

Effect of katuk (*Sauropus androgynus*) powder supplementation on the levels of progesterone (P4) and estradiol-17 β (E2) hormones in kacang goat (*Capra aegragus*)

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Abstract. Putranto HD, Hasibuan GP, Yumiati Y, Ginting SM. 2017. Effect of katuk (*Sauropus androgynus*) powder supplementation on the levels of progesterone (P4) and estradiol-17 β (E2) hormones in kacang goat (*Capra aegragus*). *Nusantara Bioscience* 9: 86-91. Kacang goat (*Capra aegragus*) is one of Indonesian endemic ruminant species which commonly retained by the community in Indonesia. This species is classified as a small ruminant which has many benefits i.e easy to be domesticated and to adapt, and has an economical value especially for saving purposes to farmers in marginal areas. This study aimed to observe the effects of the katuk powder supplementation (*Sauropus androgynus*) in the feed of kacang goats on the level of reproductive hormones (progesterone (P4) and estradiol-17 β (E2)) in female kacang goats. A total of 9 female kacang goats which meet the prerequisite samples i.e reaching the age of a sexual maturity, having a homogenous weight (12.00 ± 1.00 kg) and in healthy condition were then divided into three groups of treatments using a Completely Randomized Design method. Each treatments consisted of 3 kacang goats as a replication. The treatments consisted of: (i) feed without katuk powder supplementation (control), (ii) feed with the concentration of katuk powder supplementation was 3% of kacang goat's body weight, and (iii) feed with the concentration of katuk powder supplementation was 6% of kacang goat's body weight. After 60 days of treatments, the results showed that the supplementation of katuk powder on female kacang goats had no significant effect on the level of progesterone and estradiol-17 β in the first sampling (day-0, $P > 0.05$), however the treatment had a significant effect to the level of progesterone and estradiol-17 β on the second sampling (day-28) and the third sampling (day-56) with a significance level of $P < 0.05$. It was concluded that the supplementation of katuk powder could increase the level of progesterone and estradiol-17 β hormones in kacang goats. The katuk powder supplementation about 3% of goat weight has sufficiently increased the level of reproduction hormones in female kacang goats.

Keywords: Estradiol-17 β , kacang goat, katuk, progesterone

INTRODUCTION

Since centuries ago, Indonesia is known as one of mega source of fauna natural plasma in the world. One of them includes the local endemic fauna species, which are further domesticated for commercial purposes. A Bali cattle, for an example, is genetically derived from wild banteng (*Bos javanicus javanicus* D'Alton, 1823, syn. *Bos javanicus sondaicus* Temminck, 1839/Blyth, 1842). Recently, this cattle becomes one of the top four of largest local ruminants in Indonesia (Sutarno and Setyawan 2015). Basically, ruminants can be classified into two groups i.e large ruminants and small ruminants. From the group of small ruminants, endemic goats are one of small ruminants which their species are abundant in Indonesia. One of the potential endemic goat which are now being intensively studied is, kacang goat (Ind.: *kambing kacang*) (Putranto et al. 2014a).

Primarily, Government has intensively developed some fauna species of Indonesia for specific purposes, although; occasionally it did not meet with the principles of conservation biology which is upheld by scientists. One of government programs is meat self-sufficiency program which is continuously developed until 2014. This program is done in order to attain the need of animal protein of Indonesian society which is noted still low and lags behind its neighbor countries especially in Southeast Asia (Putranto et al. 2014a, 2014c). As an abundant goat species in most of Indonesia regions, kacang goat is deserved to be developed further for being an alternative animal protein in society (Putranto et al. (2014c).

As one of a potential meat source, generally; kacang goats eat a natural feed and residue products which are not for human consumption (Yurmiaty 2006). In addition, kacang goat (Figure 1) is mostly retained in villages because it is easy to adapt and well survive in the wide-



Figure 1. Kacang goat as one of small endemic ruminants of Indonesia (Photograph by HDP)

range of environmental condition (Putranto et al. 2014a, 2014b, 2014c; Yurmiaty 2006). However, the need of kacang goat meat, which is targetted as one of an alternative meat source in the meat self-sufficiency program was not sufficient with its availability in the natural habitat. One of these conditions can be seen from the input and output balances of kacang goat population in Bengkulu province where its growth did not meet the additional demand in the market and the consumption of kacang goat meat as protein source in the society. It is predicted that the population of kacang goats plummet each years.

