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Estradiol-17β hormone concentration and follicles number in exotic Burgo chicken supplemented by *Sauropus androgynus* leaves extract

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Abstract. *Putranto HD, Setianto J, Santoso U, Warnoto, Nurmeliasari, Zueni A. 2012. Estradiol-17β hormone concentration and follicles number in exotic Burgo chicken supplemented by* Sauropus androgynus *leaves extract. Biodiversitas 13: 1-6.* Bengkulu Province of Indonesia has an indigenous crossbreed chicken named burgo or Rejang chicken. A conservation effort in this study was represented by supplementing 4 different levels of *Sauropus androgynus* or katuk leaves extract (KLE) to improve number of fertile eggs. The purpose of study was to identify the effects of KLE supplementation on female burgo chicken's serum estradiol-17β (E2) hormone concentration profile and number of follicles. KLE was added into drinking water (0, 9, 18 and 27 g/chicken/day) during 8 weeks of treatment. The results showed that supplementation of KLE dosed 9 to 27 g/chickens/day had significantly affected E2 concentrations and number of follicles ($P < 0.05$). In contrast, the average of female burgo E2 concentration with supplemented KLE was higher than control group. The total number of small follicle yield was highest (86.5%) compared to medium follicle (7.8%) and large follicle (5.3%). Many primary follicles (primordial) and post ovulatory follicles were probably in micro size and unseen by an usual visual counting. It seems that serum E2 hormone concentration correlated to total number of preheararchal follicles. Supplemented KLE was able to improve the serum estrogen steroid hormone concentration and number of preheararchal follicle (small and medium follicles) in female burgo chicken.

Key words: burgo, follicles, katuk, serum estradiol-17β.

INTRODUCTION

Indonesia is well known as a rich biodiversity country with various species of flora and fauna (Putranto 2010; Putranto et al. 2010a), including some sub-species of native chickens. Indigenous chickens such as kedu, cemani, pelung, bekisar, merawang, balenggek and nunukan chicken are well known and famous as fancy fowls or landrace chickens among Indonesians. Previous studies (Putranto 2011a,b; Putranto et al. 2010b) reported that there are also wild sub-species of chicken, unfortunately when they are captived, this rare sub-species would easily suffered from stress, low reproductive status and could be suffered for loss of feed intake which resulted in a high mortality rate. In fact, their low reproductive and low growth rate status of those indigenous sub-species have lead into a low population growth situation.

Furthermore, in year of 1999 some scientists began to promote a new sub-species of Bengkulu native chicken called burgo chicken or also well known as Rejang chicken (Nurmeliasari 2003; Setianto et al. 2009; Putranto 2011a,b; Putranto et al. 2010b). Burgo chicken (Figure 1) is an endemic indigenous chicken of Bengkulu, a province which is located in Southern Sumatra island. They have been kept by rural communities for many generations in Rejang Lebong District of Bengkulu Province, therefore it is also known as Rejang chicken (Setianto et al. 2009; Warnoto and Setianto 2009).

Figure 1. Burgo cock profile

Burgo chicken is genetically a crossmated chicken with some specific phenotypical characteristics and beautiful feather color. Unfortunately, there are limited reports on burgo chicken reproductive physiology (Putranto 2011a,b; Putranto et al. 2010b). Previous reports informed about population of burgo chicken in Rejang Lebong District (Nurmeliasari 2003), population based on macro environment, altitude, temperature, rainfall and farming system type (Setianto et al. 2009) and the population percentage and population density in 5 regencies in Bengkulu Province (Putranto 2011a,b; Putranto et al. 2010b).

Farmers in rural area of Bengkulu keep burgo chicken live naturally by a backyard farming system. However, similar to their parental characteristics (kampung chicken and wild red forest chicken) which is low reproductive rate and low growth rate have resulted in a low population growth rate (Putranto et al. 2010b). Similar to other wild sub-species chicken, they are also difficult to domesticate and could not easily adapt to a captivity condition. This sub-species of chicken can be categorized as one of Indonesia biodiversity treasure and should be conserved immediately (Putranto 2011a, b; Putranto et al. 2010b). Therefore, conservation program would be one useful effort to increase their population by improving their reproductive performances.

