individual farmer)	Kampung Manggis	West Padang Panjang	1



$$\mathbf{Y_1 = \rho_{y1x1}X_1 + \rho_{y1x2}X_2 + \varepsilon_1 \ldots\ldots (1) \text{ and } Z = \rho_{zy1}Y1 + \varepsilon_2 \ldots\ldots\ldots\ldots\ldots} (2)$$

Direct effect of exogenous variables (X_1 and X_2) to endogenous variable (Y_1) and indirectly to Z variable with ε_1 error.

Direct effect of Y_1 variable to Z variable with ε_2 error.

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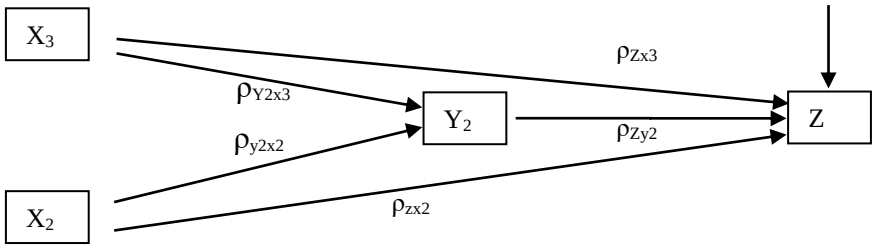

$$\mathbf{Y_2 = \rho_{y2x3}X_3 + \rho_{y2x2}X_2 + \varepsilon_3 \ldots\ldots\ldots (3) \text{ dan } Z = \rho_{zy2}Y_2 + \varepsilon_4 \ldots\ldots\ldots\ldots\ldots (4)}$$

(1) Direct effect of exogenous variables (X_3 and X_2) to endogenous variable (Y_2) and indirectly to Z variable with ε_3 error.

Direct effect of Y_2 variable to Z variable with ε_4 error.

RESULTS AND DISCUSSION

Testing result of the first model

Simultaneous effect of education level, amount of cows lactation and farm scale to the adoption rate of feed technology, which was notified as $Z = f(X_1, X_2, Y_1)$.

This model was motivated by field condition that generally, the large-scale business willing to adopt the technologies introduced. The increase of the number of lactation cow lead to the desire to expand the business scale and to increase milk production. It surely would increase farmer's income. Education level had positive correlation to the adoption rate of technology (Shiferaw et al. 2015; Saridewi & Siregar 2016).

The result of path tracking analysis to test variable: education (X_1), amount of lactating cows (X_2) and farm scale (Y_1) simultaneously, contributed to the adoption rate of feed technology (Z), showed that R^2 and probability value by 0.086 and 0.921>5%, respectively (Table 2). Therefore, the H_0 : $\rho_{zx1}=\rho_{zx2}=\rho_{zy1}=0$ was accepted. This meant that there was no direct effect from the X_1 and X_2 variables against the Z and indirect effect that was through the Y_1. In consequence, the test was continued for partial test of the X_1 and X_2 variables against Y_1 . The partial test resulted R^2 and F value by

Table 2. Testing result of direct effect model

Model	R Square	Std. Error of the Estimate	F Change	Sig. F Change
1	0.086	0.907	0.156	0.921

a) Predictors: (Constant), Education (X_1), Lactation Cow (X_2), Farm Scale (Y_1)
b) Dependent Variable: Adoption of Technology (Z)

Table 3. Coefficients of the first partial path model (1)

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-1.857	1.705		-1.089	0.318
X1.Education	1.698	0.651	0.133	2.607	0.040
X2.Lactating Cow	1.891	0.095	1.016	19.902	0.000

a Dependent Variable: Farm Scale (Y_1)

0.985 and 200.399, respectively with probability value by 0.00 <5% showed that the X_1 and X_2 simultaneously contributed against the Y_1. That meant the H0 : $\rho_{y1x1}= \rho_{y1x2} = 0$ was rejected and the alternative hypothesis, H1 : $\rho_{y1x1}= \rho_{y1x2} \neq 0$ was accepted with the value presented in Table 3.

Path coefficient value of the partial test was ρ_{y1x1} = 0.133 with lower significance value by 0.040 than the probability value by 0.05. That meant that the H_0:ρ_{y1x1}=0 was rejected or the H_1: $\rho_{y1x1}\neq0$ was accepted. It also meant that the education contributed to the farm scale level. Then the value of ρ_{y1x2} = 1.016 with significance level by 0.00 showed that the number of lactation cow was significantly affected by the farm scale. The higher education and number of lactation cow increased the willingness to increase the farm scale. Education level might have changed the mindset, even better reasoning, so it might hve been concluded that higher education level leading someone to be more rational (Narti 2016), for a better mindset resulting in a better management for their agribusiness (Ruggiero et al. 2017; Kumaran et al. 2017). Even the value showed the contribution of education level to the farm scale, as a result of the willingness to expand their business, in this study, showed there was no direct correlation between the farm scales with the adoption rate of technology. It seemed that the correlation test of the adoption rate of technology to the farm scale needed to be carried out as staed by the Rosandy et al. (2012) that one attempt to expand the business scale was improvement and adoption of the technologies. At the same time, the acceptance of information technology (IT) required special efforts. The low rate of IT adoption was influenced by many factors that the most were from the internal. Those factors were social, institutional and financial (Fauzi et al. 2017; Suhaeti & Suharni 2017; Yuwono 2017).

From the test results above, causal-empiric correlation framework between the X_1 and X_2 to Y_1 might have been formed as the following formulas:

$$Y_1 = \rho_{y1x1} X_1 + \rho_{y1x2} X_2 + \varepsilon_1.$$

$$Y_1 = 0{,}133\ X_1 + 1{,}016\ X_2 + \rho_{y1}\varepsilon_1.$$

ρ_{y1} (remind variable)= $1-R^2 = 1- 0{,}985 = 0{,}015$

$$Y_1 = 0{,}133\ X_1 + 1{,}016\ X_2 + 0{,}015\varepsilon_1.$$

Testing result of the second model

The simultaneous effect of age, amount of lactation cow and milk production against the adaptation of feed technology, was noted as $Z = f(X_2, X_3, Y_2)$.

The result of path tracking analysis to test variables: age (X_3), amount of lactation cow (X_2) and milk production (Y_2), showed a weak contribution (P=8%) to the adoption rate of feed technology (Z) (Table 4). The path coefficient tracking result showed that the age (X_3) affected directly to the adoption rate of feed technology with the path coefficient and significance level value by -0.843 and 18%, respectively. Whereas, the other factors were not significantly affected the Z (Table 5). The negative value of the coefficient ρ_{zx3} showed an inverse correlation of the age to the technology adoption rate. This indicated that the older farmer the less the interest in adopting the recommended technologies. Young stakeholders were more interested in the new technologies which meant that age was a significant effect (Gyau et al. 2014).

The partial test of the X_2 and X_3 to the Y_2 resulted in the R^2 = 0.722; F = 7.807 and was significant at the level of 2.1%. But, it was only the amount of lactating cows affecting milk production with value of ρ_{y2x2} = 0.841 and with significance by 0.008 (Table 6).

From this study result, it was expected to be a basis of selection of the target characteristics to improve the adoption rate of technology. Considering that technology adoption was expected to provide significant correlation to improve the productivity and increase the population of livestock. Besides, effective socialization was required to deliver better understanding and build the willingness to adopt the technologies.

Table 4. Testing result of direct effect model

Model	R Square	Std. Error of the Estimate	F Change	Sig. F Change
2	0.713	0.508	4.149	0.080

a Predictors: (Constant), Age of farmers (X_3), Lactating Cow (X_2), Milk Production (Y_2)
b Dependent Variable: Adoption of Technology (Z)

Table 5. Coefficient value of direct path toward Z (2)

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta	B	Std. Error
(Constant)	6.395	0.967		6.610	0.001
X_2.Lactating Cow	0.027	0.049	0.244	0.542	0.611
Y_2.Milk Production	-0.001	0.005	-0.142	-0.312	0.768
X_3.Age	-0.077	0.022	-0.834	-3.439	0.018

Dependent Variable: Adaptation of Technology (Z)

Table 6. Coefficient value of partial path toward Y_2 (2)

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta	B	Std. Error
(Constant)	39.794	83.201		0.478	0.649
X_3. Age of farmers	-0.705	1.936	-0.078	-0.364	0.728
X_2.lactating cow	8.971	2.300	0.841	3.900	0.008

Dependent Variable: Milk Production (Y_2)

Table 7. The Value of path coefficient

Variable		Testing Result		Value	
Endogenous	Exogenous	R^2	P	Standardized Coefficients $\rho_{End.Ex}$	Sig.
Z	X1	0.086	0.921	0.156	ns
	X2			0.244	ns
	Y1			0.131	ns
Y1	X1	0.985	0.000**	0.133	**
	X2			0.982	**
Z	X2	0.713	0.080	0.244	ns
	X3			-0.834	**
	Y2			0.142	ns
Y2	X2	0.722	0.021**	0.841	**
	X3			0.078	ns

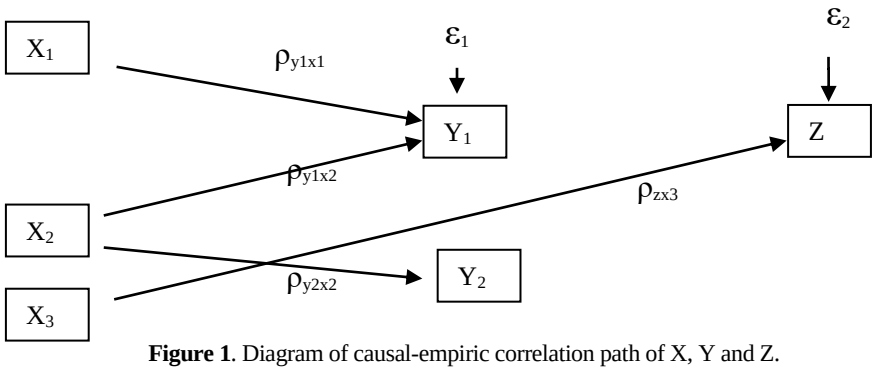

Figure 1. Diagram of causal-empiric correlation path of X, Y and Z.

CONCLUSION

The study concluded that only age of farmers that had direct effect to the adoption rate of feed technology. The older the age of farmers the more difficult for adopting recommended technology. Education and the amount of lactation cow significantly affected the farm scale. The higher education and the greater number of lactating cows the higher willingness of farmer.

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Production Performance of HyCole, New Zealand White Rabbits and Its Reciprocal

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ABSTRAK

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Kelinci New Zealand White (NZW) dikenal sebagai kelinci pedaging yang telah beradaptasi baik di Indonesia. Kelinci HyCole merupakan kelinci unggul yang terseleksi reproduksi baik dan laju pertumbuhan tinggi. Peningkatan produktivitas kelinci NZW dapat dilakukan dengan menyilangkan pada kelinci HyCole yang diimpor dari Perancis. Penelitian ini bertujuan untuk mengevaluasi produktivitas HyCole dan New Zealand White serta persilangan timbal baliknya sebagai dasar pembentukan rumpun kelinci pedaging unggul adaptif iklim tropis. Sejumlah 40 induk betina (P_BP_B) dan 17 pejantan (P_AP_A) kelinci HyCole, dan 30 induk betina dan 6 pejantan kelinci New Zealand White (NN). Ransum penelitian diberikan sesuai standar Balai Penelitian Ternak (Balitnak; PK 18 persen, EM 2500 kkal/kg dan SK 14 persen). Ransum berbentuk pellet diberikan pagi dan sore hari, air minum diberikan *ad libitum*. Pengukuran dilakukan pada kinerja reproduksi induk (litter size lahir, litter size sapih, mortalitas dan bobot badan induk) dan kinerja pertumbuhan anak (bobot badan mingguan dari lepas sapih sampai umur 20 minggu). Data dianalisis dengan menggunakan program SAS (SAS 2001). Data penimbangan ternak kelinci secara periodik dianalisis dengan pendekatan kurva pertumbuhan non linier model Gompertz. Hasil evaluasi menunjukkan bahwa kelinci HyCole yang dikembangkan di Balitnak memiliki performa produksi yang lebih baik dibandingkan kelinci NZW dan persilangan pejantan HyCole dengan NZW betina (P_AN) berpotensi dikembangkan sebagai materi genetik calon induk kelinci unggul adaptif iklim tropis karena memiliki hybrid vigor yang baik pada jumlah anak sekelahiran dan pertumbuhan anaknya.

Kata Kunci: Kelinci HyCole, Kelinci New Zealand White, Hasil Persilangan

ABSTRACT

Brahmantiyo B, Raharjo YC, Prasetyo LH. 2017. Production performance of HyCole, New Zealand White Rabbits and its reciprocal. JITV 22(1): 16-23. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1590

New Zealand White rabbits (NZW) has been known as broiler rabbit that has been well adapted in Indonesia. HyCole rabbits were imported from France that were selected for high reproduction and growth rate. This study was aimed to evaluate the productivity of HyCole and New Zealand White rabbits and their reciprocal as the basis to develop broiler rabbit which adaptive to tropical climate. Forty heads of doe (P_BP_B) and 17 heads of buck (P_AP_A) of HyCole rabbit, and 30 heads of doe and 6 heads of buck of New Zealand White rabbits (NN) were used. The ration was given according to IRIAP standard ration (18 % CP, 2500 kcal ME/kg and 14 % CF). The diet was provided in the morning and the evening, and drinking water was provided *ad libitum*. Reproductive performance of does (litter size at birth, litter size at wean, mortality and weekly does body weight) and the kit's growth performance (weekly body weight from weaning until the age of 20 weeks) were evaluated. Data were analyzed using the SAS program (SAS 2001). Rabbits growth data were periodically analyzed by Gompertz model (Blasco & Gomez 1993). HyCole rabbit which was bred in Indonesia had production performance better than NZW rabbit and P_AN crossbred (HyCole bucks x NZW does) had the potential to be bred as superior rabbit adaptive to tropical climate because they had hybrid vigor of the number of litter size at birth and kit's growth rate.

Key Words: HyCole Rabbit, NZW Rabbit, Crossbred

INTRODUCTION

Generally, a breeding rabbit proram in the developed countries, follows three-way crossbreeding method, where the first crossing is performed between two selected breeds to produce dam with superior reproduction, is crossed with a sire that has good growth performance (Baselga et al. 2003). It improves the performance of growth and body weight its crossbred. From the economic point of view, daily body weight gain (pre/post-weaning), feed consumption and feed conversion ratio are crucial properties in the livestock productivity.

The Indonesian Resarch Institute for Animal Production (IRIAP) imported 40 heads of dam and 17 heads of sire of HyCole rabbit from France. The rabbit has been well known to have good production and reproduction performances of litter size of more than 9

kits, and more than 50% of dam population has 5 pairs of the nipple and the adult weight may reach 2.5 kg at 10 weeks of age. The observation were on HyCole rabbit parent stock sire A and parent stock dam B (Lenoir et al. 2012). The HyCole rabbit became parental in the crossing with the New Zealand White rabbit.

New Zealand White (NZW) the rabbit has been developed in the United State of America. In 1916, WS Preshaw was the first person who breeds the NZW rabbit to create superior meat producer rabbit. Its origin was unknown, however, it was believed that the Angora rabbit played a role in its forming. Lebas et al. (1986) described that this rabbit was plain white with red eyes and adult weight by 4.1-5.0 kg. The first matting age was 144 days with average litter size by 8.5 kits, life litter size by 8.0 kits and weaned litter size by 6.5 kits. Cheeke et al. (1987) said that the New Zealand White rabbit was known as commercial meat producer. It had fast growth rate, good carcass quality, high fertility and good maternity. Raharjo et al. (1986) described that the NZW rabbit had pregnancy rate by 89.9%, pregnancy time by 31.6 days, calving interval by 37.8 days, dam body weight at the first birth by 3.1 kg, litter size by 9.1 heads, weaned litter size at 5 weeks by 7.2 heads, weaned body weight by 550 g/head, mortality from the birth to weaned by 16.9%.