It is widely known that katuk plants, also known as star gooseberry or sweet leaf (*Sauropus androgynus*) have been used as additives either on the feed or drinking water of livestock (Putranto et al. 2012, 2014a, 2014b, 2014c). Furthermore, Agustal et al. (1997) has reported that katuk leaves contain 6 main compounds i.e, monomethyl succinate, cis-2-methyl cyclopentanol acetate, benzoic acid, phenyl malonic acid, 2-pirolidinon, and methyl piroglutamat. In the reproduction process, benzoic acid contained in the katuk leaves will be converted to estradiol benzoate in the body. According to Putranto et al. (2015), supplementation of Katuk plants can increase the levels of reproductive hormones in the kacang goats female pre-weaning although there was no evident statistically for significant impacts. Estradiol benzoate is known to improve the performance of the reproductive system of female animals and to stimulate follicle growth. Therefore, the application of katuk supplementation, hopefully; can improve the performance of reproductive system in the female kacang goats leading to the increase population of kacang goats in society.

The objectives of the research was to compare the effect of katuk powder supplementation in the natural feed toward the levels of reproductive hormones i.e progesterone (P4) and estradiol-17 β (E2) in the female kacang goat. The expected results obtained in this study were of important information to the development of science and technology,

especially in the field of reproductive physiology of kacang goats. In advance, this will be highly contributed to the increase population of kacang goats in the future.

MATERIALS AND METHODS

Time and study site

This serial study was started in the year of 2014 at female kacang goat's farms belonging to farmer (a single research partner in Sidomukti village, Padang Jaya sub-district, Bengkulu Utara district, Bengkulu province, Indonesia. Research was continued in the female kacang goats belonging to members of group of livestock farmers "Rukun Warga II" in Lokasi Baru village, Air Periukan sub-district, Seluma district, Bengkulu province in 2015. However, the results shown in this report were collected in the year of 2015 research.

Equipment and materials

Equipment used in this study were analytical balance, individual feed container, individual bucket of water, and enclosure stage with individual cages measuring 1x2 m², injections 5 mL (Terumo), cotton, alcohol 70%, as well as stationeries. Meanwhile, the materials used in this study were 9 of female kacang goats with the the initial weight average of 12,00 \pm 1,00 kg and an average age ranged from 7-10 months as the minimum age treshold of sexual maturity. All kacang goats were in the same reproductive status which have reached the sexual maturity.

Research design

The 9 of female kacang goats were divided randomly into 3 treatments with each of them consisted of 3 individual kacang goats as replication. Those treatments were: (i) H₁ = Feeding the goats with natural green materials and concentrate *ad libitum* without katuk powder supplementation (control); (ii) H₂ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 3% of the goat's weight, and (3) H₃ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 6% of the goat's weight.

Katuk powder production

The production of Katuk powder was modified from Putranto et al. (2014a) methods. The leaves and pliant young stems from katuk plants were dried naturally in ranged of 28.0 \pm 1.0 $^{\circ}$ C for 4 x 24 hours in the shedding rooms far away from sun expose. The use of young stems and leaves were in accordance with results from Putranto et al. (2014b) who found that those part of katuk plants mostly contained the steroid compounds. The room temperatures (28.0 \pm 1.0 $^{\circ}$ C) would perfectly dried the katuk leaves leading to be destroyed easily, while the pliant young stems would be dry for 4 days followed by subsequent drying process under sun rise for 6 hours with ranged of temperatures between 33 to 35 $^{\circ}$ C for perfect drying purposes, thus; it was easy to be break (Putranto et al. 2014c, 2013a). After drying process, katuk would be

processed further become powder with the blender machine and ready to be supplemented in the feeds.

Katuk powder supplementation

The supplementation of katuk powder in the feed was conducted 2 times which were at 9 am and additional feed at 5 pm. The water drinking was conducted *ad libitum*.