Katuk leaves extract (KLE) or leaves extract of *Sauropus androgynus* (LESA) is already well known as a natural supplementation for some mammals (Agusta et al. 1997). Katuk leaves contains precursor, which is mostly important in eicosanoids biosynthesis and involved in reproduction process and milk production in lactating sheep. It is also containing 17-ketosteroid, androstan-17-one, 3-ethyl-3-hydroxy-5alpha which are involved in female steroid hormone synthesis. KLE which was supplemented in broiler diet have improved the meat quality and increased number of chicken egg production (Santoso et al. 2005).

Katuk leaves contains benzoate acid which would converted into estradiol-17β benzoate (Santoso et al. 2005). Estradiol-17β benzoate has a role function in reproduction and stimulate follicles growth and development. Furthermore, it is reported that 17-ketosteroid, androstan-17-one, 3-ethyl-3-hydroxy-5alpha contents of katuk leaves are also involved in female progesterone (P4) and estradiol-17β (E2) steroid hormones secretion.

Steroid hormones such as E2 and P4 are important in follicles growth and development. When the primordial follicle grows until the stage of de Graff follicle, those hormones are mutually interacted. Previous studies also reported that sexual steroid hormones such as P4, E2, estrone (E1), immunoreactive pregnanediol glucoronide (PdG), estradiol glucoronide (E2G) of female can be used widely to monitor natural ovarian activity such as functional luteal activity and follicular maturation and to confirm pregnancy (Adachi et al. 2011; Kusuda et al. 2006, 2007a, b; Putranto 2011c; Putranto et al. 2007a, b, c).

In this study, domestication effort was represented by keeping burgo chicken lived in a small postal cage with adequate space to enable chickens move freely. Furthermore, the conservation effort was represented by supplementing burgo chicken with 4 different levels of KLE to improve number of fertile eggs. The purpose of this study was to identify the effect of KLE supplementation on female burgo chicken's serum estradiol-17β hormone concentration profile and number of follicles.

MATERIAL AND METHODS

Burgo chickens

Sixteen female burgo chickens age ranged between 10 to 12 months of age were used in this study. Suryana and Hasbianto (2008) reported that generally Indonesia indigenous chicken reaches sexual maturity at 8.5 months of age. In addition, burgo chicken begins its reproduction cycle earlier than other Indonesia indigenous chicken species (Setianto et al. 2009). Moreover, it is reported that burgo chicken reaches their sexual maturity at 4.5 months of age. Based on those reports, we assumed that female burgo chickens used in this study were sexually matured.

Procedures

This study was conducted at Commercial Zone and Animal Laboratory, Department of Animal Science, Faculty of Agriculture, University of Bengkulu, Indonesia for 66 days of treatment (May to July 2010). Each female burgo chicken was housed in an individual postal cage size 1.0 x 0.8 m^2 facilitated with feeding and drinking tools. Cage and tools were sprayed by disinfectant a week prior the study. During the first 10 days, chickens were in their adaptation period for the cage, tools and formulated chicken feed.

Completely randomized design (CRD) was used as research design with 4 treatments. There were 16 females burgo chickens distributed into 4 treatments. The treatments were:

H1 : female burgo chicken with no supplementation as control group (0 g/chicken/day).

H2 : female burgo chicken supplemented KLE in drinking water dosed 9 g/chicken/day.

H3 : female burgo chicken supplemented KLE in drinking water dosed 18 g/chicken/day.

H4 : female burgo chicken supplemented KLE in drinking water dosed 27 g/chicken/day.

Those KLE supplementation treatment was conducted for 8 weeks. KLE supplementation dose was justified from previous report (Santoso et al. 2005). Furthermore, Santoso et al. (2005) reported that in broiler chicken, the supplementation of 27 g KLE/kg ration resulted in an increase of egg production.