The crossing with the CAL was carried out and produced a good result. The crossbred of NZW and CAL had higher growth rate from the weaning to slaughter age compared to its pure breed. Body weight at 70 days of the crossbred of NZW x CAL was higher than the crossbred of CAL x NZW and its CAL pure breed. The body weight of CAL rabbit was the lowest (Maj et al. 2009). The crossbred of NZW x CAL consumed smaller diet per unit body weight gain than the NZW, CAL and the CAL x NZW and reached 2.5 kg/head of slaughter weight at the earlier age followed by CAN x NZW, NZW and CAL. The crossing of NZW x CAL has positive effect on growth rate and the slaughter characteristics. Ozimba & Lukefahr (1991) studied the NZW, CAL and it's reciprocal which showed lower slaughter and carcass weight.

Forming of meat producer rabbit as conducted by 3 crossing of several lines. The dam from crossbred lines was selected for litter size and the terminal sire from a line that was selected for high growth performance (Piles et al. 2000). This study was the beginning of the forming of Superior Rabbit Adaptive to Tropic (KUAT) breed where the HyCole, NZW, and HyLa rabbit were used as the material. Characteristic of hybrid HyCole (sire P_AP_A and dam P_BP_B became P_AP_B) and its crossing with NZW was required as a standard of determination of prospective KUAT breed forming.

This study was addressed to evaluate the productivity performance of HyCole and New Zealand White and its reciprocal as a basic of breeding activity, the three ways cross informing the superior rabbit adaptive to the tropical climate.

MATERIALS AND METHODS

This study was conducted at Indonesian Research Institute for Animal Production (IRIAP) for 18 months. This study was used 40 dams (P_BP_B) and 17 sires (P_AP_A) of HyCole rabbit, and 30 dams and 6 sires of New Zealand (NN) rabbit. The matting process resulted in 49 mates of HyCole (P_AP_B), 29 mates of New Zealand White (NN), 30 mates of HyCole x New Zealand White (P_AN) and 22 mates of New Zealand White x HyCole (NP_B). Those mates resulted in P_AP_B, NN, P_AN and NP_B respectively by 389 heads, 185 heads, 212 heads and 181 heads. The matting diagram was presented in Figure 1.

The matting with a ratio of one sire for 4-5 dams was conducted until three parities. The dams were matted after 5-6 months old and 8 months of age. The dams were matted when it showed estrus signs by evaluating the vulva. Redden vulva showed sign of estrus. The palpation was conducted on the 12^{th} day after mating to determine the pregnancy. The dams, which was not pregnant were re-matted. The net for pregnant dams was prepared on the 28^{th} days of pregnancy. The matting was immediately performed on two weeks after giving birth and showed estrus sign. The sire was mated with 4-5 dams. The mating system was adjusted to pedigree note.

The cages were individual wire cage with the size according to the age. The cage height from the floor was 100 cm. Each cage was equipped with diet trough made from pottery by 15 cm x 12 cm x 60 cm and drinking water was provided in the nipple made from metal. The dam cages size was 60 cm x 75 cm x 40 cm while the sire cage size was 75 cm x 45 cm x40 cm.

The nesting cage size was 40 cm x 30 cm x 25 cm. The sawdust was used as flooring. The dams would shed their hairs that would be used as a nest for their kits. The nesting cage was cleaned when the kits reached 4-5 weeks old and prepared for the next kits. The kits were weaned at 5 weeks old and maintained in 45 cm x 75 cm x 45 cm cage made from wood.

The diet in this study contained crude protein by 18%, metabolic energy by 2500 kcal/kg and crude fiber by 14%. The diet was made in the form of pellet that was delivered in the morning and the evening while the drinking water was provided *ad libitum*.

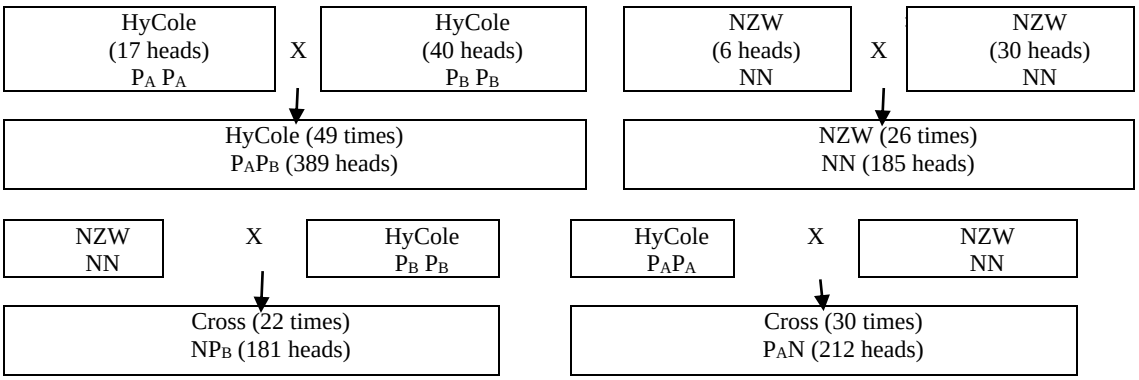

Figure 1. Diagram of mate of the HyCole and New Zealand White Rabbit.

The parameters measured in this study were reproduction performance (birth liter size, weaned litter size, mortality) and dam body weight (from the beginning of giving birth up to next five weeks) and kits growth performance (weekly body weight from after the weaned up to 20 weeks). The whole productivity and reproductivity data were analyzed using SAS program with the general linear model (SAS 2001).

Development of weight data was periodically analyzed using nonlinear growth curve of Gompertz model with SAS package program (SAS 2001). It was considered as a model, based on Blasco & Gomez (1993), which had been proved as the best model to describe the rabbit growth. The Gompertz was also used in the analysis of the development of mice (Kurnianto et al. (1998), lamb (Lambe et al. 2006) and cattle (Forni et al. 2009).

Kurnianto et al. (1998) said that Gompertz model was a good model in comparing data of body weight with logistic and asymptotic models. For more general utilization, Lenart (2012) reported that Gompertz model was also applied in demography and actuarial. Estimation of maximum-likelihood was used to determine parameters in Gompertz distribution. Goshu & Koya (2013) stated that Gompertz was one of growth curves that had been used widely along with another growth curves.

The mathematical formula was:

$$Y_t = A\exp(-B\exp-kt)$$

Description:
Y_t = body weight at the t age
A = adult body size (asymptote) for body weight
B = scale of variables (constant)
exp = basic logarithm (2.178282)
k = growth rate of kits until reached adult body
t = time unit of age

To obtain estimation value which could be accounted when the biologic point of a growth. P'tak et al. (1994) used a simple model obtained from nonlinear equation derivative. The mathematic model had been used well in estimating the inflection point location when the age and body weight of rabbit experienced the first puberty. To determine the inflection point of body weight, the quotient of A value and exponential number [A/exp] were used, while the inflection point of age was [(lnB)/k].

RESULTS AND DISCUSSION

Performance of kits

The growth of kits from the matting of HyCole (P_AP_B), NZW (NN) and its reciprocal (P_AN and NP_B) was presented in Table 1. The P_AP_B rabbit had the highest growth rate than the NN, P_AN and NP_B. Birth and weaned body weight (6 weeks old) of P_AP_B was 54.3±11.4 g/head and 752.7±161.4 g/head, NN was 53.4±11.8 g/head and 565.9±121.7 g/head), P_AN was 56.6±10.8 g/head and 604.4±200.4 g/head and NP_B was 50.4±8.7 g/head and 622.1±175.8 g/head. The P_AP_B reached slaughter weight (2.0 kg/head) at 14 weeks values in the same row with the different suppercript are significant diference ($p < 0.05$) old by 2,111.9±462.3 g/head, while the P_AN rabbit was 16 weeks. The NP_B and NN rabbit was at 17 weeks old by 2,086.0±511.9 g/head and 2,027.7±251.3 g/head respectively. Maj et al. (2009) reported that crossing of California (CAL) with New Zealand White (NZW) rabbit resulted crossbred that had shorter slaughter age compared its elders by 97±14 days of NZW, 105±17 of CAL, 96±13 days of CAL x NZW and 94±12 days of NZW x CAL.

Table 1. Growth performance of kits of HyCole (P_AP_B), New Zealand White (NN) and its reciprocal (P_AN and NP_B)

Variables	P_AP_B	P_AN	NP_B	NN
bb0	54.3±11.4^b	56.6±10.8^a	50.4±8.7^c	53.4±11.8^b
bb3	290.1±109.7^a	221.2±66.2^{bc}	230.1±59.4^b	201.5±56.2^c
bb6	752.7±161.4^a	604.4±200.4^{bc}	622.1±175.8^b	565.9±121.7^c
bb10	1453.9±324.9^a	1174.82±324.1^{bc}	1076.6±298.9^b	985.0±234.4^c
bb11	1588.4±371.5^a	1316.20±338.9^b	1213.0±301.8^{bc}	1147.3±208.6^c
bb12	1724.6±399.2^a	1466.74±381.0^b	1348.7±321.6^{bc}	1297.3±199.2^c
bb13	1855.3±402.7^a	1670.34±435.6^b	1473.0±368.6^c	1383.1±205.1^c
bb14	2111.9±462.3^a	1819.70±427.1^b	1573.4±430.9^b	1465.7±164.4^c
bb15	2288.1±495.1^a	1955.3±427.7^b	1756.9±426.8^{bc}	1687.1±227.6^c
bb16	2394.9±539.2^a	2075.8±472.9^b	1953.2±453.1^b	1860.7±222.8^b
bb20	3095.4±434.0^a	2734.4±561.6^b	2617.9±380.8^{bc}	2483.5±272.4^c

Description: bb0-bb20: body weight of 0 – 20 weeks old kits
Values in the sama row with the different superscript are significantly difference ($P < 0.05$)

The difference of growth rate was influenced by environment, such as temperature, diet, maintains management (Rao et al. 1978; Gupta et al. 1992), genetic of growth performance of each breed (Sartika & Diwyanto 1986; Gupta et al. 1992; Lakabi et al. 2004) and interaction between the genetic and environment (Sartika & Diwyanto 1986). The HyCole rabbit was a selected rabbit for high growth performance of male line and high reproduction of female line which were resulted in hybrid rabbit (final stock) that had combined properties of its pure breeds. The crossing of the CAL and NZW rabbit resulted in better kits growth and carcass performance compared to its elders (Maj et al. 2009; Ozimba & Lukefahr 1991).

Estimation of growth curve

The estimation of curve formula of growth of HyCole (P_AP_B), New Zealand White (NN) and its reciprocal (P_AN and NP_B) using Gompertz model as followed:

$Y = 4{,}260.61 \exp(-3.88 \exp -0.12t)$ for P_AP_B.....(1)

$Y = 4{,}301.85 \exp(-3.71 \exp -0.09t)$ for NN.....(2)

$Y = 3{,}827.29 \exp(-3.80 \exp -0.12t)$ for P_AN ..... (3)

$Y = 4{,}666.30 \exp(-3.61 \exp -0.09t)$ for NP_B.....(4)

Using those formulations, the graphic of the growth curve for each P_AP_B, P_AN, NP_B and NN may be made. The nonlinear growth curve of Gompertz was presented in Figure 2. It showed that the growth of crossbred was higher compared to the NN rabbit both the P_AN and NP_B

The Gompertz model might have also be used to estimate inflection point of body weight and age of rabbit as showed in Table 2. The inflection point of body weight was a point where the animal experiences a decrease of growth rate at the time unit of the inflection point of age or when the body weight reached its puberty period. Goshu & Koya (2013) described that the inflection point was a trait of growth curves such as Richard Model, Von Bertalanffy, Brondy, logistic and Gompertz. The inflection point of the growth curve was a point in a curve where the growth rate reaches a maximum value and showing a crucial physical interpretation (Goshu & Koya 2013).

The inflection point was related to age and body weight. On this point, the auto acceleration step was replaced by autoretardation step (Setiaji et al. 2013). The inflection point of body weight of the HyCole, NZW, P_AN and NP_B was 1,955.9 g/head, 1,974.9 g/head, 1,757.0 g/head and 2,142.2 g/head. This infection point was highly influenced by breed. Brahmantiyo et al. (2010) and Brahmantiyo & Raharjo (2011) reported that Rex and Satin rabbit reached adult age by 8.6 weeks and 10.3 weeks respectively.

The inflection point in growth curve had high statistical attractiveness (Goshu & Koya 2013). The HyCole, NZW and its reciprocal were interesting to be observed. The growth rate of kits of the P_AN and NP_B reached inflection point of age which was influenced by both elders. The inflection point of HyCole by 10.6 weeks increased u to 11.1 weeks after its sire mated with dam NZW (P_AN), while its dam mated with sire NZW (NP_B) increased into 14.3 weeks and the NZW itself had age inflection point by 14.6 weeks.

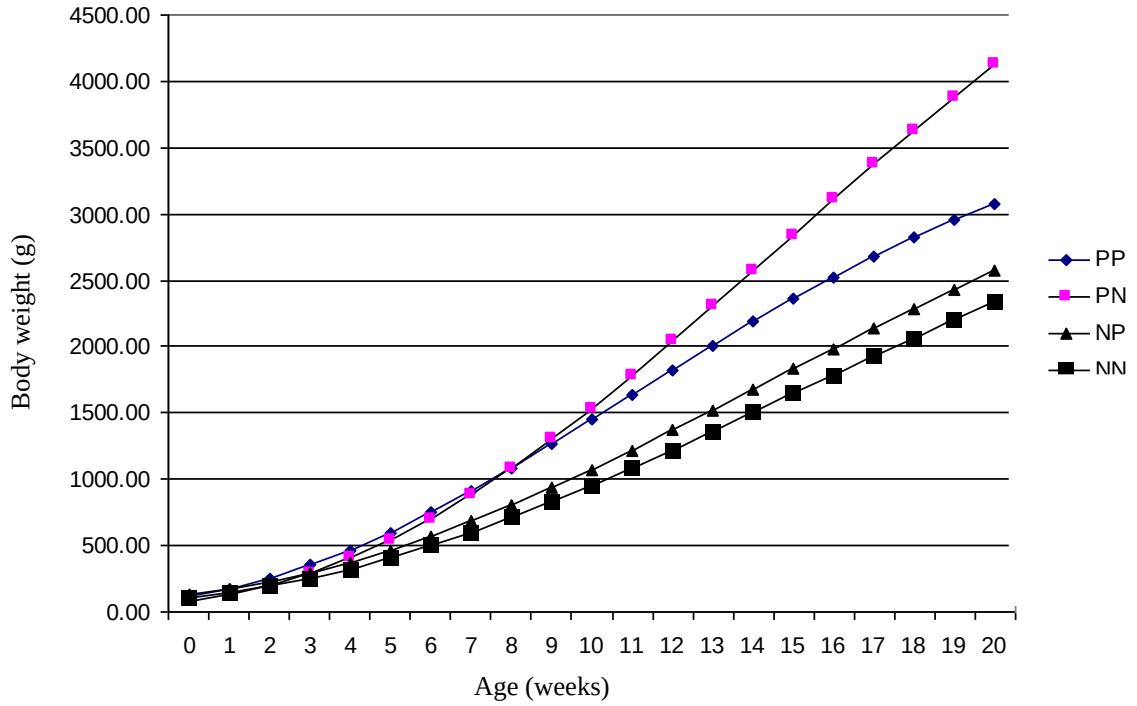

Figure 2. Graphic of growth curve estimation of Gompertz model. PP = pure HiCole, NN = pure New Zealand White

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Brahmantiyo & Raharjo (2011) reported that change of growth curve caused by selection of weaning weight of Satin rabbit (SS), increased its puberty weight from 1,301.12 g to 1,451.05 g at the same age of 10.3 weeks. While selection of weaning weight of Rex rabbit (RR) and Reza rabbit (RS) increased its puberty weight and time from 1,135.87 g at 8.6 weeks of age to 1,446.22 g at 10.1 weeks of age of RR and from 1,080.79 g on 8.0 weeks of age to 1,571.86 g on 10.5 weeks of age of RS. Larzul & de Rochambeau (2004) stated that selection on growth rate would decrease adult age when the animal was slaughtered on the appointed weight (constant), which was reached in the shorter time. They also stated that selection of a growth rate influenced growth path of the rabbit.