Steroid hormone analysis

The measurement of progesterone (P4) and estradiol-17 β (E2) levels were done by protocol as follows: blood was taken about 5 mL of blood vessels jugular vein in the back of the ears in 3 sampling periods which were at day-0, the first oestrus (day-25 through day-30), and the second oestrus (day-55 to day-60). Blood sampling was conducted in the morning at 07.00 pm. Blood was then stored in the tube without any mixture of EDTA or other chemicals. Tubes containing blood samples were then put into a flask containing ice at temperatures ranging from 4 - 10°C. This samples were then sent to Kimia Farma laboratory for analysing the hormone concentrations of P4 and E2 using a single method of radioimmunoassay (RIA) (Putranto et al. 2012).

In this case, P4 antiserum will react mainly with P4 (100%), 5 α -pregnanedione (62.2%), pregnenolone (6.3%), 11-deoxycorticosterone (3.9%), 17 α -hydroxyprogesterone (2.3%), and 11 α -hydroxyprogesterone (1.2%) (Putranto et al. 2011). Meanwhile, E2 antiserum will react mainly with E2 (100%), estrone-2-sulfate (8%), 16-epiestriol (5.3%), estrone (3.2%), and estriol (1.8%) (Putranto et al. 2012).

Data analysis

The levels of reproductive hormones in kacang goat treated with the supplementation of katuk powder were then analyzed using analysis of variance followed with LSD (Least Significant Difference Test) prior to knowing the levels of significancy data.

RESULTS AND DISCUSSION

The assessment of levels of reproductive hormone was done in some of wild animals such as Sumatera tigers and Siberia tigers (Putranto et al. 2007a, 2007b), Sumatera Orang Utan, Sambar deer (Putranto et al. 2011) and Burgo chicken (Putranto et al. 2012). In this study, the measurement of levels of reproductive hormone in the kacang goats treated by the katuk powder supplementation in the animal feed was the first research paper published in scientific journal.

The level of progesterone hormone (P4)

The levels of progesterone hormone (P4) in the female kacang goat treated with katuk powder supplementation was shown in the Table 1. The analysis of variance showed that the 3 different treatments of katuk powder supplementation did not have a significant effect on the level of P4 hormone in the first blood sampling (day-0) ($P > 0.05$). Interestingly, it showed a significant result of that

hormone level in the second (day-28) and third (day-56) blood sampling with the level of significant confidence 95% ($P < 0.05$). These results were different with the level of progesterone hormone in the pre-weaning phase of kacang goat reported by Putranto et al. (2015, 2013b). In the female kacang goat which was in the age of sexual immaturity, there was no significant different among treatments toward the level of progesterone hormone. This might happen due to the female reproductive system begins to work optimally when the individu reaches the age of sexual maturity.

In the initial research, most of female kacang goats used as samples were in the same of reproductive status (Figure 2). It could be seen from the level average of the progesterone hormone, which were almost similar (7.947 – 8.297 ng/mL/individuals). After treating with katuk powder, result showed that the level of progesterone hormone in the female kacang goat increased significantly from 8.297 ng/mL/individuals to 13.423 ng/mL/individuals in the H2 treatment. The same results were also obtained in female kacang goat after H3 treatment which increased significantly from 7.947 ng/mL/individuals to 13.260 ng/mL/individuals. Compared to the control (without katuk powder supplementation), both katuk powder supplementation in treatment H2 and H3 increased the level of progesterone hormone higher (11.630 ng/mL/individuals and 11.467 ng/mL/individuals) than the control (9.092 ng/mL/individuals).

The average of progesterone hormone level was presented in Table 1. Result showed that the katuk powder supplementation in ranged of 3% of the sample's weight (Treatment H2) showed the best average (11.630 ng/mL/individuals) of all treatments although it was not significantly different compared to other treatments. The result also showed that the percentage number of katuk powder supplemented into the feed had effect on the level of progesterone hormone in female kacang goat.

The number of katuk powder supplementation treated to female kacang goats increased the level of progesterone production. It can be seen from the profile of progesterone levels, which showed in the increase trend (Figure 2). The progesterone hormone produced in each sampling rate from 3 sampling periods showed that the longer the sampling rate, the higher the concentration of the progesterone hormone would be produced.

The levels of estradiol-17 β (E2) hormone

The result analysis of estradiol-17 β (E2) levels in female kacang goats treated with katuk powder supplementation was presented in Table 2. The results of analysis of variance showed that the 3 treatments of katuk powder supplementation in the feeds of female kacang goats had no significant effect on the levels of estradiol-17 β hormone observed in the first blood sampling (day-0, $P > 0.05$). However, in the second (day-28) and third (day-56) blood sampling, it showed that the katuk powder supplementation had a significant effect on levels of estradiol-17 β hormone with a significance level $P < 0.05$.