Animal feed

Chickens were fed formulated chicken feed contains 16% crude protein (CR) and 2750 kcal/kg metabolic energy (ME) with non-supplemented antibiotic (Santoso et al. 2005). Table 1 below is shown a basal feed animal formulation. Animal feed and drinking water were given ad libitum.

Table 1. Basal feed animal formulation (kg/100 kg)

Diet materials	Volume (kg/100 kg)
Milled corn	50.0
Concentrate	24.0
Green bean	4.0
Soy bean	4.0
Peanut	6.0
Fish meal	7.0
Coconut oil	1.0
Bone meal	2.2
Calcium carbonate	1.0
Premix	0.5
Composition	
CR (%)	16.5
ME (kcal/kg)	2752.0

Note: Modified from previous report (Santoso et al. 2005).

Katuk leaves extraction protocol and supplementation method

Fresh katuk leaves were obtained from some traditional farms in Bengkulu Province. The katuk leaves extraction protocol was modified based on previous reports (Santoso et al. 2005). One kg of fresh katuk leaves and 6 l of water were boiled 60°C for 30 min, filtered and the dregs were boiled ones more time. This extraction procedure was frequently repeated for 3 times. Then, the filtrates were warm-over 50°C for 48 h.

KLE was supplemented by mixed up a dose of extract in 100 mL drinking water at 0700 h in the morning. When morning drinking water was all consumed, another 100 mL of fresh drinking water was added between 1300 h to 1500 h in the afternoon.

Hormonal analysis and follicles counting

The parameters measured in this study were the concentration of serum estradiol-17β hormone (E2), number of large follicle yield (LFY), number of medium follicle yield (MFY) and number of small follicle yield (SFY). The serum steroid hormones were determined by a single radioimmunoassay (RIA) procedure conducted in Kimia Farma Laboratory. In day 60, blood samples were collected from female chickens of treatment group for E2 hormone measurement. At 0700 h in the morning, the amount of 5 mL blood was taken from vena blood vessel and directly transferred into a non EDTA tube, and than kept in a cooler pot and sent to laboratory immediately. The E2 anti-serum mainly cross-reacts with E2, estrone-3-sulfate, 16-epiestriol, estrone and estriol at 100, 8.0, 5.3, 3.2 and 1.8 %, respectively.

In the end of study period, female chickens were slaughtered and the ovary and connective tissue were collected for LFY, MFY and SFY counts. The LFY or tertiary follicles and de Graff follicles (preovulatory) size varied between 5 to 8 mm or > 8 mm, MFY or secondary follicles size varied between 1 to 5 mm, SFY or primary follicles (primordial) sized < 1 mm (Johnson 1990; Zueni 2011). The counting method was by using a hand counter and repeated 3 times for each female ovary.

Data analysis

Total counted number of follicles was expressed as follicles per chicken unit. All collected data was analysed by using analysis of variance, any significant results would be further tested by using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The diversity of phenotypic or genotypically exotic native and wild chicken in Indonesia are varied and unique, which makes a specific and most interesting reason for a further investigation. Different to other exotic native chickens in Indonesia, burgo chicken has received a less attention because there was limited scientific information on burgo chickens (Putranto 2011a,b; Putranto et al. 2010b). This report would be the first reproductive physiology status of burgo chicken information.

This study is also perform a local wisdom treatment by using an KLE or in Indonesia is well known as *daun katuk*. Indonesian is familiar with katuk leaves as one of nutritious vegetables included in their daily meals. Katuk leaves is widely used for antibacterial and antiprotozoa, and traditionally used as a supplement for a precursor of milk initiation (Zueni 2011). In general, katuk leaves contains Fe, provitamin A in β-carotene form, vitamin C and protein. Some previous reports found that katuk leaves contains 10.8% water, 20.8% fat, 15% crude protein, 31.2% crude fiber and 10.2% BETN (Santoso et al. 1999), 12% water, 26.32% fat, 23.13% protein, 29.64% carbohydrate, 372.42 mg/100g β-carotene and 447.96 kcal in katuk leaves powder, 75.28% water, 9.06% fat, 8.32% protein, 4.92% carbohydrate, 165.05 mg/100g β-carotene and 134.10 kcal in fresh katuk leaves (Yuliani and Marwati 1997).