According to the estimation of the growth curve of Gompertz model, it might have been estimated that the growth rate was in accordance with the age. The P_AP_B, NN, P_AN and NP_B reached puberty age at 10.6 weeks, 14.6 weeks, 11.1 weeks and 14.3 weeks, respectively. The does should be separated to prevent an early mating. Ouyed & Brun (2008) stated that the formation of broiler rabbit should be following the three ways cross with the dam of crossbred Californian x New Zealand White (CA x NZ) which was mated with the sire Giant Blanc du Bouscat (GB). It then the evaluation result of productivity of the P_AP_B and P_AN and NP_B with NN might have been a basis of broiler rabbit formation by adding the 3rd the HyLa breed.

Performance of dams

The broiler rabbit was widely formed from selected pure rabbit for meat productivity through crossing (Maj et al. 2009). In this study, the evaluation on pure rabbit (HyCole and NZW) and crossing (the reciprocal of HyCole and NZW) were aimed to form broiler rabbit which provided optimal performance. The performance of dams of the HyCole, New Zealand White and its reciprocal was presented in Table 3. Those dams had no different litter size (birth LS) and the number of weaned kits (weaned LS). The HyCole showed lower reproduction performance compared to the report of Lenoir et al. (2012) who reported that the birth of LS was more than 9 kits. The body weight of HyCole dams had higher birth weight compared to the NZW and its reciprocal at the age of giving birth (BB0) until weaning the kits (BB5).

Mortality of kits of the P_AP_B, P_AN, NP_B and NN was 30.4±21.1%, 34.7±24.8%, 32.9±22.8% and 34.6±21.7%, respectively. This was higher than research result of Sartika & Diwyanto (1986) which showed average mortality from birth until weaning period at five weeks old was 11.1% of native rabbit. In Java Island, mortality of kit until weaning age was.

Table 2. Estimation of inflection point of body weight and age of P_AP_B, P_AN, NP_B and NN

Breed	Description	Inflection point of body weight (g)	Inflection point of age (weeks)
PAPB	HyCole	1955.9	10.6
PAN	HyCole x NZW	1757.0	11.1
NPB	NZW x HyCole	2142.2	14.3
NN	New Zealand White	1974.9	14.6

Table 3. Production performance of dam of HyCole HyCole (P_AP_B), New Zealand White (NN) and its reciprocal (P_AN and NP_B)

Parameter	P_AP_B	P_AN	NP_B	NN
Birth LS	8.1±1.9^a	7.2±1.9^a	8.1±1.7^a	7.3±1.9^a
Weaned LS	5.8±1.6^a	4.7±2.3^a	5.3±2.1^a	5.5±2.3^a
Mortality	30.4±21.1^a	34.7±24.8^a	32.9±22.8^a	34.6±21.7^a
BB0	3969.8±387.6^a	3706.2±452.8^b	3605.3±362.4^{bc}	3478.2±355.4^c
BB1	4106.0±450.6^a	3830.8±671.7^{ab}	3746.2±344.3^b	3545.4±327.5^b
BB2	3959.3±367.9^a	3548.7±462.9^b	3532.3±393.2^b	3261.9±471.7^c
BB3	3684.1±324.3^a	344.3±514.3^{ab}	3456.9±426.5^{ab}	3290.5±435.3^b
BB4	3530.4±431.5^a	3396.4±478.5^{ab}	3397.6±426.5^{ab}	3136.0±402.6^b
BB5	3660.9±331.1^a	3344.7±373.2^b	3413.8±514.8^{ab}	3207.0±486.9^b

Description: Different superscript in the same row means significant difference ($P<0.05$).

around 14.7-23.3% (Sastrodihardjo 1985) and the mortality around 20-25% was fair (Cheeke et al. 1987). Olowofeso et al. (2012) reported that mortality of kits from the crossed rex (RX), Flemish Giant (GF) and Chinchilla (CH) rabbit, reared in the Southwest Nigeria, was 18.26 % (CHxGF) to 30.03% (GFxRX). While, Raharjo (1994) stated that high mortality (23-43%) still occured in the breast feeding period. The post-mortem evaluation proved that the highest case of mortality was influenced by enteritis.

The interaction between genetic and environment influenced the mortality of kitHigh mortality in this period was allegedly due to environment effect (climate, wind, and temperature), aeration and hygiene inside the cage and its surrounding which might have caused stress to the kits. Environmental hygiene was a crucial factor influencing the mortality of kits in this study. The death in this study was highly caused by diarrhea and mastitis. Rabbit rearing needed highly hygiene sanitation, smooth airflow and appropriate treatment. Cheeke (1986) described that rabbit development boundary in a tropical region was due to easy experience stress from temperature. Appropriate management was required to monitor the mating, earlier diseases outbreak, patience and attention and much knowledge about the rabbit.

Body weight of dam at the first giving birth of P_AP_B, P_AN, NP_B and NN was 3,969.8±387.6 g/head, 3,706.2±452.8 g/head, 3,605.3±362.4 g/head and 3,478.2±355.4 g/head which was influenced by breed. Their body weight was continuous to decrease since the breast feeding entered the fourth week of age. This was due to the dams that had pregnant with new kits. It was required to have known that the dams were re-matted after 14 days of giving birth, so that at the fourth week, the dams had one week pregnant. This required higher nutrient which would be used not only for basic live and kits, but also for the fetus. Sartika & Diwyanto (1986) said that the dams with high litter size had heavier body weight leading to highest body weight which would e decreasing during the breast feeding period. This study showed that the dams with more kits required better diet quality (Cheeke et al. 1987).

CONCLUSION

HyCole rabbit hich was developed in Indonesia had better reproduction performance compared to the NZW rabbit. The crossed HyCole sire and NZW dam (P_AN) was potential to be developed as the genetic resources of dam adaptive to tropical climate due to its good heterosity (hybrid vigor) in litter size and its growth. In

the formation program of Superior Rabbit Adaptive to Tropical (KUAT) with the genetic source of HyCole, Hyla, and NZW rabbit, and performance of the P_AN rabbit might have been assigned as dam strain.

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Weight Estimation of Empty Carcass and Carcass Cuts of Female SenSi-1 Agrinak Chicken

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ABSTRAK

Hidayat C, Iskandar S. 2017. Estimasi berat karkas dan potongan karkas ayam SenSi-1 Agrinak betina. JITV 22(1): 24-29. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1626

Ayam SenSi-1 Agrinak adalah ayam lokal Sentul yang telah diseleksi untuk pertumbuhan selama enam generasi. Tujuan dari penelitian ini adalah untuk menghitung bobot karkas dan potongan karkas ayam SenSi-1 Agrinak betina berdasarkan data umur dan bobot hidup. membangun model matematik untuk menduga bobot karkas kosong dan potongan karkas ayam SenSi-1 Agrinak betina tanpa harus dilakukan proses pemotongan. Sebanyak 128 ekor ayam SenSi-1 Agrinak betina diambil secara acak dari populasi pengamatan, kemudian dipotong secara Islami pada umur 5, 8 dan 15 minggu, untuk diamati bagian karkasnya. Data dianalisis menggunakan metode analisis korelasi dan regresi. Hasil percobaan menunjukkan bahwa bobot hidup ayam SenSi-1 Agrinak betina memiliki korelasi positif tinggi dengan bobot karkas kosong dan potongan karkas ayam umur 5, 8 dan 15 minggu. Pendugaan bobot hidup, karkas, potongan karkas, rempela, hati dan lemak abdominal dengan menggunakan model matematik, menunjukan perbedaan yang rendah (0,09% – 4.43%) dari bobot aktual hasil pengukuran. Disimpulkan bahwa pada ayam SenSi-1 Agrinak betina bobot karkas dan potongan karkas dapat dihitung dengan menggunakan data umur (hari) dan bobot hidup (g) tanpa harus melakukan penyembelihan.

Kata Kunci: Ayam SenSi-1 Agrinak Betina, Karkas, Potongan Karkas

ABSTRACT

Hidayat C, Iskandar S. 2017. Weight estimation of empty carcass and carcass cuts of female SenSi-1 Agrinak chicken. JITV 22(1): 24-29. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1626

SenSi-1 Agrinak chicken is Indonesian local chicken that was selected for growth rate for six generations. The aim of this study was to estimate of carcass weight and carcass cuts of female SenSi-1 Agrinak chicken, based on age and live weight. The chicks were reared intensively in colony wire cages and they were slaughtered with Islamic method when they reached age of 5, 8 and 15 weeks. Empty carcass and carcass cuts were weight in fresh. Data were analyzed using correlation and regression analysis method. Results showed that the live weight had a high positive correlation to carcass weight and carcass cuts weight of female SenSi-1 Agrinak chickens aged 5, 8 and 15 weeks. Estimation of live weight, carcass, carcass cuts, gizzard, liver and abdominal fat of female SenSi-1 Agrinak, using mathematical model, showed small value differences (0.09 - 4.43%) from the actual data. It was concluded that female SenSi-1 Agrinak chicken's carcass weight and carcass cuts, could be estimated based on of age in days and live-body weight in gram without slaughtering.

Key Words: Female SenSi-1 Agrinak Chicken, Empty Carcass, Carcass Cuts

INTRODUCTION

Indonesian Research Institute for Animal Production (IRIAP) has carried out research on improving breeds of native chicken since 15 years ago. One of the improved breeds was SenSi-1 Agrinak, which was selected from native Sentul breed. SenSi-1 Agrinak was then released officially by Ministery of Agriculture of the Republic of Indonesia decree early in 2017. SenSi-1 Agrinak was the results of seletion of native Sentul chicken for 10 weeks growth rate and it was a candidate for one of local breed for male line incorporated in local chicken crossing program (Iskandar et al. 2012; Hasnelly et al. 2012; Iskandar & Sartika 2015). Sulandari et al. (2007) and Sartika & Iskandar (2007) reported that native Sentul chicken was a dual purpose type chicken with having medium body size.

Although SenSi-1 Agrinak chicken has been introduced to Indonesian local chicken industry, continue examination on every aspect of economic value of the breed has to be carried out. Carcass quality is one aspect that has to be observed, as the breed is utilized for local chicken meat supply. Holcman et al. (2003) reported that economically, carcass quality was determined by the portion of empty carcass and carcass cuts preferred by the consumers. Empty carcass, breast part, thigh both upper and lower parts were important

economically parts, determining quality marketed products. However, since invasive like slaughtering in order to gather data, has been little bit time consuming and disposing valuable selected chicken, estimation through noninvasive method, like mathematical method, is worth pursuing.

Furthermore, Khosravinia et al. (2006) indicated that the noninvasive methods provide an opportunity to collect slaughter value information from live birds for selection while still alive. The regression models can be used to predict carcass, breast and leg weights utilizing data on body conformation traits and weight at different ages. Raji et al. (2010) also suggested that since slaughter value parameters were difficult to obtain in the live animal except after slaughter, simple, reliable and indirect methods for the estimation was a necessity. The aim of this study was to estimate of weight of empty carcass and carcass cuts of female SenSi-1 Agrinak chicken based on age and live weight.

MATERIALS AND METHODS

A total of 128 of female SenSi-1 Agrinak chickens were taken from a population used in an massive nutritional experiment (Hidayat & Iskandar 2017), which was applying intensive husbandry, where the chicks were kept in colony wire cages and fed rations, containing 17-20% crude protein with energy of 2800-3000 kcal ME/kg. When they reached age of 5, 8 and 15 weeks, they were randomly taken to be slaughtered according to Islamic slaughter method. Fresh empty carcass, carcass cuts (head, neck, wings, whole back, whole breast, upper and lower thighs, shanks), gizzard, liver and fat pad were then weighed.

Data were analyzed using correlation and regression analysis method. Mathematical model for estimating live body weight was using: $y = a + bx$, where y was body live weight (g/bird); x was age in days; a was constanta; and b was the slope of the line. Whilst in estimating empty carcass and carcass cuts weight, data were subjected to regression equation of $y = a + b_1x_1 + b_2x_2$, where y was the estimated weight of empty carcass or carcass cuts (in g/bird); x1 was actual body live weight (g/bird); x2 was the age when the variable measured (days), b1 was the slope of the x1 line; and b2 was the slope of x2 line.

Data were subjected to ANOVA (analysis of variance) and correlation between two observed variables as suggested by Steel & Torrie (1993). Multiple regression analysis for any change in the fixed variable (age and live weight), influenced unfixed variable (weight of empty carcass or carcass cuts), were analyzed using the SPSS statistical software (Levesque 2007). For the particular measurement such as percentage values, data were examined for normality before running with ANOVA.

RESULTS AND DISCUSSIONS

Live weight, empty carcass weight, and carcass cuts weight of the analyzed chicks at three different ages are presented in Table 1. As it was expected, that the increase in weight of all measured variables increased as the age increased, which indicated that they were in good keeping management.

Table 1. Average weight of empty carcass and carcass cuts

Variables (g/bird)	Five weeks of age	Eight weeks of age	Fifteen weeks of age
Live weight	237±10.3	388±11.7	1247±17.1
Empty carcass[1)]	130±6.9	221±7.7	803±11.3
Head and neck	25.0±0.8	38.7±0.9	103±1.6
Whole back	34.2±1.7	59.9±2.1	201±3.4
Two wings	22.1±1.1	34.6±1.1	109±1.7
Whole breast	33.3±1.6	53.6±2.1	218±3.9
Two upper thighs	21.4±1.0	37.2±1.4	144±2.3
Two lower thighs	21.2±1.1	35.9±1.3	131±2.3
Two shanks	12.1±0.5	19.1±0.6	50.0±1.0
Gizzard	8.63±0.4	12.4±0.4	24.0±0. 6
Liver	6.55±0.3	8.70±0.3	23.1±0.5
Abdominal fat	0.20±0.1	2.54±0.5	17.3±2.2

[1)] Empty carcass is the carcass without head, neck, legs, and oval

Table 2. Analysis of variance on empty carcass and carcass cuts as the percentage of live-body weight of female SenSi-1 Agrinak chicken

Age (weeks)	EC (%)	HN (%)	WB (%)	TW (%)	WBr (%)	TUT (%)	TLT (%)	TS (%)	G (%)	L (%)	AFP (%)
5	54.38$^{b1)}$	10.70^{a}	14.37^{c}	9.32^{a}	14.01^{b}	9.00^{c}	8.86^{b}	5.13^{a}	3.68^{a}	2.77^{a}	0.08^{a}
8	56.59^{b}	10.06^{b}	15.38^{b}	8.93^{b}	13.56^{b}	9.51^{b}	9.19^{b}	4.91^{b}	3.25^{b}	2.24^{b}	0.68^{a}
15	64.50^{a}	8.24^{c}	16.16^{a}	8.72^{b}	17.48^{a}	11.59^{a}	10.54^{a}	4.02^{c}	1.90^{c}	1.83^{c}	1.33^{a}
SEM	0.69	0.15	0.13	0.07	0.24	0.14	0.10	0.06	0.10	0.05	0.09

1) Values in the same column with the same superscript are not significantly difference (P>0.05)
EC = Empty carcass
HN = Head and neck
WB = Whole back
TW = Two wings
WBr = Whole breast
TUT = Two upper thighs
TLT = Two lower thighs
TS = Two shanks
G = Gizzard
L = Liver
AFP = Abdominal fat pad

As shown in Table 2, which was the result of statistical analysis (ANOVA) on the empty carcass and carcass cuts variables measured as the percentage of live weight of the same observed of the individual chicken, indicated that the simultaneous development of every carcass cut was not the same as the bird aging.