Table 1. The levels of progesterone hormone (P4) in female kacang goats (ng/mL/individuals) treated with Katuk powder supplementation

Treatments	Blood sampling quantities			The average of progesterone hormone level (ng/mL/individuals)
	1 st	2 nd	3 rd	
H ₁	8.296 ^a	9.630 ^b	9.350 ^b	9.092 ^b
H ₂	8.297 ^a	13.170 ^a	13.423 ^a	11.630 ^a
H ₃	7.947 ^a	13.193 ^a	13.260 ^a	11.467 ^a
Probability	ns	*	*	*

Note: The numbers followed by the same letter in the same column were not significance among treatments, while the numbers followed by the different letter showed a significant results among treatments. H₁ = Feeding the goats with natural green materials and concentrate *ad libitum* without katuk powder supplementation (control); H₂ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 3% of the goat's weight; H₃ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 6% of the goat's weight. ns = not significance (P>0.05), * = significant data with the level of confidence 95% (P<0.05)

Table 2. The level of estradiol -17 β (E2) hormone in the kacang goats (pg/mL/individuals) supplemented with katuk powder

Treatment	Blood sampling quantity			The average of estradiol-17 β hormone level (ng/mL/individuals)
	1 st	2 nd	3 rd	
H ₁	91.567 ^a	93.987 ^b	96.413 ^b	93.989 ^b
H ₂	91.730 ^a	112.323 ^a	127.483 ^a	110.512 ^a
H ₃	91.720 ^a	112.483 ^a	126.707 ^a	110.303 ^a
Probability	ns	*	*	*

Note: Similar to Table 1.

Based on the previous research conducted by Putranto et al. (2012), it has reported that katuk plants contain the steroid compound shown in the fitonutrient screening. Katuk leaves contain benzoic acid which will be converted to estradiol benzoic in the animal body system. Estradiol benzoic has important function to increase the reproductive function and trigger the follicle growth. In the Figure 3, it can be seen that levels of estradiol-17 β hormone increased because of the katuk powder supplementation. The highest increase of estradiol-17 β hormone was obtained in the treatment H2 (katuk powder supplementation 3% of goat weight) about 110.512 pg/mL/individuals (Table 2). On the other hand, the increase number of katuk powder supplementation from 3% to 6% of goat weight had no significant effect on the increase levels of estradiol-17 β production. It can be seen from the increase of estradiol-17 β level in treatment H2 (3% katuk powder supplementation) was higher than that in treatment H3 (6% katuk powder supplementation). Furthermore, the time duration for katuk powder addition during this research had effects on the increase of estradiol-17 β level, especially in the treatment H2 (3% katuk powder supplementation).

Results from estradiol-17 β analysis in the day-0 (the first blood sampling) showed lower than that in the day-28 (the second blood sampling) and day-56 (the third blood sampling). This was correlated with the oestrus cycle, which stated by Putranto et al. (2013b) who further reported that the oestrus cycle of kacang goats was in range of 18 -24 days with an average of 21 days. Besides, katuk powder added in the feed contains the estradiol benzoic. This chemical compound has a function to trigger the biosynthetic pathway of steroid hormone from cholesterol

(Putranto et al. 2012) leading to the positive increase of estradiol-17 β level. In Leghorn hens, the supplementations of katuk leaf extract had a positive effect on egg production, both in percentages of grain and weight, as well as increase the amount of egg production (Santoso et al. 2005; 2003). In the animal reproductive system, the benzoic acid contained in katuk leaves will be converted to estradiol benzoat inside the body. Estradiol benzoat has a function to increase the reproductive system and trigger the follicle growth, thus; the egg production will be higher and more efficient.

Several research papers mentioned that katuk leaves has five basic substances derived from the group of unsaturated fatty acids (poly-unsaturated fatty acids). This fatty acid has a function as a precursor in the biosynthesis of eicosanoids (prostaglandin, prostacycline, thromboxane, lipoxins and leukotrienes) and also involved in the reproduction process and physiology (Suprayogi 2000; Ganong 1993). In addition, the content of the 17-ketosteroid, androstan-17-one and 3-ethyl-3-hydroxy-5 α on katuk leaves play an important role in the biosynthesis of the female steroid hormone (progesterone and estradiol) (Despopoulos and Silbernagi 1991).