The most recent non-invasively method to access the steroid hormones status of some mammals is by using fecal or urine samples. However, for majority application, serum steroid hormones still performs an accurate result for a steroid hormone profile (Putranto et al. 2007a, b, c). In this study, the collection of blood samples was at 0700 h in the morning conducted by a well-trained technician.

The result showed that the supplementation of KLE dosed 9 to 27 g/chickens/day had significantly affected the serum E2 hormone concentration and number of follicles in female burgo chickens ($P < 0.05$, Table 2). The result for the average of E2 hormone concentration and the average of number of female burgo follicles after 8 weeks treatments are shown on Table 2.

Data on Table 2 is shown that female burgo chicken with non-supplementation (control group) has the lowest average of serum E2 hormone concentration (175.600 ng/mL) and burgo chicken supplemented by KLE dosed 18 g/chicken/day has resulted the highest average of serum E2 hormone concentration (457.020 ng/mL). Furthermore, female burgo chickens which were supplemented by KLE, have a varied serum E2 hormone concentration above 400 ng/mL or 3-times higher than non-supplemented KLE which was ranged from 410 to 457 ng/mL (Figure 2).

Table 2. The average of E2 hormone concentration and number of female burgo follicles after 8 weeks treatment

	Katuk Leaves Extract (KLE) Treatments			
	H1	**H2**	**H3**	**H4**
Steroid hormone				
E2 (ng/mL)	175.600[a]	410.455[b]	457.020[b]	442.640[b]
Follicles number				
LFY[1)]	9.0[a]	2.0[b]	5.5[ab]	4.5[ab]
MFY[2)]	4.0[b]	6.0[ab]	8.0[ab]	12.0[a]
SFY[3)]	77.0[b]	73.5[b]	107.5[a]	83.5[b]

Note: H1: female burgo chicken with no supplementation as control treatment, H2: female burgo chicken supplemented KLE dose 9 g/chicken/day, H3: female burgo chicken supplemented KLE dose 18 g/chicken/day, H4: female burgo chicken supplemented KLE dose 27 g/chicken/day, [1)]: large follicle yield, [2)]: medium follicle yield, [3)]: small follicle yield, [a,b,ab]: superscribe with different letters in the same column indicate the group mean is significantly different ($P < 0.05$).

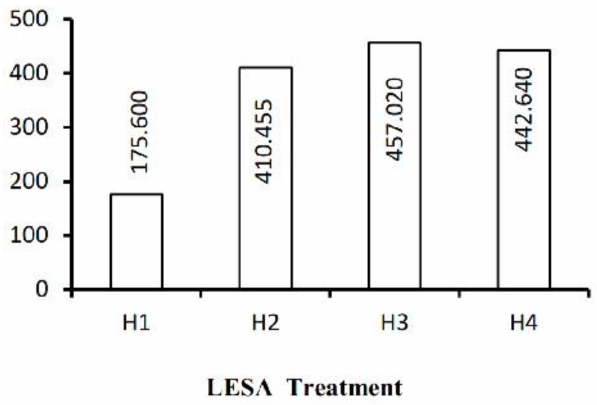

Figure 2. The effect of KLE (LESA) on serum E2 hormone concentration in female burgo after 8 weeks treatment.

The average concentration of E2 hormone of control group was presumably low. It is because female burgo chicken only synthezed steroid hormone by using their natural cholesterol in cortex adrenal and liver (Zueni 2011). In contrast, the average of female burgo E2 hormone concentration with supplemented KLE was higher than control. KLE supplementation may have involved in increasing process of the concentration of serum E2 hormone in female burgo chickens (Zueni 2011). KLE contains lipoprotein which is involved in estrogen steroid hormone development.