As seen in Table 2, percentage of empty carcass at the age of five and eight weeks was not significantly different (P>0.05), but it significantly increased (P<0.05) by the age of 15 weeks. The same trends were also showed by the other variables measured unless head and neck, two wings, two shanks, gizzard and liver, which were declining at the older age. The abdominal fat pad, which also increased at the age of 15 weeks (P>0.05), which was due to a large differences between individual of female chickens. The uneven acceleration of growth of organs as a proportion to body mass was also reported by Tickle et al. (2014), showing that the heart, lungs, and intestines decreased in size from hatch to slaughter weight when considered as a proportion of body mass.

Correlation between two variables

The correlation between two parameters measured of female SenSi-1 Agrinak chicken at the age of 5, 8 and 15 weeks is presented in Table 3. Body live weight of female SenSi-1 Agrinak chicken had considerable (r = 0.99 – 0.67) relation with empty carcass and carcass cuts as an impact of growth status, which was at the prime status in their live to build the body mass as much as the genetic capacity and conducive environment. Rymkiewicz & Bochno (1999) reported this closed relation in chicken, Kleczek et al. (2006) in duck and Vali et al. (2005) in quail. However, Raji et al. (2010) and Shafey et al. (2013) found out the correlation (r) varied from 0.98 to 0.26 in modern broiler chicken. The different might have been due to different in breed (Choo et al. 2014).

Therefore, body live weight is a good indicator that can be used to estimate empty carcass and carcass cuts weight of native chicken, although in this experiment, low correlation was shown in abdominal fat pad, as it was also reported by Guo et al. (2011) in 13th generation of broiler chicken selected for divergent adipose tissue. However, it was in contrast with modern broiler chicken reported by Musa et al. (2006); Ojedapo et al. (2008).

Linear regression

The results of analysis by the linear regression are presented in Table 4. The coefficient determination were high (R^2 = 0.99- 0.88) except for Abdominal fat pad was low (0.453), showing that the relationship between Y (estimated weight of empty carcass) with A (actual age of individual) and LW (actual live weight of an individual), were high except for abdominal fat pad.

The R^2 is also known as coefficient of determination, measuring the goodness of fit of the mathematical model, giving value to unfixed variables proportionally to fixed variable. The value of R^2 stands between 0 and 1. The fit model has the R^2 closed to value of 1.

The models presented in Table 4 worked well in estimating the weight of carcass of live female SenSi-1 Agrinak chicken. This finding will certainly good information to genetic researchers in estimating how much carcass will be yielded by the selected chicken. Moreover, the information will be useful for the culinary in estimating how much they can obtained the carcass and carcass cuts of the female SenSi-1 Agrinak chicken in term of economic. Actually, such models had been reported on the different breed of chicken, which

showed the good fit for every particular breed or species observed (Raji et al. 2010; Banerjee 2011; Shafey et al. 2013). The reported models, however, those vary for every breed and species lead to examine the models that reported in this research, to see how accurate the models that can be calculated for the actual value. The model were then examined, using the actual weight of female SenSi-1 Agrinak chicken at the age of 15 weeks.

The test results of the models are presented in Table 5, showing that there were very little variation in gap between actual values and estimated values (0.09-4.43%). Therefore the mathematical models in estimating the carcass and carcass cuts of female SenSi-1 Agrinak chicken could be applied covering the age of 5 to 15 weeks.

Table 3. Correlation between two measured variables of female SenSi-1 Agrinak chicken at the age of 5, 8 and 15 weeks

Variables	Body-live weight	Empty Carcass	Head & neck	Whole back	Two wings	Whole breast	Two upper thighs	Two lower thighs	Two shanks	Gizzard	Liver	Abdominal fat pad
Body-live weight	1	0.997**	0.990**	0.994**	0.993**	0.991**	0.993**	0.994**	0.984**	0.930**	0.974**	0.701**
Empty carcass		1	0.989**	0.996**	0.995**	0.995**	0.997**	0.996**	0.983**	0.927**	0.970**	0.698**
Head & neck			1	0.986**	0.987**	0.982**	0.982**	0.988**	0.983**	0.924**	0.958**	0.702**
Whole back				1	0.989**	0.986**	0.991**	0.992**	0.980**	0.934**	0.969**	0.710**
Two wings					1	0.987**	0.989**	0.995**	0.988**	0.924**	0.969**	0.678**
Whole breast						1	0.993**	0.986**	0.973**	0.912**	0.959**	0.682**
Two upper thighs							1	0.989**	0.976**	0.924**	0.963**	0.701**
Two lower thighs								1	0.984**	0.924**	0.972**	0.699**
Two shanks									1	0.929**	0.968**	0.637**
Gizzard										1	0.923**	0.645**
Liver											1	0.686**
Abdominal fat pad												1

** Highly correlated (P<0.01)

Table 4. Mathematical models of estimating live weight, empty carcass and carcass cuts of female SenSi-1 Agrinak chicken

Variables(g/bird)	Mathematical models	Coeff.determination (R^2)
Body live weight	Y = -139.87 + 12.70 A[1)]	0.947
Empty carcass	Y = - 45.22 + 0.485 A + 0.638 LW	0.992
Head & neck	Y = 3.14 + 0.198 A + 0.063 LW	0.980
Whole back	Y = - 8.64 + 0.168 A + 0.154 LW	0.986
Two wings	Y = 0.054 + 0.069 A + 0,081 LW	0.983
Whole breast	Y = - 13.57 – 0.021 A + 0.187 LW	0.980
Two upper thighs	Y = -13.21 + 0.178 A + 0.11 LW	0.984
Two lower thighs	Y = - 6.643 + 0.041 A + 0.107 LW	0.984
Two shanks	Y = 3.43 + 0.018 A + 0.036 LW	0.967
Gizzard	Y = 3.408 + 0.101 A + 0.008 LW	0.886
Liver	Y = 3.24 – 0.030 A + 0.018 LW	0.951
Abdominal fat pad	Y = - 4.38 + 0.026 A + 0.015 LW	0.453

[1)] It stands for age (day); LW stands for live weight (g/bird)

Table 5. Actual *versus* estimated values of observed variables of female SenSi-1 Agrinak chicken

Variables	Actual (g)	Estimated (g)	Actual *versus* estimated values	
			(g)	(%)
Body live weight	1247	1193.63	52.93	4.43
Empty carcass	803	801.291	2.07	0.26
Head & neck	103	102.491	0.09	0.09
Whole back	201	201.038	0.35	0.17
Two wings	109	108.306	0.25	0.23
Whole breast	218	217.414	0.35	0.16
Two upper thighs	144	143.897	0.46	0.32
Two lower thighs	131	131.091	0.20	0.15
Two shanks	50	50.212	-0.14	0.28
Gizzard	24	23.989	-0.31	1.29
Liver	23	22.536	0.33	1.44
Abdominal fat pad	17	17.055	-0.35	2.03

CONCLUSION

In female SenSi-1 Agrinak chicken, estimation of weight of carcass and carcass cut can be calculated using the data of age (day) and live-body weight (g). The study also informed that estimation of body-live weight can be calculated based on the data of age (day). Furthermore, there is a high correlation relationship between the body-live weight with carcass cut.

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Effectiveness of DMSO Concentration on Recovery Rate and Viability of Primordial Germ Cell of Gaok Chicken

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ABSTRAK

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Perkembangan teknologi terbaru untuk memproduksi *germline chimera* dengan transfer *primordial germ cell* (PGC) ke dalam embrio penerima telah memungkinkan konservasi dan pengambilan kembali sumber daya genetik ayam dalam bentuk yang lengkap. Penelitian dilakukan untuk mendapatkan persentase krioprotektan dimethyl sulfoxide (DMSO) yang paling efektif terhadap *recovery rate* dan viabilitas PGC ayam Gaok sesudah pembekuan yang nantinya layak untuk ditransferkan. Dalam penelitian ini, telur fertil ayam Gaok diinkubasi selama sekitar 2,5 - 3 hari untuk mendapatkan embrio pada tahap 14 - 16. Pengambilan darah embrio dilakukan melalui aorta dorsalis dengan menggunakan mikropipet di bawah mikroskop. Prosedur isolasi PGC ayam Gaok dengan gradien sentrifugasi menggunakan nycodenz. Krioprotektan yang tersedia secara komersial digunakan untuk pembekuan PGC. PGC ayam Gaok hasil isolasi dan yang layak dibekukan diencerkan dengan krioprotektan yang mengandung 2,5; 5; dan 10 % DMSO dalam *fetal bovine serum* (FBS). Nilai *recovery rate* dari perlakuan DMSO 2,5; 5; dan 10 % berturut-turut adalah 36,4; 48,2 dan 48 %. Viabilitas PGC setelah pembekuan secara signifikan lebih tinggi untuk DMSO 5 % dibandingkan dengan perlakuan DMSO 2,5 % (P<0,05), akan tetapi tidak berbeda dengan perlakuan DMSO 10 %. Dari hasil penelitian dapat disimpulkan bahwa konsentrasi DMSO sebanyak 5 % pada pembekuan PGC ayam Gaok merupakan tingkat konsentrasi efektif.

Kata Kunci: Krioprotektan, DMSO, PGC, Ayam Gaok

ABSTRACT

Kostaman T, Yusuf TL, Fahrudin M, Setiadi MA. 2017. Effectiveness of DMSO concentration on recovery rate and viability of primordial germ cell of Gaok chicken. JITV 22(1): 30-37. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1799

Recent technological developments to produce germ line chimeras with primordial germ cell (PGC) transfer into the recipient embryo provide an opportunity to conserve and retrieval of chicken genetic resources in complete form. The study was conducted to obtain the most effective DMSO percentage to recovery rate and viability of Gaok chicken PGC after freezing which will later be feasible to be transferred. In this study, the eggs of Gaok chicken were incubated for about 2.5 - 3 days to obtain embryos at stages 14 - 16. Blood retrieval was done through the dorsal aorta using micropipettes under microscope. The procedure of PGC isolation of Gaok chicken with centrifugation gradient was using nycodenz as a substance. Commercially available cryoprotectants (dimethyl sulfoxide = DMSO) were used for PGC freezing. Isolated and frozen PGCs of Gaok chicken were diluted with cryoprotectants containing 2.5; 5 and 10% DMSO in *fetal bovine serum* (FBS). The recovery rate of 2.5; 5 and 10% DMSO concentration were 36.4; 48.2 and 48 % respectively. The viability of PGC after freezing was significantly higher for 5% DMSO compared with 2.5% DMSO (P<0.05), but not different from 10% DMSO. It can be concluded that the concentration DMSO of 5 % was effectve contration in freezing Gaok chicken PGC.

Key Words: Cryopreservation, DMSO, PGCs, Gaok Chicken

INTRODUCTION

It is reported by the International Union for Conservation of Nature (IUCN 2015) that more than 13% of bird species were endangered. Recent adopted strategies to conserve the bird biodiversity both *in situ* and *ex situ* were not effective (Sawicka et al. 2015). Therefore, the development of bird biodiversity conservation was indispensable.

In chicken, freezing sperm was one of the methods commonly used on genetic diversity (Blesbois et al. 2007; Santiago-Moreno et al. 2011), but might not guarantee the reconstruction of poultry species (Sawicka et al. 2015). Furthermore, freezing embryo and oocyte might not be performed due to its big size and high lipid content. As an alternative, primordial germ cell (PGC) freezing had been developed to preserve female and male animal germplasm (Liu et al.

2010; Silversides et al. 2013). PGC had been reported as a valuable starting material for cell-based genetic engineering, germplasm expansion and genetic preservation (Tonus et al. 2017). Furthermore, with the PGC cryopreservation as the genetic material seems effective for the conservation strategy.

Chicken PGC collected from embryo blood and gonad might be maintained in the form of frozen PGC in the liquid nitrogen using a medium containing dimethyl sulfoxide (DMSO) (Setioko et al. 2007; Nakamura et al. 2011) without changing its biological characteristics (Nandi et al. 2016; Tonus et al. 2016). Notman et al. (2006) reported that the DMSO that was used as cryoprotectant on phospholipid membranes caused cell membrane become more floppy and it was able to facilitate membrane fusion process. The cell became better in preparation of recieving the effect of stress caused by cryopreservation, thus reducing the molecule transport obstacles and helps the formation of a pore.

Freezing PGC of commercial chicken in a medium consisting of 10% serum and 5-10% DMSO had been reported to have various recovery rates (RR) and viabilities of PGC. Research of Moore et al. (2006) resulted in the viability of White Leghorn (WL) chicken PGC with DMSO addition by 10%, was 76.5%. Setioko et al. (2007) reported that percentage of RR and viability with DMSO addition by 10% on WL chicken PGC each was 49.9% and 83.5%, respectively. While, Nakamura et al. (2011) reported that addition of the same concentration of DMSO on Barred Plymouth Rock (BPR) showed recovery rate and viability by 54.3% and 86.8%, respectively. Kohara et al. (2008) reported the vitrification and slow freezing methods of RR value by 36.8 and 56.7 % respectively.

This study was expected to obtain optimal concentration of DMSO for RR and viability of Gaok chicken PGC after freezing and further feasible to be transferred.

MATERIALS AND METHODS

Isolation and collection of the primordial germ cell (PGC) on 14-16th level

As much as 60-70 fertile eggs of Gaok chicken were used in this study. Those eggs were incubated under 37.8°C with 60-65% of humidity using portable incubator (P-008B Biotype; Showa Furanki, Saitama, Japan). After reaching 14-16 stadium of 50-59 hours of incubation, the egg shell was cracked and the embryos were transferred into a petri dish (90 x 15 mm, LBS60001PT, BIOLAB).

Embryo blood collection through aorta dorsalis used micropipette under a microscope (Olympus SZ30, Japan). The collected blood was placed into eppendrof 1.5 ml tube which was filled by 1000 µl phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS-) in 10% fetal bovine serum (FBS, 26140 Gibco). Isolation procedure of Gaok chicken PGC with centrifugation gradient used nycodenz (Prod. No. 1002424, Axis-Shield Pos AS) (Kostaman 2014).

Selection of the primordial germ cell (PGC)

Evaluation of amount and quality of morphology of the PGC resulted from PGC purification process obtained from Gaok chicken embryo blood, was performed under microscope (Olympus CKX41, Japan). The PGC morphology is divided into two categories: feasible to be frozen and not feasible to be frozen. The PGC criteria that are feasible to be frozen were round; non-defective; equally large; symmetrical and transparent, while for the not feasible one were not symmetrical and not in the same size (Kostaman 2013) (Figure 1).

Stages of PGC freezing were (1) entering straw into methanol pool of embryo freezing tools under 25 ºC – (-7)°C with temperature decrease speed of 1 ºC per minute (for 32 minutes), (2) maintaining the temperature of -7°C for 10 minutes. Seeding was conducted in 2-3 minutes after reaching -7°C of temperature. It was performed to initiate the forming of ice crystal by pressing the of a dipped nipple into liquid nitrogen to the end of the straw to freeze the whole straw content, (3) re-entering straw into freezing and cooling tools until reaching -30°C of temperature with temperature decrease speed by 0.5°C per minute (for 46 minutes), and (4) entering and keeping the -30°C straw into liquid nitrogen with -196°C of temperature (Kostaman et al. 2011).

Thawing process of the frozen primordial germ cell (PGC)

Thawing process of the frozen PGC was performed by carrying out the straws from the liquid nitrogen container and then placed in the 39°C water (Setioko et al. 2007). As soon as the cryoprotectant media in the straw melted (± for 20 seconds), circulated-PGC was removed from the straw and then put into eppendorf tube and added by 1000 µl PBS-FBS 10% then was centrifuged under 1200 rpm for 7 minutes.