From these results it can be concluded that katuk powder supplementation can increase levels of reproductive hormones (progesterone and estradiol-17 β) in the blood of a female kacang goats. Furthermore, the application of micronutrients of katuk powder in the dried feeds of female kacang goats with concentration of 3% of its body weight could increase the levels of reproductive hormones in female kacang goats.

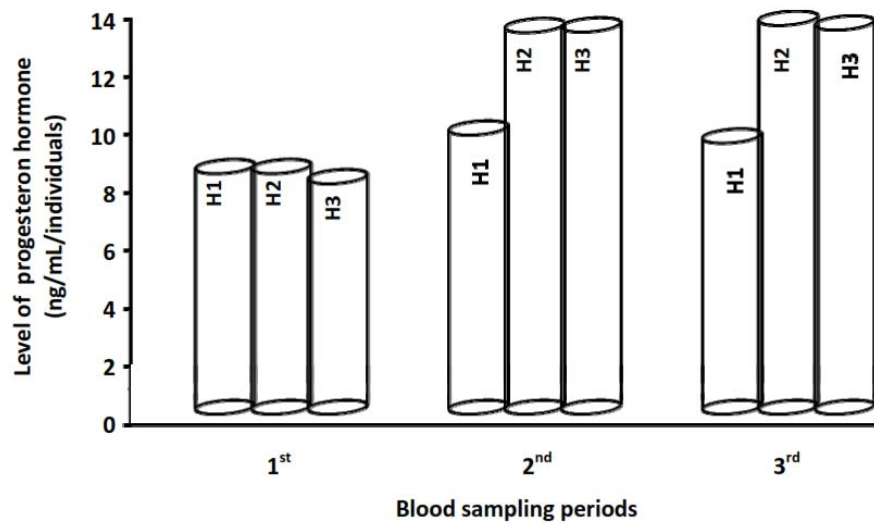


Figure 2. The levels of progesterone hormone in female kacang goats treated with katuk powder supplementation. H₁ = Feeding the goats with natural green materials and concentrate *ad libitum* without katuk powder supplementation (control); H₂ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 3% of the goat's weight; H₃ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 6% of the goat's weight.

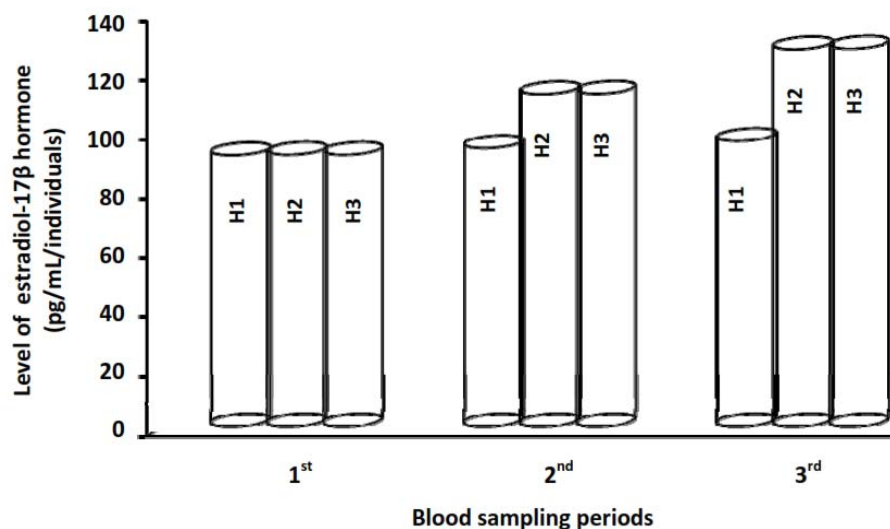


Figure 3. The level of estradiol-17β hormone in the female kacang goats treated with katuk powder supplementation. H₁ = Feeding the goats with natural green materials and concentrate *ad libitum* without katuk powder supplementation (control); H₂ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 3% of the goat's weight; H₃ = Feeding the goats with natural green materials and concentrate *ad libitum* supplemented with katuk powder 6% of the goat's weight.