The higher concentration of serum E2 hormone in this study reflected that female burgo chicken produces more estrogen steroid hormone not only by using their natural cholesterol, however, the cholesterol from KLE with its high lipoprotein and fatty contents might also affect ovary to be more active and contribute a higher amount of estrogen in serum. Ovary of Aves species begins to develop and cause secretion of steroid hormones such as estradiol when the individual reached sexual maturity. Previous report reported that the majority of estrogens are formed as estrone, E2 and estradiol-17α (Sturkie 2000). Furthermore, E2 hormone will increase as the follicles developed.

Most of follicles development activities in Aves are conducted in inner cortex of ovary and it are different to mammals (Jamieson 2007). The post primordial phase of chicken follicles is easy to observe and distinguish from stroma ovary tissue, a binded grape-like. In this study, the follicle counting was based on follicle size classification by previous report (Johnson 1990). It is reported that a classification of follicles sized less than 1 mm were categorized as primary follicles (primordial), medium size or secondary follicles size were ranged from 1 to 5 mm and large size or tertiary follicles and de Graff follicles (preovulatory) size were ranged from 5 to 8 mm or > 8 mm (Zueni 2011).

The total number of small follicle yield (SFY) has been more populated (86.5%) than medium (7.8%) and large size follicle yield (5.3%, Table 2). Naturally, a sexually matured kampung chicken has approximately 3000 to 4000 follicles in its ovary (Supriyatna 2009). However, total number of follicles in this study was approximately only 5 to 10% of normal populated follicles in mature kampung chicken. Many primary follicle (primordial) and post ovulatory follicles in this study probably were in micro size and unseen by an usual visual counting.

Another consideration is body size of the sub-species chicken. Burgo chicken body size is smaller than kampung chicken in common and bigger than the wild red forest chicken (Setianto et al. 2009). Female body weight is between 0.60 to 1.25 kg and 15 to 20 cm in height. With its smaller size and compact body, it may cause the number of follicles of burgo is less populated than a normal kampung chicken as reported by Supriyatna (2009).

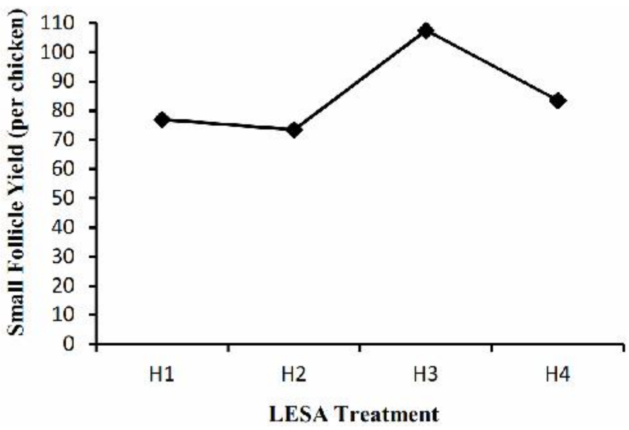

Figure 3. The effect of KLE (LESA) on the number of small follicle size in female burgo after 8 weeks treatment

Figure 3 (small follicles) and Figure 4 (large and medium follicles) showed that KLE has affected the average number of follicles in various sizes of female burgo chicken. It seems that serum E2 hormone concentration correlated to total number of preheararchal follicles. As we can see that the higher concentration of serum E2 hormone, the more total number of follicles found. During vitelogenesis, the follicles development is a chain process from fatty acid synthesis in liver and this process is controlled by estradiol (Jamieson 2007). KLE with high content of lipoprotein and fat affected the digestion of crude fatty inside the body, therefore fat absorption, fat component and fat derivates such as cholesterol, LDL, HDL and triglyceride are also automatically changed. This situation is supported by previous report; revealed that high protein level in diet improved the follicle development and growth (Firdaus 2010).