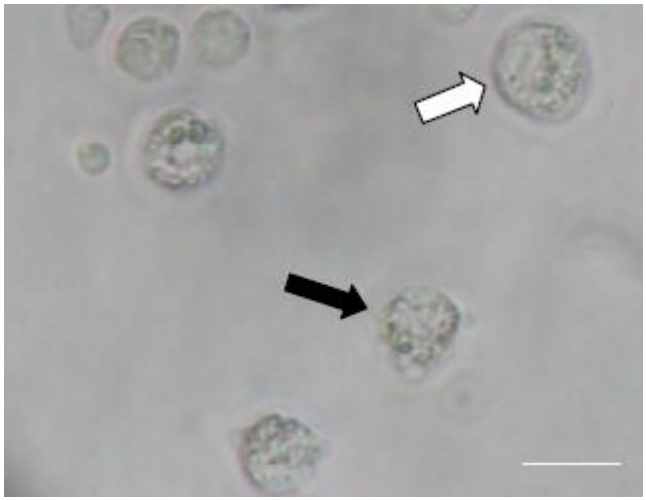

Figure 1. Morphology characteristic of Gaok chicken PGC. The feasible PGC to be frozen (showed by white arrow). Frozen PGC (showed by black arrow). Bar=40 µm. **Source:** Kostaman (2013).

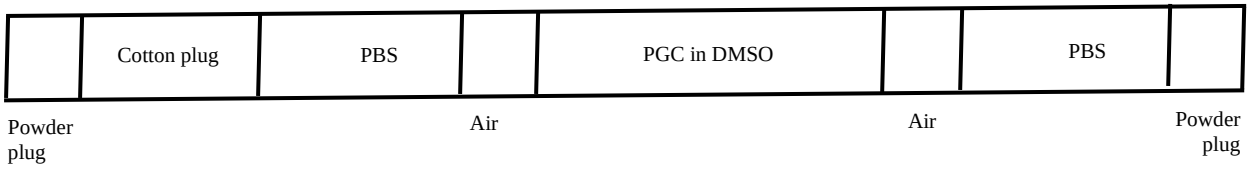

Figure 2. PGC in the mini straw

Viability of the primordial germ cell (PGC)

Frozen PGC that had been evaluated was then assessed for its viability using trypan blue staining (Freshney 2005): ± 20 µl cell PGC was mixed with 10 µl trypan blue 0.4% (Sigma-Aldrich Corporation, St. Louis, MO, USA) then stirred until homogeneous and aged for 2 minutes at room temperature. Then, its viability was measured under a microscope (Olympus CKX41, Japan). Life PGC was the PGC that did not absorb the blue stain and the dead one was the PGC that absorb the blue stain.

Variables observed

Variables observed were:

1. *Recovery rate* (RR) of circulated-PGC after thawing was the number of PGC that could be re-activated after being frozen divided by the number of PGC before being frozen multiplied by 100%.
2. PGC viability after thawing was the number of life PGC reduced by dead PGC divided by life PGC multiplied by 100%.

Analysis

Data were analyzed using complete randomized design (CRD) with concentration of DMSO as treatment and repeated five times. Data were also analyzed using ANOVA and continued by Duncan test when there was a difference between treatments based on Steel & Torrie (1995).

RESULTS AND DISCUSSION

The primordial germ cell (PGC) of Gaok chicken after

The quality of purified PGC that would be used in the freezing process was evacuated based on morphological characteristics. The evaluation of the morphological characteristics of feasible fresh PGC to be frozen was assessed according to round shape, no defect, in the same size and symmetrical and transparent, while the not feasible one was not symmetrical and not in the same size (Figure 1).

The number of PGC collected was 2,125 cells, where 1,513 cells (71.2%) were feasible to be frozen and the 612 cells (28.8%) were not feasible to be frozen. The feasible to be frozen PGC were divided into

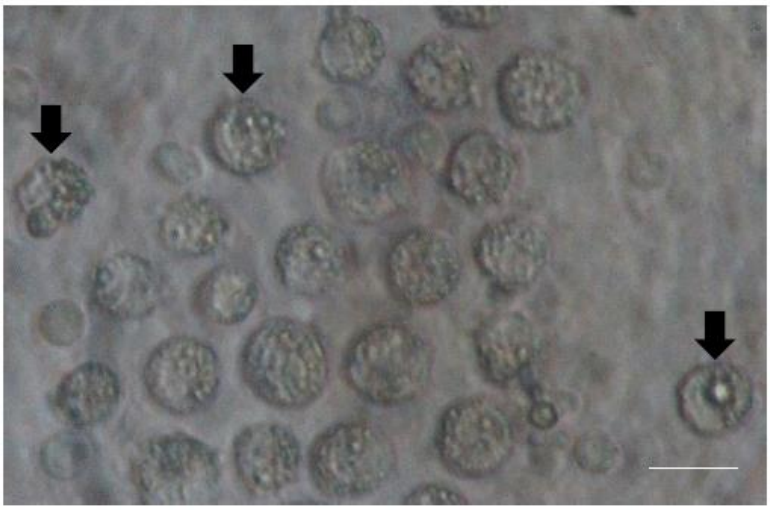

Figure 3. Morphological characteristics of Gaok chicken PGC after thawing. Dead PGCs are shown by the black arrow. Bar=40 µm. Source: Private documentation.

three treatments, where each treatment was repeated five times. PGC packaging used mini straws and was filled with 100 cells.

The success of a method in cryopreservation process might be determined through microscopic evaluation of PGC morphology. The PGC after freezing had almost similar morphology with the one before freezing. The cells were still intact, in a large round shape and the edge looks like a bright ring beneath the cell membrane. Meanwhile, the dead PGCs were dark with very little cytoplasmic mass (Figure 3). It is believed due to several changes and damages of membrane and organelles of PGC cytoplasm. Mohr & Trounson (1981) reported that structural changes following cryopreservation process were nucleus damage and plasma membrane.

Kohara et al. (2008) reported that the morphology of WL chicken did not show a difference between the PGC before and after being frozen. Life PGC looked like fresh PGC and cannot be distinguished from unfrozen PGC. The cells were still intact and had similar size and shape with the fresh PGC. In the other word, the morphology of frozen PGC was normal.

The comparison of concentration of DMSO on recovery rate (RR) of Gaok chicken primordial germ cell (PGC) after freezing

According to the result of morphological selection, the feasible to be frozen PGC then was observed its influence to concentration of DMSO resulted in difference effect on the RR percentage. Hafez (2000) reported that RR was observed to evaluate the effect of cryoprotectant on cell viability after cryopreservation process.

Average RR percentage of PGC after freezing in this study was relatively low. However, RR percentage with DMSO treatment by 5% showed a better role by the RR value of 48.2% (241 cells), which was higher compared to 10% and 2.5% DMSO concentration each by 48% (240 cells) and 36.4% (182 cells), respectively (Figure 4).

Statistical analysis result showed that DMSO concentration by 5 and 10% was not significantly different ($P>0.05$), while the 2.5% DMSO concentration was significantly lower ($P<0.05$). It was allegedly that the addition of 2.5% DMSO could not protect PGC during washing and centrifugation process leading to PGC damage. Damage cells during washing and centrifugation process would affect the RR. This condition was allegedly due to some changes and damages of membrane and PGC cytoplasm organelles. Damage level of PGC was showed by the damage of membrane, marked by intracellular ice crystals formation in the fast cooling process, as the osmotic effect and the attack mechanism of extracellular ice crystals during the slow cooling process. The formation of ice crystals during cryopreservation process lead to electrolyte buildup in the cell causing mechanical cell damage, in which the accumulated electrolyte would damage the cell wall, so that permeability of plasma membrane at the thawing process would change and the cell would die (Watson 2000).

Meanwhile, PGC treated by 5 and 10% of DMSO on the freezing medium was able to protect Gaok chicken PGC from any adverse effects. It could protect PGC by preventing ice crystals formation during the

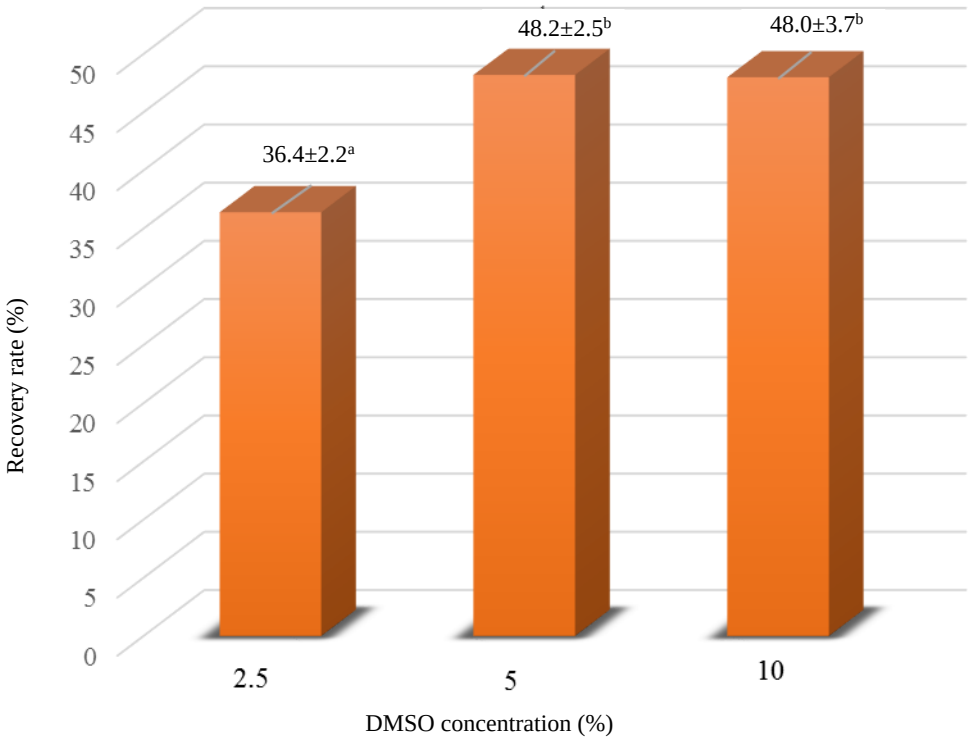

Figure 4. Recovery rate (RR) of circulated-PGC of Gaok chicken after freezing treated with DMSO.

freezing process due to excessive dehydration of cells and stabilizing cell plasma membrane that may prevent physical and functional damages during the freezing process and modify ice crystal structure.

It might prevent damage to PGC cytoplasm organelles. DMSO's cryoprotectant work process was by replacing the water in the cell so that dangerous ice crystals were not be formed (Valerdi et al. 2009). DMSO was able to penetrate the cell before the liquid cell was fully compacted during the freezing process, in which the electrolyte concentration inside and outside the cell was reduced. Meanwhile, the humidity inside the cell wold not be too much to penetrate outside and the dehydration could be avoided. In another word, DMSO served to protect the cell from damage lead by high concentration due to freezing process (Yan et al. 2016).

Average RR percentage of PGC after thawing in this study was lower than the PGC of WL (49.9%) and BPR (54.3%) chickens reported by Setioko et al. (2007) and Nakamura et al. (2011). The PGC in this study had allegedly experienced a lysis during the freezing process.

Rosato & Iaffaldano (2013) explained that cryoprotectant that penetrated to the cell membrane was needed to improve fluidity of membrane and partially dehydrated cells. DMSO would provide effective protection to the PGC when its concentration in the freezing medium was optimum. Not optimum DMSO concentration in the freezing medium leads to quality decease of the PGC. Administration of 2.5% DMSO into freezing medium in this study resulted in the lowest RR percentage after thawing. Therefore, the 2.5% DMSO treatment had not been able to provide optimal protection for PGC freezing of Indonesian native chickens.

Cell freezing optimization to maximize RR value after thawing depended on the formation of intracellular ice crystals and cryogenic damage due to the high concentration of solute when the intracellular water freezed. This could be achieved using hydrophilic cryoprotectant to absorb the water and fast thawing to minimize ice crystal forming (Freshney 2005).

As a comparison, Gautam (2008) reported that by using the slow freezing method, the addition of DMSO cryoprotectant on buffalo oocyte, the damage of RR was less than the addition of ethylene glycol (EG) or propylene glycol. Meanwhile, for the zebrafish embryo stadium, DMSO toxicity was not as much as glycerol, EG, methanol and N,N-dimethylacetamide (Lahnsteiner 2008).

The comparison of concentration of DMSO on viability of Gaok chicken primordial germ cell (PGC) after freezing

In this study, the highest percentage level of PGC viability after thawing was on 5% DMSO by 79.2 % (191 cells) followed by 10% DMSO concentration by 79 % (190 cells) and 2.5% DMSO concentration by 75.7% (138 cells) (Figure 5). This study result was in accordance with the study conducted by Moore et al. (2006) which showed that gonadal germ cell (GGC) of WL chicken frozen by DMSO cryoprotectant addition and ethylene glycol (EG) was less than 5% and resulted in low viability percentage.

Statistical analysis result showed that the viability level of 5% DMSO was not different significantly ($P>0.05$) from the 10% DMSO concentration. Those two concentrations were significantly different with the 2.5% DMSO, which described optimum cryoprotectant concentration in protecting PGC viability on the cryopreservation process. Use of the proper cryoprotectant concentration was a necessity in cryopreservation to avoid cell damage. Therefore, the addition of 5 and 10% DMSO on the freezing medium was able to protect PGC from cold stress during the freezing process. Its protection effect was by managing the balance of intracellular and extracellular electrolyte, so that biochemical process occurring on the PGC persisted and reduced the excessive dead of PGC. The existence of DMSO on the freezing medium was expected could increase electrolyte concentration to avoid adverse damages.

During the freezing process, PGC experienced changes in osmotic pressure due to the cryoprotectant addition or temperature change. A drastic change of temperature occured during circulated-PGC from room temperature to storage temperature (-196°C) or when the thawing process at 39°C lead to damage of PGC plasma membrane. The damage to plasma membrane lead to loss of necessary enzymes in the metabolic process so there was no energy generated which resulted in low viability (Rizal et al. 2003). The whole change of osmotic pressure or temperature would directly influence the quality of PGC. The difference of temperature and osmolarity between freezing medium and PGC caused a huge different in the water volume in the cell that lead to stress mechanism in the cell (Watson 2000).

Pegg (2002) reported that osmotic stress during thawing was caused by the effect of excessive cryoprotectant, causing the cell to swell and busted. It was allegedly due to the extortionate osmotic pressure of the medium liquid, so the water in the cell would break out and cause dehydration. (Best 1990). Furthermore, Best (1990) reported that cell water consisted of bulk water that filled about 90% of the cell and bound water which only filled 10% of the cell. Bulk water is the water that can be frozen and will come out due to osmotic pressure change. Whereas, bound water is water molecule which 20-100 times more viscous than bulk water. Its hydrogen bond is very tightly bound to hydrophilic surface and macromolecule. During the freezing process, the exterior cell will freeze first and will draw the water into the cell out.

Generally, results of this study both the RR percentage and the viability of PGC were lower than study reported by Setioko et al. (2007) who obtained RR percentage and viability by 49.9% and 83.5%, respe and Nakamura et al. (2011) by 54.3% and 86.8%, respectively. It is strongly allegedly due to the different method used in each study. The method used in this study was slow freezing with freezing speed setting.

Total time needed to freeze the PGC was 1 hour and 28 minutes to reach -30°C, before entered into liquid nitrogen (-196°C). Meanwhile, the freezing method used by Setioko et al. (2007) and Nakamura et al. (2011) was freezing PGC for 24 hours to reach -80°C, to enter into liquid nitrogen (-196°C). Slow freezing with freezing speed setting had an advantage of control at every step and limiting the effect of cryoprotectant toxicity using low concentration (Santos et al. 2006).