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REFERENCES

- Agustal A, Harapini M, Chairul. 1997. Chemical analysis of Katuk leaves (*Sauropus androgynus* (L.) Merr) extract by GCMS. *Warta Tumbuhan Obat* 3 (3): 31-33. [Indonesian].
- Despopoulos A, Silbernagi L. 1991. *Color atlas of Physiology*. 4th Edition. George Thieme Verlag, New York.
- Ganong WF. 1993. *Review of medical Physiology*. 6th ed. Prentice-Hall International Inc., San Fransisco.
- Putranto HD, Yumiati Y, Santoso U. 2015. Revenue analysis on Burgo chicken farming in Bengkulu. *Jurnal Agribis* 7 (1): 480-489.
- Putranto HD, Nurmeliarsari, Ginting SM. 2014a. Leukocyte profiles of pre-weaning kacang does supplemented by Katuk powdered-feed. *Jurnal Sain Peternakan Indonesia* 9 (1): 1-12.
- Putranto HD, Nurmeliarsari, Ginting SM. 2014b. Metabolite steroid screening of Katuk and Banana flower for reproductive hormone. *Jurnal Peternakan Indonesia* 16 (1): 20-25.
- Putranto HD, Yumiati Y, Ginting SM. 2014c. Economical analysis of Katuk powdered-feed utilization on pre-weaning female kacang goat (*Capra aegagrus*) for reproductive purpose. In: Ratriyanto A, Nuhriawangsa AMP, Indreswari R et al. (eds). *Proceeding of 2014 Animal Science National Seminar Based on Innovative Research*. Universitas Sebelas Maret, Surakarta, 22-23 October 2014.
- Putranto HD, Ginting SM, Nurmeliarsari. 2013. Phytonutrien study and traditional supplement utilization to optimalize ruminant reproductive status (Year-1). *Hibah Bersaing Research Report*. Lembaga Penelitian Universitas Bengkulu, Bengkulu.
- Putranto HD, Setianto J, Santoso U. 2012. Estradiol-17 β hormone concentration and follicles number in exotic burgo chicken supplemented by extract of katuk leaves (*Sauropus androgynus*). *Biodiversitas* 13 (1): 1-6.
- Putranto HD, Soetrisno E, Nurmeliarsari et al. 2011. A non-invasive identification of hormone metabolites, gonadal event and reproductive status of captive female tigers. *Biodiversitas* 12 (3): 131-135.
- Putranto HD, Kusuda S, Inagaki K et al. 2007a. Ovarian activity and pregnancy in the Siberian tiger, *Panthera tigris altaica*, assessed by fecal gonadal steroid hormones analyses. *J Vet Med Sci* 69 (5): 569-571.
- Putranto HD, Kusuda S, Ito T et al. 2007b. Reproductive cyclicity based on fecal steroid hormones and behaviors in Sumatran tigers, *Panthera tigris sumatrae*. *Japanese J Zoo Wildl Med* 12 (2): 111-115.
- Putranto HD, Kusuda S, Hashikawa H et al. 2007c. Fecal progestins and estrogens for endocrine monitoring of ovarian cycle and pregnancy in Sumatran orang utan (*Pongo abelii*). *Japanese J Zoo Wildl Med* 12 (2): 97-103.
- Santoso U, Setianto J, Suteki T. 2005. Effect of *Sauropus androgynus* extract on egg production and lipid metabolism in layers. *Asian Australas J Anim Sci* 18 (3): 364-369.
- Santoso U, Setianto J, Suteki T. 2003. The utilization of Katuk leaves extract to improve Leghorn egg production and quality. *Hibah Bersaing Lanjutan Research Result*. Lembaga Penelitian Universitas Bengkulu, Bengkulu.
- Suprayogi A. 2000. *Studies on the Biological Effects of Sauropus androgynus* (L) Merr.: Effects on Milk Production and the Possibilities of Induced Pulmonary Disorder in Lactating Sheep. [Dissertation]. University of Gottingen, Germany.
- Sutarno, Setyawan AD. 2015. Genetic diversity of local and exotic cattle and their crossbreeding impact on the quality of Indonesian cattle. *Biodiversita* 16: 327-354.S
- Yurmiaty H. 2006. Relationship between male kacang goat slaughtered weight and raw skin quality. *Jurnal Ilmu Ternak* 6 (2): 121-125.