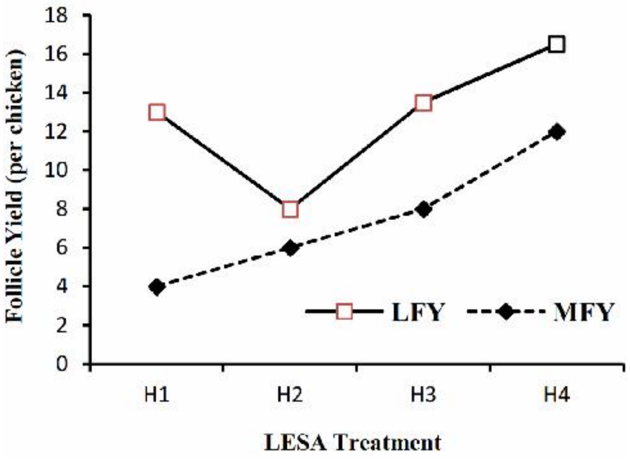

Figure 4. The effect of KLE (LESA) on the number of medium (MFY) and large follicle (LFY) size in female burgo after 8 weeks treatment

Data from Table 2 and Figure 4 also showed that for largest size of follicles, the supplementation of KLE did not significantly improve the number of follicles. In preovulatory phase and ovulation phase, the supplementation of KLE might have blocked the follicle development. Katuk leaves contain high level of active compounds and crude fiber which are able to block the cholesterol absorption. The blocked absorption affected the estrogen synthesis in ovary and also disturbs the follicle development and growth from preheararchal follicle phase (secondary and tertiary follicles) to preovulatory and ovulation follicle phase (de Graff follicle) (Sturkie 2000; Zueni 2011).

This study described a conservation effort through a combination of local wisdom utilization and reproduction technology which will give a positive contribution to increase a future population by improving the number and quality of follicles of Bengkulu Province burgo chicken sub-species. KLE supplementation treatment was positively improved the serum E2 hormone concentration and an increase in number of follicles which will improve the egg production from a single female burgo chicken.

CONCLUSION

It can be concluded that KLE supplemented in drinking water of female burgo chicken is an effort to reveal the reproductive status of this exotic burgo chicken. Supplementation of KLE has a positive impact to improve the concentration of serum E2 steroid hormone level and mobilizing ovary to release more number of follicles in preheararchal follicle phase (small and medium follicles) in exotic female burgo chicken.

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REFERENCES

Adachi I, Kusuda S, Kawai H, Ohazama M, Taniguchi A, Kondo N, Yoshihara M, Okuda R, Ishikawa T, Kanda I, Doi O. 2011. Fecal progestagens to detect and monitor pregnancy in captive female cheetahs (*Acinonyx jubatus*). J Reprod Dev 57 (2): 262-266.

Agusta A, Harapini M, Chairul. 1997. Katuk leaves extract (*Sauropus androgynus* (L) Merr) chemical analysis by using GCMS. W Tumb Obat 3 (3): 31-33.

Firdaus AA. 2010. Application for anatomy and physiology of female reproduction organ. www.google/penerapan anatomi dan physiologi alat reproduksi betina.

Jamieson, BGM. 2007. Reproductive Biology and Phylogeny of Birds Volume 6A of Series: Phylogeny, Morphology, Hormones, Fertilization. Science Publishers, School of Integrative Biology, University of Queensland. St. Lucia, Queensland, Australia.

Johnson AL. 1990. Steroidogenesis and actions of steroids in the hen ovary. Poult Bio 2: 319-346.

Kusuda S, Nagami H, Ueda K, Nishikaku T, Nakagawa D, Takida T, Kurita D, Uemichi K, Fukai M, Kubota H, Ueda K, Ooe T, Okuda K, Kusunoki H, Doi O. 2006. Characterization of the reproductive cycle in female bharals (*Pseudois nayaur*) based on the changes in serum progesterone concentrations and parturition season. J Vet Med Sci 68 (8): 847-851.