Apart from the different methods of freezing, the other factor affecting RR percentage and viability was packaging material used for PGC storage. In this study, the PGC was packed in the mini straw, while the previous study was packed in cryovial. Mohamad et al (2005) reported that the type packaging would influence temperature decrease and re-liquefaction rate of the cell. Straw packaging was more practical to provide clearer sample identification (Benesova & Trefil 2016). Kostaman et al. (2011) reported that PGC freezing packed with straw 0.5 ml resulted in the highest recovery rate and viability of 44.9 and 7.4%, respectively. Then Riesco et al. (2012) reported that zebrafish PGC packed in straw showed viability by 70%, better than PGC packed in microcapsule that showed viability by 20%.

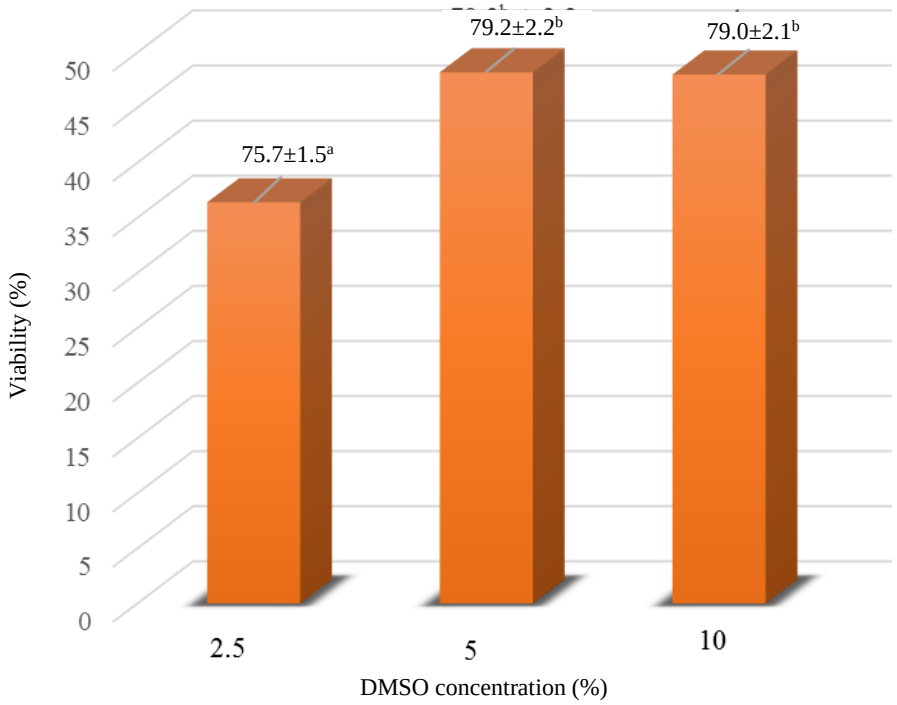

Figure 5. Viability of Gaok chicken PGC after freezing, treated with DMSO concentration.

CONCLUSION

It can be concluded that DMSO concentration by 5 or 10% on Gaok chicken PGC showed better role in protecting PGC quality (recovery rate and viability) during the freezing process compared to the 2.5% DMSO. The morphology characteristics of Gaok chicken after the freezing and thawing were similar with the fresh PGC.

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Genetic Variability of ESAG 6/7 Gene Isolat *Trypanosoma evansi*

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ABSTRAK

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Trypanosoma evansi (*T. evansi*) sebagai agen penyakit Sura merupakan salah satu penyakit parasitik penting untuk diperhatikan karena dapat menimbulkan kerugian ekonomi yang sangat besar di Indonesia. Parasit ini memerlukan zat besi untuk fase propagasi yang diperoleh dari pejamu melalui reseptor transferrin (Tf) yang dikode oleh *Expression Site Associated Genees* (ESAGs). ESAG6/7 dilaporkan mengkode reseptor transferrin pada afinitas yang berbeda pada pejamu yang berbeda.Adanya perbedaan patogeneitas *T. evansi* diduga menyebabkan variabilitas pada gene ESAG 6/7. Penelitian ini bertujuan untuk melihat variabilitas gene ESAG6 *T. evansi* dengan virulensi yang berbeda pada mencit. Penelitian ini dilakukan dengan 2 tahap yaitu uji patogeneitas *T. evansi* pada mencit dan analisis sekuen gene ESAG6/7. Uji patogeneitas dilakukan dengan melihat median lama hidup mencit setelah masing-masing kelompok diinfeksi dengan 25 *T. evansi* asal kerbau dari berbagai kondisi geografi. Hasil penelitian uji patogeneitas menunjukkan adanya perbedaan virulensi pada 25 isolat T.evansi pada mencit. Hasil analisis sekuensing gene ESAG6/7 dari 25 isolat *T. evansi* asal Indonesia cenderung homogene pada daerah pengikatan transferin (Tf) tetapi ditemukan adanya variabilitas pada hiper variable site. Perubahan tersebut mampu memisahkan isolat *T. evansi* virulensi tinggi dan rendah. Hasil analisis pohon filogenetika terbentuk 11 clade dari 25 isolat *T. evansi*. Isolat dengan virulensi tinggi termasuk dalam clade 7 dan 10. Isolat dengan virulensi rendah masuk dalam clade 5 dan 11. Isolat dengan virulensi moderat terbagi dalam dua *clade* tersebut.

Kata Kunci: *T. evansi*, Gen ESAG6/7, Variabilitas, Virulensi

ABSTRACT

Sawitri DH, Wardhana AH. 2017. Genetic variability of ESAG6/7 gene *Trypanosoma evansi*. JITV 22(1): 38-50. DOI: http://dx.doi.org/10.14334/jitv.v22i1.1638

Trypanosoma evansi as an agent of Surra is one of the crucial parasitic diseases that cause great economic losses in Indonesia. These parasites need iron for growth and propagation phase which is obtained by receptor-mediated uptake of host transferin. The transferrin receptors are encoded by Expression Site Associated Genees (ESAGs). ESAG6/7 encodes transferrin receptors which reported have different affinities of a different host. The distinction of *T. evansi* pathogenicity is supposed to cause variability in the ESAG6/7 gene. This research was aimed to investigate the variability of genes ESAG6/7 *T. evansi* with different virulence in mice. This research was conducted in two steps: bioassay pathogeneicity in mice and analysis of ESAG6/7 gene sequences. The median survival time of mice was investigated after each group of mice infected by 25 *T. evansi* isolates from buffaloes where its geographically differ. The test results showed a difference of pathogenic virulence on 25 *T. evansi* isolates in mice. Sequence analysis of the ESAG6/7 gene from 25 *T. evansi* isolates origin from Indonesia tended to be homogeneous on the transferrin binding site but there was variability in the hypervariable site. These changes are able to separate high and low virulence of the *T. evansi* isolates. Phylogenetic tree analysis was formed 11 clades of 25 *T. evansi*. High virulence *T. evansi* was included in clades 7 and 10, while low virulence *T. evansi* was included in clade 5 and 11 and the moderate virulence was divided into those four clades.

Key Words: *T. evansi*, ESAG6/7 Gene, Variability, Virulence

INTRODUCTION

Trypanosomiasis caused by *T. evansi* has widely spread South America, Africa and Asia (Sarkhel et al. 2017). This blood protozoon is the cause of Surra disease which is one of the crucial parasitic diseases on the animal in Indonesia. Almost in all area in Indonesia is an endemic area of Surra. The disease has caused huge economic losses on the late case in Sumba during 2010-2012 (direct communication with East Sumba Livestock Services 2012) and in Pandeglang, Banten during 2013-2014 (direct communication with Indonesian Research Center for Veterinary Sciences, 2014).

Various biochemical and molecular typing on *trypanosomiasis* have been widely developed to describe the correlation between species and subspecies (Amer et al. 2011). *Trypanosoma evansi* consists of a

large number of morphologically identical populations that significantly different on various biological characters such as host range, virulence, pathogenicity and drug sensitivity (Sarkhel et al. 2017). Reid (2002) stated that there are different clinical manifestation and geography differences of isolate that supposed to show high genetic diversity between isolates, especially related to pleiotropism phenomenon (single gene that causing various phenotypic). This was supported by study result of Masiga et al. (2006) who found a genetic diversity of *T. evansi* from Kenya according to the amplified fragment length polymorphism/AFLP test (Masiga et al. 2006). A genetic variable of *T. evansi* was also reported based on the *expression-site-associated* gene *e* 6/7 (ESAG6/7) as a marker (Mekata et al. 2009; Villareal et al. 2013). Trypanosome is able to avoid the immune attack of the host by changing the expression of Variant Surface Glycoprotein (VSG) genes. Variant Surface Glycoprotein expressed was located in the end part of the telomere, the polycistronic transcription unit called VSG expression site (VSG-ESs) (Hutchinson et al. 2016). The polycistronic VSG ESs consisted of a number of Expression Site Associated Genes (ESAGs) which one of it had been characterized as ESAG6/7 involved in the nutrient acquisition (Schell et al. 1991).

Trypanosome takes iron in the blood circulation through high-affinity receptor mediated by an iron-bound transferrin endocytosis called holo-Tf. This receptor was encoded by homolog ESAG6 and 7 genes (Sarkhel et al. 2017). These genes were reported able to detect *T. evansi* genetic diversity in South America (Mekata et al. 2009). As much as 20 variants have been identified (Witola et al. 2005). Variable Surface Glycoprotein Expression Site (ES) forms complex nucleotide pattern as long as 40-60kb. The upstream part of the VSG gene is repeated unit as long as 70bp which is suspected to play a role in the gene conversion. At the part of ES promotor and upstream part of the VSG, there is minimal six different open reading frame (ORF) called expression-site-associated genee (ESAG). In the blood circulation, *T. evansi* used Tf receptor to take the whole iron (Fe) from the host blood for growth and multiplication **(**Kabiri & Steverding 2001; Sarkhel et al. 2017**).**

Transferrin is a protein from a beta globulin group that binds and distributes iron in the blood serum. Transferrin receptor is encoded by two homolog expression-site-associated genees (ESAG6 and ESAG7) located in VSG area. Transferrin receptor was heterodimer receptor linked by Glycosylphosphatidylinositol (GPI) (Mehlert et al. 2012). As much as 20 ESAG 6/7 variants were equivalent with the number of VSG expression site but there was only one that active sometimes (Isobe et al. 2003; Witola et al. 2005). That two ESAG only showed the different of a sequence about 1-10% (Witola et al. 2005; Mekata et al. 2009). Despite that small difference, it was reported to be able to cause affinity difference of transferrin host (Bitter et al. 1998; Steverding et al. 1995). Bitter et al. (1998) reported that natural variability of ESAG6/7 might affect the ability of trypanosome to take Tf molecule from various species of Mammalia as host. Isobe et al. (2003) and Witola et al. (2005) reported that on *Trypanosoma equiperdum*, where this parasite only infected horse, has known as ESAG6 gene, which was less diverse compared to *T. brucei and T. evansi* infecting wide range host (Isobe et al. 2003). This indicated that the diversity of ESAG6/7 gene had an important role in the ability of parasite to adapt the different hosts (Mekata et al. 2009).

Nevertheless, a hypothesis of a chance of *T. brucei* to express different Tf receptor related to the Tf difference obtained on various mammalian species (Gerrits et al. 2002). Recent results indicated that iron storage caused an increase of 3-10 times from Tf area along with redistribution of the receptor from flagellar pocket to entire parasite surface (Mehlert et al. 2012). On the cronical infected animal, the Tf level of host decreased then trypanosome developed an ability to grow on low iron concentration by increasing the level of expression area of Tf so that increase iron infusion (van Luenen et al. 2005). Therefore, activity or inactivity of transcription from VSG Es gene was a mechanism of very active regulation causing the parasite quickly responses the environment change. Therefore, the ESAG6/7 sequence had high polymorphism on site which related to Tf binding part contributed on iron requirement fulfilling and its adaptation on the different host (Gerrits et al. 2002). This gene was also suspected to be able to describe genetic diversity of *T. evansi* that allegedly related with pathogeneicity difference (Witola et al. 2005)**.**

The result of pathogeneity level study on mice showed that nine Indonesian *T. evansi* isolated from different geographic had different pathogeneicity level on mice (Wardhana et al. 2011). This pathogenetic level difference is allegedly to cause genetic diversity of ESAG 6/7 *T. evansi* gene of Indonesian isolates that have not been studied so far. This study was conducted to observe the variability of ESAG6/7 gene on various *T. evansi* isolates from the same host that had different virulence on mice. The existence of variability on this gene is expected able to be used as marker to distinguish the virulence of local *T. evansi* isolate. This marker then can be used to help determine the spread of *T. evansi* isolate in Indonesia according its virulence.

MATERIALS AND METHODS

Ethics committee

This research has been approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia with the number of Ethics 24 / H2.F1 / ETIK / 2013.

Trypanosoma evansi isolate

As much as 25 Trypanosome sp isolates were used in this study were the Culture Collection of the Indonesian Research Center for Veterinary Sciences (BCC) for 1985-2008 and osilates from outbreak site during 2012-2014 (Table 2). Those isolates were stored as stabilate in the form of cryopreservation using glycerol as the cryoprotectant.

Table 1. Samples of *T. evansi* isolates used in this study

Isolate code	BCC code	Isolate source (Sub District, District, Province)	Year of isolation	Type of animal	Information
Bang 85		Bangkalan, Bangkalan East Java	1988	Buffalo	BCC
Bang 87	P0176	Bangkalan, Bangkalan, East Java	1988	Buffalo	BCC
Pml 287	P232	Pemalang, Pemalang, Central Java	1996	Buffalo	BCC
Pml 291	P233	Taman, Pemalang, Central Java	1997	Buffalo	BCC
Sbw 340	P202	Lape Lopok, Sumbawa Besar, West Nusa Tenggara	1998	Buffalo	BCC
Sbw 341	P203	Lape Lopok, Sumbawa Besar, West Nusa Tenggara	1998	Buffalo	BCC
Sbw 366	P029	Lape Lopok, Sumbawa Besar, West Nusa Tenggara	1999	Buffalo	BCC
Smi 68	P169	Surade,Sukabumi, West Java	1985	Buffalo	BCC
Smi 369	P125	Surade, Sukabumi, West Java	2008	Buffalo	BCC
Smb 370	-	Wajelo, Sumba Timur, East Nusa Tenggara	2012	Buffalo	Outbreak, Dept of Paracitology
Smb 371	-	Wajelo, Sumba Timur, East Nusa Tenggara	2012	Buffalo	Outbreak, Dept of Paracitology
Smb 372	-	Wajelo, Sumba Timur, East Nusa Tenggara	2012	Buffalo	Outbreak, Dept of Paracitology
Smb 373	-	Wajelo, East Sumba, NTT	2012	Buffalo	Outbreak, Dept of Paracitology
Smb 374	-	Wajelo, East Sumba, East Nusa Tenggara	2012	Buffalo	Outbreak, Dept of Paracitology
Smb 375	-	Wajelo, East Sumba, East Nusa Tenggara	2012	Buffalo	Outbreak, Dept of Paracitology
Lbk 376	-	Lebak, Banten	2013	Buffalo	Outbreak, Dept of Paracitology
Munt377	-	Muntilan, Central Java	2013	Buffalo	Endemic, Dept of Paracitology
Pdg 378	-	Cisata, Pandeglang, Banten	2013	Buffalo	Outbreak, Dept of Paracitology
Pdg 379	-	Cisata, Pandeglang, Banten	2013	Buffalo	Outbreak, Dept of Paracitology
Pdg 380	-	Cisata, Pandeglang, Banten	2013	Buffalo	Outbreak, Dept of Paracitology
Pdg 381	-	Cisata, Pandeglang, Banten	2013	Buffalo	Outbreak, Dept of Paracitology
Pdg 382	-	Carita, Pandeglang, Banten	2014	Buffalo	Outbreak, Dept of Paracitology
Pdg 384	-	Carita, Pandeglang, Banten	2014	Buffalo	Outbreak, Dept of Paracitology
Pdg 386	-	Cisata,Pandeglang, Banten	2014	Buffalo	Outbreak, Dept of Paracitology
Pdg 388	-	Cisata, Pandeglang, Banten	2014	Buffalo	Outbreak, Dept of Paracitology

Experimental animals

As much as 80 male mice of DDY of 10-12 weeks old with body weigh around 25-30 g were obtained from the National Agency of Drug and Food Control, Jl. Percetakan Negara, Jakarta. Those mice were fed with commercial pellets (Indofeed) in the morning and evening as much as 5-10 g per head/day. The drinking water was offered *ad libitum*. Twenty mice were used for *T. evansi* multiplication and those 60 mice were used for biological test.

***T. evansi* multiplication on mice**

Isolates stored with cryopreservation in the liquid nitrogen was thawed first then diluted using Phospat Buffer Saline Glucose (PBSG) up to 0.2 mL and injected (0.1 mL/mice) intraperitoneally (IP) to multiply the parasite infection material. Parasitemia level of experimental animal was evaluated every two days. When the parasitemia level reached the peak (about 10^7-10^8 *trypanosoma*/mL blood), the experimental animal were sacrificed and the blood was collected. The blood containing *T. evansi* was used as the source of infection to be injected to the experimental animal by 10^4 parasites per mice intraperitoneally (IP) and partially used for DNA isolation.

***T. evansi* counting**

Parasite counting was conducted to obtain parasite concentration by 1x 10^4 parasite/ml blood to be tested its virulence on mice and for observation of parasitemia level every two days. For the parasitemia tested in every two days, the blood samples were collected from vena tail: the end of tail was cleaned using alcohol 70% then wounded. The dripping blood was collected by micropypet (± 10 µL), then mixed with SDS 1% (1 : 1) until homogeny. The dilution was then diluted immediately using PBSG with 1 : 100 or 1 : 1000 ratio according to its patacytemia level and examined using hemocytometer (Naubeuer Improved) (Subekti et al. 2013). The number of parasite was counted in leucocyte counting room using the following formula:

The number of parasite/mL = A x B X 10^4

A = The number of *trypanosoma* counted on the leucocyte room
B = dilution factor

Pathogenicity test of *T. evansi* on mice

Determination of pathogenicity level was to identify *T. evansi* isolates which were categorized as high, moderate and low virulence. As much as 25 *T. evansi* isolates were infected to mice by 10^4 parasite/0.3 mL dose. Each isolate was infected into 3 male mice. The observation of the experimental animal death was performed twice a day along with the feeding time.

Criteria of virulence level were in accordance with Wardhana et al. (2011):

- High virulence: when the tested isolates were able to kill mice within less than one week.
- Moderate virulence: when the tested isolates were able to kill the mice in 8 days-2 weeks
- Low virulence: when the tested isolates were able to kill the mice in more than 2 weeks.

DNA extraction

DNA extraction of *Trypanosoma sp* on the mice blood was performed using Genomic DNA Mini Kit (Geneaid, Taiwan) following the procedure offered in the kit. The extracted DNA of each isolate was filled in the 1.5 mL tube, be labeled and stored at -20°C for further analysis.

PCR optimization of ESAG 6/7 *T. evansi* gene

PCR amplification was performed using Primer ESAG 7 Forward (F): 5'-CATTCCAGCAGGAGTTGGAGG-3' and ESAG 6 Reverse (R): 5'-TTGTTCACTCACTC TCTCTTTGACAG-3' and ESAG 6 Reverse (R): 5'-TTGTTCACTCACTC TCTCTTTGACAG-3' on ABi GeneAmp Thermal Cycler 9700 machine. Each reaction has total volume by 25 µl using KAPA2G^{TM} Fast PCR kit (KAPA BIOSYSTEMS, USA). PCR condition in this study was pre-denaturation (95°C, 3 minutes, 1 cycle); denaturation (95°C, 10 seconds, 35 cycles); primer attachment (58°C, 15 seconds, 35 cycles); DNA fragment extension (72°C, 15 seconds, 35 cycles) and the last DNA fragment extension (72°C, 10 minutes, 1 cycle). Amplification results were visualized on 1.5% agarose gel using SYBR® Safe gel staining (Invitrogene^{TM}) with electrophoresis technique (100 volt 20 minutes). The visualization and vaccinated DNA bands analysis were performed in the GelDoc Transluminator (Cleaver).

Sequencing and phylogenic tree analysis of ESAG 6/7 *T. evansi* gene

Sequencing process was performed using ABI Prism 3.1.1 sequencing machine. Sequence of gene was edited using DNAbaser version 4 (BioSoft 2013) software. Translation of DNA sequence into protein was performed using translation program (http: //tw expasy.org/tools/dna.html). Sequence alignment and

phylogenic tree were constructed using MEGA program version 6 (Tamura et al. 2013). Calculation of genetic distance value used neighbor joining genetic distance method with 2 parameters Kimura substitution model (K2P) with 2000 replications.

Table 2. Sequence reference of *Trypanosoma* on ESAG6/7 site from gene bank for amino acid analysis

Access number	Species/Isolate	Host	Location	Reference
AB 551909	*T. evansi*	Camel	Egypt	Amer et al. 2011
AB551912	*T.evansi*	Camel	Egypt	Amer et al. 2011
AB551914	*T. evansi*	Camel	Egypt	Amer et al. 2011
AB551917	*T.evansi*	Camel	Egypt	Amer et al. 2011
AF068704	*T. brucei rhodesiense*	-	-	Pedram and Donelson, unpublished
AF068705	*T. brucei rhodesiense*	-	-	Pedram and Donelson, unpublished
EU726388	*T. brucei rhodesiense*	-	-	(Young et al. 2008)
EU726354	*T. brucei gambiese*	-	-	(Young et al. 2008)
EU726385	*T. equiperdum*	-	-	(Young et al. 2008)
EU726387	*T.equiperdum*	-	-	(Young et al. 2008)
EU726388	*T.equiperdum*	-	-	(Young et al. 2008)
EU726389	*T.equiperdum*	-	-	(Young et al. 2008)
EU726392	*T.equiperdum*	-	-	(Young et al. 2008)
EU726431	*T.brucei brucei*	-	-	(Young et al. 2008)
XM 840855	*T. brucei brucei*	-	-	-
KR 858296	*T. evansi*	-	-	-
KR 858299	*T.evansi*	-	-	-

RESULTS AND DISCUSSION

Pathogenicity test on mice

Pathogenicity is generally determined by infectivity and virulence. Infectivity is defined as the ability of parasite to multiply and stay alive on the host, while virulence is the capacity of parasite to damage and cause a disease. Study of T. evansi from Brazil showed the virulence heterogeneity of isolate and laboratory animal pathogenicity (Queiroz et al. 2000; de Menezes et al. 2004). Herrera et al. (2001) reported that artificial infection on the domestic and wild animal showed virulence difference and pathology change.

This study results showed that life time of mice in each group that infected by 25 *T. evansi* isolates from Indonesia was around 4-28 days. Those results could be divided into three categories: high, moderate and low. High virulence was when the mice death less than one week, moderate virulence was when the mice death between 7-14 days and the low virulence was when the mice death more than two weeks (Table 4). Those categories was consistent with the research result of Wardhana et al. (2011).

According to virulence of T. evansi isolate and the number of mice death on the 30 days observation, it may be categorized into three groups: group I (high virulence), group II (low virulence) and group III (moderate virulence) with life time median by 0+ 0.25 day: 21.0+0.47-28.4+0.68 days and 7.6+0.71- 9.0 + 0.64 days (Table 4). This result was similar with the T. evansi isolates from Philippine reported by Mekata et al. (2013) who reported that the virulence of T. evansi isolate from Philippine was divided into three categories: high virulence when the life time median + standard error (SE) was less than 12.86 days after infection (hpi) and low virulence when the life time median + standard error (SE) was more than 14.56 hpi. Isolates that did not be categorized into those two categories was considered as moderate virulence.

This research result also showed that isolates from the same location might have same or different virulence. As an example, Sumba isolate from same location had two virulence categories as high virulence (Smb372) and moderate virulence (Smb370, 371, 373, 374, 375). This was similar with T. evansi isolate from Philippine. Verdillo et al. (2012) stated that T. evansi isolate from Philippine collected from Luzon, Visayas

and Mindanao Islands, showed different virulence. Branda˜ et al. (2002) and O'Garra (1998) stated that the pathogenicity of Surra depended on strain virulence, infection route and individual sensitivity of host.

Table 3. Sequence reference of *Trypanosoma* on ESAG6/7 site from gene bank for clade determination

Access number	Species/Isolate	Location	Reference
AB179565	*T. evansi*	Thailand	Witola et al. 2005
AB179576	*T. evansi*	Thailand	Witola et al. 2005
AB179578	*T. evansi*	Thailand	Witola et al. 2005
AB179594	*T. evansi*	Thailand	Witola et al. 2005
AB179599	*T. evansi*	Thailand	Witola et al. 2005
AB179604	*T. evansi*	Thailand	Witola et al. 2005
AB179607	*T. evansi*	Thailand	Witola et al. 2005
AB179617	*T. evansi*	Thailand	Witola et al. 2005
AB179613	*T. evansi*	Thailand	Witola et al. 2005
AB496616	*T. evansi*	Peru	Mekata et al. 2009
AB496621	*T. evansi*	Peru	Mekata et al. 2009
AB496622	*T. evansi*	Peru	Mekata et al. 2009
AB496623	*T. evansi*	Peru	Mekata et al. 2009
AB496626	*T. evansi*	Peru	Mekata et al. 2009
AB496627	*T. evansi*	Peru	Mekata et al. 2009
AB496629	*T. evansi*	Philippine	Mekata et al. 2009
AB496630	*T. evansi*	Philippine	Mekata et al. 2009
AB496631	*T. evansi*	Philippine	Mekata et al. 2009
AB496633	*T. evansi*	Philippine	Mekata et al. 2009
AB496638	*T. evansi*	Philippine	Mekata et al. 2009
AF068702	*T. brucei rhodesiense*	-	Alarcon, Pedram, and Donelson 1999
AF068703	*T.brucei rhodesiense*	-	Alarcon, Pedram, and Donelson 1999
AJ007021	*T. brucei brucei*	-	Ansorge et al. 1999
AJ007023	*T. brucei brucei*	-	Ansorge et al. 1999
AJ007026	*T. brucei brucei*	-	Ansorge et al. 1999
AY152684	*T. equiperdum*	-	Isobe et al. 2003
AY152688	*T.equiperdum*	-	Isobe et al. 2003

Sequencing Analysis of ESAG6/7 *T. evansi* gene

To determine the genetic variability of ESAG6/7 of Indonesian *T. evansi* isolate, as much as 25 *T. evansi* isolates with different virulence were analyzed along with *T. evansi*, *T. brucei* and *T. equiperdum* from GeneBank in this study. Multiple Sequence Alignment and phylogeny tree analysis of amino acid sequence was confirmed that there were 4 variants of the total 25 *T. evansi* isolates (Figure 2 and 3). Only a small part of the amino acid sequence showed any variability. However, Witola et al. (2005) and Mekata et al. (2009) reported that ESAG6/7 gene only showed sequence difference about 1-10%. Despite its small difference, but its reported to cause affinity difference of different transferrin host (Bitter et al. 1998). In contrast to the *T. evansi* isolates from Thailand, Indonesian *T. evansi* isolates tend to be homogeneous on the transferring binding site located on box II and III (Figure 3) (Salmon et al. 1997). Isobe et al. (2003) stated that

Table 4. Median of long life, death and virulence of *T. evansi* on mice

Isolate	Median of long life ± SE day)	Dominant mice death	Virulence
Bang85	4±0.00	< 7 hpi	High
Bang87	4±0.00	< 7 hpi	High
Pml287	21±0.47	>15 hpi	Low
Pml291	21±0.62	>15 hpi	Low
Smi68	4±0.00	< 7 hpi	High
Smi369	4±0.00	< 7 hpi	High
Sbw 340	5±0.00	< 7 hpi	High
Sbw 341	4±0.00	< 7 hpi	High
Sbw 366	4±0.00	< 7 hpi	High
Smb370	7.6±0.71	7-14 hpi	Moderate
Smb371	9±0.66	7-14 hpi	Moderate
Smb372	5±0.00	7-14 hpi	High
Smb373	9±0.62	7-14 hpi	Moderate
Smb374	9±0.64	7-14 hpi	Moderate
Smb375	11±0.00	7-14 hpi	Moderate
Lbk376	4±0.25	< 7 hpi	High
Mun377	4±0.00	< 7 hpi	High
Pdg378	10.6±0.62	7-14 hpi	Moderate
Pdg379	11±0.33	7-14 hpi	Moderate
Pdg380	8.3±0.58	7-14 hpi	Moderate
Pdg381	11±0.63	7-14 hpi	Moderate
Pdg382	24.4±0.51	>15 hpi	Low
Pdg384	28.4±0.68	>15 hpi	Low
Pdg386	4+0.00	>15 hpi	High
Pdg388	4+0.00	>15hpi	High

ESAG6/7 variant of *T. equiperdum* that had been cloned and characterized, was less varied on the transferrin binding site compared to the *T. brucei*. The *T. equiperdum* only infect horse; so that the genetic diversity disappeared along with the absence of selection (Moran 2002). It was allegedly that ESAG6/7 gene became more homogeny on the area that was not influenced by positive selection including the Tf binding site (Witola et al. 2005). Low viability value in this study could be explained due to the *T. evansi* used in this study, which was from the same host, buffalo. Nevertheless, it was found amino acid variability located on the hyper variable site (HV). Variation of amino acid residual on the HV site and/or on the Tf binding site might help the parasite to avoid immune response. The HV site had played a role in anticipating antigenic surface variation and not involved in the Tf binding site (Salmon et al. 1997).

Even though the 25 *T. evansi* isolates in this study were from the same host, but it showed different virulence on mice from various location with different geographic. Therefore, the amino acid change on that position might affect antigenic characteristic of the residue located on the receptor surface and would be exposed to the outside environment

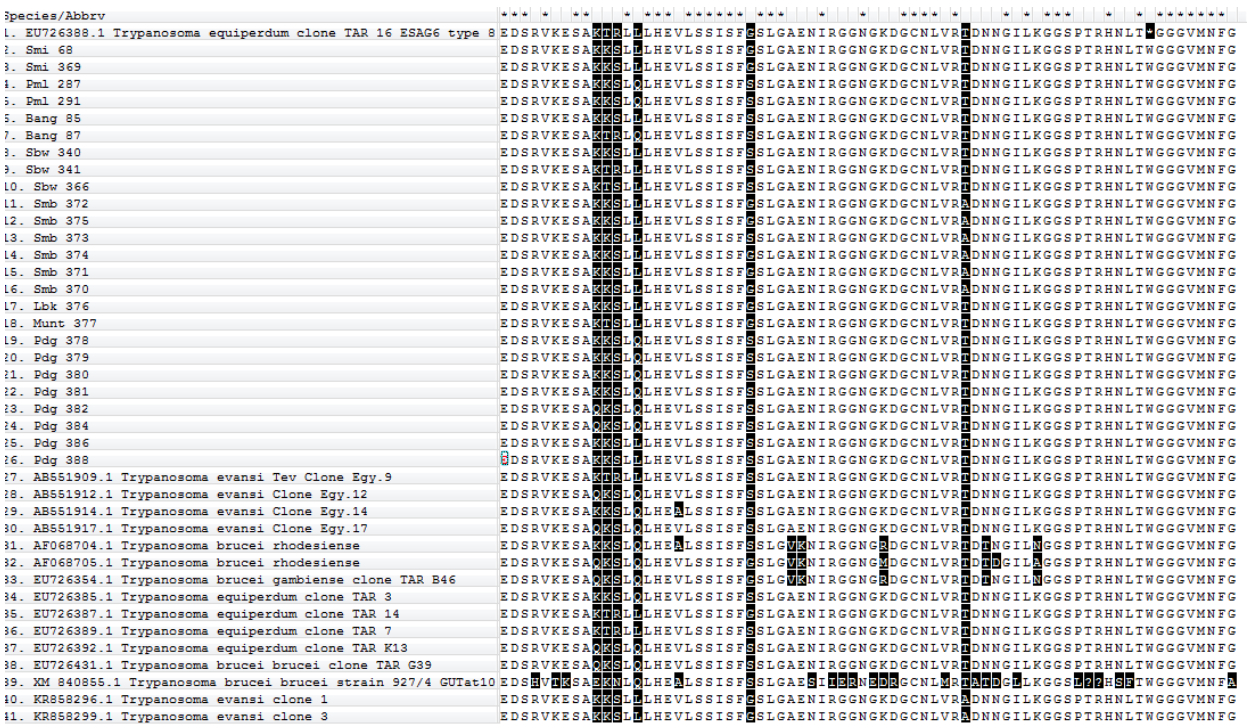

Figure 2. Sequence alignment of amino acid of ESAG6/7 on 25 Indonesian *T. evansi* isolates compared to *T. evansi, T. brucei* and *T.equiperdum* sequences form GeneBank. Two protein locations of ESAG6 which is a part of transferrin improvement site (I and II , Salmon et al. (1997) that showed by the box.