Kusuda S, Ikoma M, Morikaku K, Koizumi J, Kawaguchi Y, Kobayashi K, Matsui K, Nakamura A, Hashikawa H, Kobayashi K, Ueda M, Kaneko M, Akikawa T, Shibagaki S, Doi O. 2007a. Estrous cycle based on blood progesterone profiles and changes in vulvar appearance of Malayan tapirs (*Tapirus indicus*). J Reprod Dev 53 (6): 1283-1289.

Kusuda S, Morikaku K, Kawada K, Ishiwada K, Doi O. 2007b. Excretion patterns of fecal progestagens, androgen and estrogens during pregnancy, parturition and postpartum in okapi (*Okapia johnstoni*). J Reprod Dev 53 (1): 143-150.

Nurmeliasari. 2003. Burgo chicken population, distribution and the interaction with various ecological factor. J Raflesia UMB 5 (2): 52-55.

Putranto HD. 2010. The description of intensively captived sambar deer's reproductive behavior. Indo Anim Sci J 5 (2): 129-134.

Putranto HD. 2011a. Introduction of Indigenous Bengkulu Chicken, Population Distribution, Female Production and Reproductive Organs Description. The 19th J-AREA Annual Meeting, Himeji, Japan, 6-7 July 2011.

Putranto HD. 2011b. Effect of katuk leaves supplementation on burgo's ovarium and oviduct size and egg production performance. Indon Anim Sci J 6 (2): 103-114.

Putranto HD. 2011c. 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, Kumagai G, Ishii-Tamura R, Uziie Y, Doi O. 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, Hashikawa H, Kimura K, Naito H, Doi O. 2007b. Fecal progestins and estrogens for endocrine monitoring of ovarian cycle and pregnancy in Sumatran orangutan (*Pongo abelii*). J J Zoo and Wildlife Med 12 (2): 97-103.

Putranto HD, Kusuda S, Ito T, Terada M, Inagaki K, Doi O. 2007c. Reproductive cyclicity based on fecal steroid hormones and behaviors in Sumatran tigers, *Panthera tigris sumatrae*. *J* J Zoo and Wildlife Med 12 (2): 111-115.

Putranto HD, Soetrisno E, Nurmeliasari, Zueni A, Gibson B. 2010a. Recognition of seasonal effect on captive sumatran sambar deer reproductive cyclicity and sexual behaviors. Biodiversitas 11 (4): 200-203.

Putranto HD, Warnoto, Hakim N, Santoso U, Fenita Y, Zueni A, Gibson B. 2010b. A study on population density and distribution pattern of domesticated Bengkulu native burgo chicken. Med Ked Hew 26 (2): 198-204.

Santoso U, Setianto J, Suteki T. 2005. Effect of *Sauropus androgynus* extract on egg production and lipid metabolism in layers. Asian-Aust J Anim Sci 18 (3): 364-370.

Setianto J, Warnoto, Nurmeliasari. 2009. The phenotypic characteristic, population and the ecological factors of Bengkulu's burgo chicken. International Seminar The Role and Application on Livestock Reproduction and Products, Bukittinggi, Indonesia, June 2009.

Sturkie PD. 2000. Avian Physiology 5th ed. Springer-Verleg, New York.

Supriyatna. 2009. Manifestation of protein level in diet during growing period on reproduction organ development and the effect on medium type chicken production performances. [Dissertation]. Diponegoro University, Semarang. [Indonesia].

Suryana, Hasbianto A. 2008. Local chicken farming in Indonesia. J Litbang Pert 27(3): 75-83.

Warnoto, Setianto J. 2009. The characteristic of egg production and reproduction of various crossbreeding offspring between Burgo chicken with nature chicken. International Seminar The Role and Application on Livestock Reproduction and Products, Bukittinggi, Indonesia, June 2009.

Yuliani S, Marwati T. 1997. Katuk as nutritious additive food. W Tumb Obat 3 (3): 55–56.

Zueni A. 2011. The effect of katuk leaves extract supplementation on Bengkulu burgo chicken sexual hormone and follicles. [M.Sc. Thesis]. University of Bengkulu, Bengkulu. [Indonesia].