Salmon et al. (1997) stated that the sequence variation on transferrin binding site on the ESAG6/7 gene clearly seen on *T. equiperdum* and *T. brucei* did not play a role on improvement of the transferrin on antigenic variation on the receptor surface due to a mutation in this site but did not significantly influence the Tf improvement. Tf I receptor, which was look very much like its structure with VSG. Amino acid sequence on the very strategic position on the Tf receptor indicated that its residue was on the loop which would be exposed to the outside environment (Salmon et al. 1997). Even it was a site with high variability and located on the end molecule, but it was not yet certain whether it would be exposed and antigenic. Antigenic variation influenced was the one that was located on the receptor surface (Isobe et al. 2003). Even the *T. equiperdum* was closely related to the *T. brucei*, due to no selection pressure for telemeric maintenance of VSG ES. The number of copy of genetic variability and the selection power of this telemeric sequence would also decrease (Isobe et al. 2003).

The control of antigenic variation was influenced by two main things: the virulence and avoiding strategy of immune response, and expression of mono-allele gene (Lyons et al. 2013). Glover et al. (2013) described that trypanosome used about one-third of nucleus gene and all sub telomere for activating and inactivating the VSG gene and the sequence related to the VSG. Variation of antigenic needs a single VSG transcription by polymerase RNA I (Pol-I) and periodically change of gene expression, especially through DNA recombination by duplicating the translocation into new VSG on the new active site. Furthermore telomere location, epigenic control and mono-allele transcription by Pol-I on the extra nucleolar site is important for the VSG expression by telomere, chromatin structure. Nucleus organization played a role on the contribution to the control and expression activity change of mono-allele VSG.

Analysis of phylogenic tree of *T. evansi* according to amino acid sequence of ESAG 6/7 gene from GeneBank

The phylogeny tree of amino acid sequence of ESAG 6/7 gene of 25 Indonesian *T. evansi* isolates was compared to some reference isolates from Gene Bank produced three groups (Figure 3). Bitter et al. (1998) reported that there was 20 pairs of ESAG 6 gene (20 gene variants) equivalent to VSG expression site number, but it was only one pair that active sometimes. The difference of amino acid between isolates was only 1-10% (Bitter et al. 1998; Steverding et al. 1995). This was affected by incompatibility of the number of copies

of genes due to the effects of homogenization through gene and telomere conversion mechanism (Robinson et al. 1999). Glover et al. (2013) described that VSG ES was the polycistron key unit involved in antigenic variation on trypanosome. Function and expression of this unit highly influences the virulence. In trypanosome maturation from mRNA transcription occurs through trans-slicing process, there were addition of 39 capped sequences and polyadenilasi ESAG6 / 7.

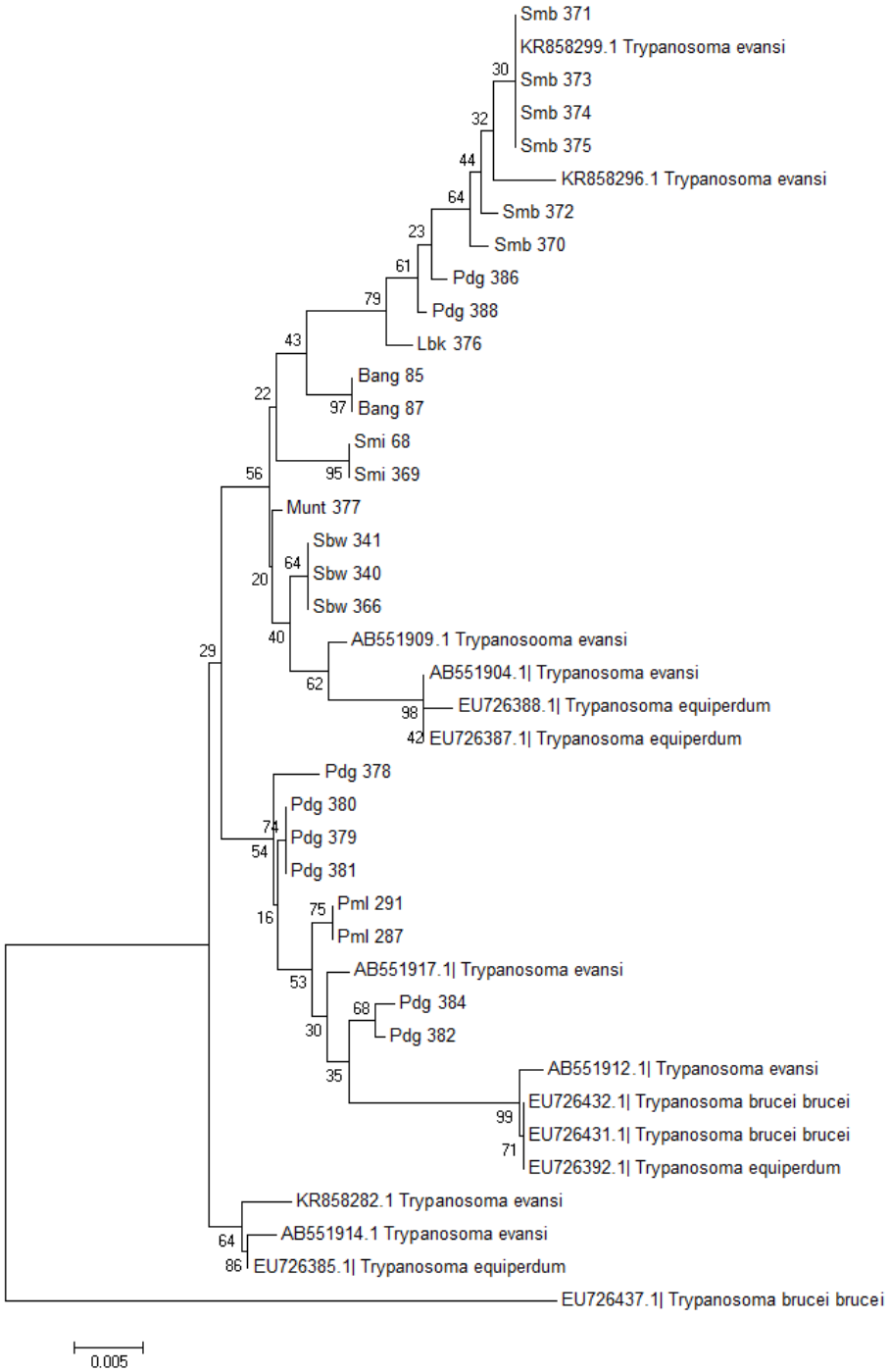

Figure 3. Neighbour joining phylogenic tree based on amino acid sequence of ESAG6/7 *T.evansi, T.brucei T. equiperdum* genes from GeneBank (access number is behind the isolates). Genetic distance between sequences is showed by number.

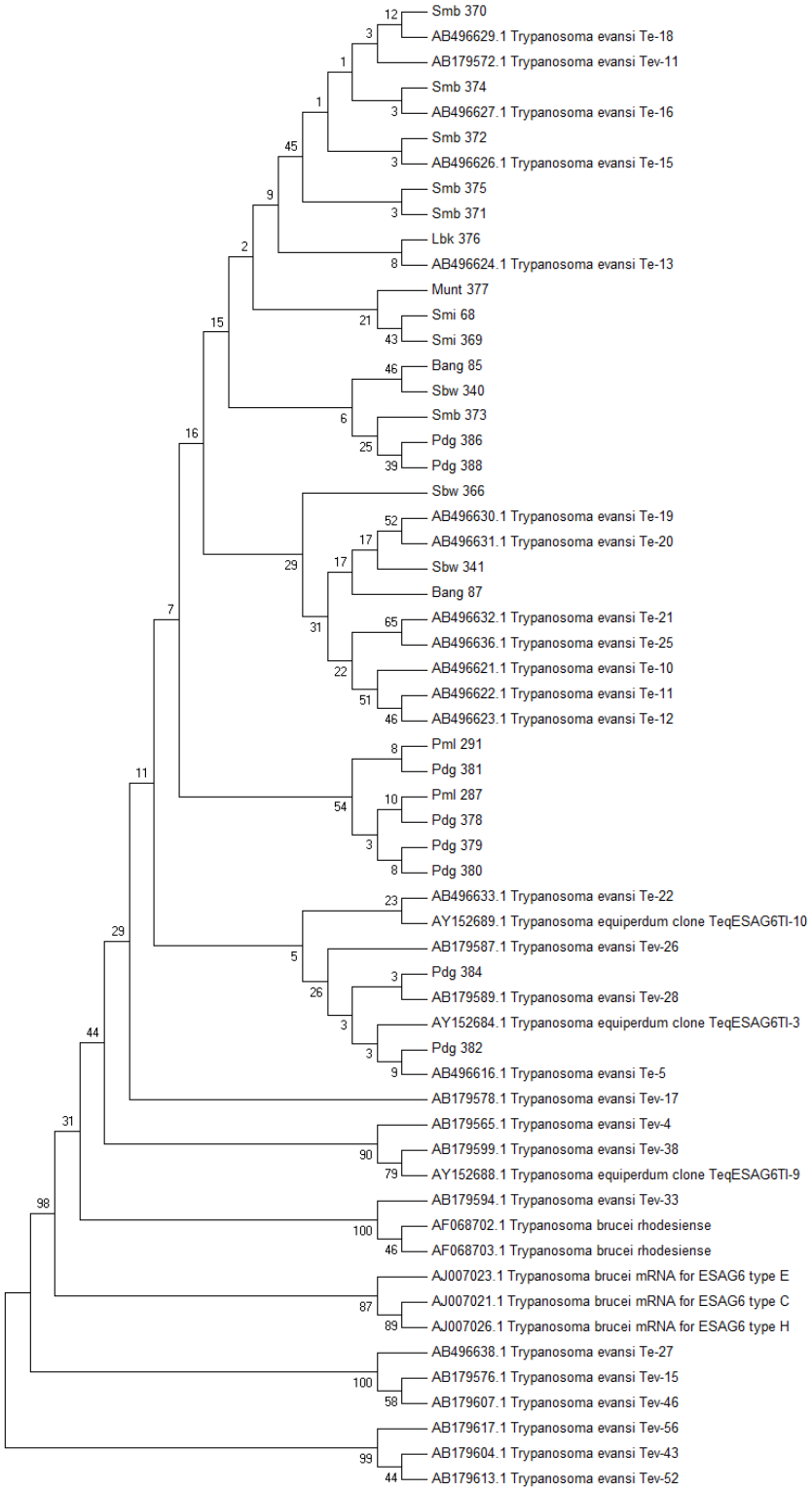

Figure 4. *Neighbour joining* of phylogeneic tree based on amino acid sequence of variant ESAG 6 *T.evansi (Tev), T.brucei brucei, T.brucei gambiense, T.brucei rhodesiense, T. equiperdum* genees (Tbb, Tbg, Tbr, Teq respectively) from Gene*eBank* (access number is behind the isolates). Geneetic distance between squences is showed by number.

Even though, isolates showed high homology but had a separate position in the phylogenetic tree. That pattern showed heterogeneity on the sequence although very small.

Analysis of phylogenic tree of *T. evansi* according to amino acid sequence of ESAG 6/7 gene for clade determination

Phylogenic tree construction was built according to mode used by Mekata et al. (2009). This construction was built according to 10 categories of ESAG 6/7 gene clade (Figure 3). As much as 25 Indonesian *T. evansi* isolates from various geographic areas with different virulence divided into 4 clades (*Clade* 5, 7, 10, and 11). The most Indonesian *T. evansi* isolates were from ESAG 6 and 7 genes of *T. evansi* from Southeast Asia (Witola et al. 2005) and South America (Mekata et al. 2009) (Figure 2). As much as 13 Indonesian *T. evansi* isolates were clade 7 and two isolates were clade 5. However, there were 6 isolates not included in 10 clades categorized in the previous study (Mekata et al. 2009; Barghash et al. 2016). Those isolates were Pdg 378, Pdg 379, Pdg 380, Pdg 381, Pdg 287 and Pdg 291. Those six isolates created new clade (clade 11). This new clade 11 was found on the Indonesian *T. evansi* isolates. Research result of Witola et al. (2005) on the *T. evansi* phylogenic tree analysis from Thailand showed 7 clades, while the research result of Mekata et al. (2009); Barghash et al. (2016) and Sarkhel et al. (2017) on *T. evansi* each from United State, Egypt and India showed 10 clades.

ESAG6gene as pathogenicity marker of *T. evansi*

Analysis result of phylogenic tree showed that isolates categorized to low virulence, was apart from the high virulence (Figure 3). However, moderate virulence isolates were spread in to low and high virulence. Dominant low virulence isolates would be merged with the low virulence isolates. While, dominant moderate virulence similar with high virulence isolates were merged with the high virulence isolates. Therefore, ESAG 6/7 gene was potential to be used as virulence marker. As well as the analysis result of phylogenic tree on the clade determination showed that high virulence isolates were categorized as different clade with low virulence isolates (Figure 4). High virulence was categorized into clade 7 and 10, while low virulence isolates was in clade 5 and 11. Bitter et al. (1998) described that natural variability of ESAG 6/7 was able to influence the trypanosome ability to take transferrin molecule from some species that was different from mammalian host. The variation of sequence, especially on HV site was related to its role on antigenic variation on the receptor surface to avoid immune response of host (Borst 1991). This was suspected that the sequence was playing a role in chronic diseases, related to parasite pathogenicity and its ability to widely infect host (Pays et al. 2006).

Some studies were expected that the variation on receptor development site was one of factors that contributed on parasite pathogenicity difference and its ability to widely infect the host (Pays et al. 2006). According to the amino acid diversity, among those, 25 *T. evansi* isolates were monophyletic group. It might be caused due to the only certain species that were infected by *T. evansi* in that site. It was suspected that the *T. evansi* on the site was stuck in the buffalo for a long time, so that it genetic diversity on the ASEG 6/7 gene was loss and became homogeny and there was no polymorphism on the transferrin development site (Mekata et al. 2009). Bitter et al. (1998) stated that sequence diversity could be also caused by the number of this gene copy. Variability of ESAG 6/7 allowed trypanosome to express the receptor with different affinity on the different host (Salmon et al. 1997) indicating the ability of parasite to adapt on the host (Amer et al. 2011).

CONCLUSION

As much as 25 Indonesian *T. evansi* isolates were divided into 4 clades of EASG 6/7 clades. Low virulence isolates were categorized as clade 7 and 10. Several low virulence isolates was in clade 11 (the new clade). Genetic variability occurred in the hipervariable site (HVR) but not in the Transferin binding site of ESAG 6/7 gene. According to this ESAG 6/7 gene, the Indonesian *T. evansi* was able to be distinguished into high and low virulence isolates. While the moderate virulence was spread on that two groups.

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