

Effects of supplemented *Sauropus androgynus* leaves powder on reproductive traits of Indonesian indigenous hens

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ABSTRACT

The aim of this study was to analyze of *Sauropus androgynus* (SA) leaves powder supplementation on kampung hens reproductive traits. Forty kampung hens aged 4-5 months were distributed randomly into 4 treatments with 10 replications. A completely randomized design (CRD) was used as research design. Samples were placed in individual postal-litter system cages. The four groups were fed diets supplemented with 0, 4, 8, and 12% SA leaves powder for 8 weeks, respectively. The supplementation of SA leaves powder affected serum steroid hormone progesterone (P4) metabolite ($P < 0.05$), serum steroid hormone estradiol-17 β (E2) metabolite concentration and a number of medium follicles yield significantly ($P < 0.01$). Concentration levels of serum steroid hormone E2 and P4 metabolites were 1 to 5-fold and 2 to 14-fold higher in supplemented hens than non-supplemented, respectively. In conclusion, supplementation of SA leaves increased serum steroid hormone E2 and P4 metabolite concentration, number, and weight of follicles. The role of precursor contained in SA leaves powder detected on Indonesia kampung hen's reproductive traits.

Keywords: Follicle, Kampung hen, Katuk leaves, Reproduction, a steroid hormone

INTRODUCTION

Kampung chicken (*Gallus domesticus*) is the most populated indigenous or local chicken in Indonesia and is widely identified as a descendant of red jungle fowls (*G. gallus*) and green jungle fowls (*G. varius*). They were inhabited by tropical rain forest along the equator (Soenarsih and Hoda, 2019), then domesticated and proliferated by rural communities. These local chickens are common to be found until now in Indonesia because they are relatively easy to raise, do not require large capital and it plays a role in utilizing household waste and agricultural products (Soenarsih and Hoda, 2019; Mariandayani *et al.*,

2013). This function role is also found in Asia Africa region local chicken (Habteslasie *et al.*, 2019; Teinlek *et al.*, 2018).

A previous study reported kampung hens can produce about 103 eggs/hen/year (Mariandayani *et al.*, 2013). Furthermore, kampung chickens rearing their offsprings after hatching for a certain time, their egg productions reach only about 52 eggs/hen/year. However, when offsprings are separated since hatching, their production could reach 115 eggs/hen/year. This egg production is far below compared to egg production in layers. It is similar to the result of Soenarsih and Hoda (2019) and Mariandayani *et al.* (2013) which stated productivity of kampung

chickens in producing meat and eggs is lower compared to the productivity of broilers and layers. Researchers (Soenarsih and Hoda, 2019; Mariandayani *et al.* 2013) assumed the body weight and body size of kampung chicken which are lighter and smaller than broilers and layers became the main cause of this problem.

Several studies (Santoso, 2018; Putri *et al.*, 2013) have attempted to improve kampung chicken egg productions, including crossbreeding methods, improving feeding material and composition methods, or application of medicinal plants to reveal its reproductive trait potency. One of the potential method to apply in this study would be the supplementation of medicinal plants.

Medicinal plants have economic value, both in the local community who live in rural areas and modern society who live in urban areas (Hayati *et al.*, 2016). Studies on the role of medicinal plants show that medicinal plants can improve production and reproduction performance (Nurmeiliasari *et al.*, 2020). Medicinal plants have active compounds that play a role in supporting animal productivity and health (Abou-Elkhair *et al.*, 2018; Ding *et al.*, 2017). In Indonesia, *Sauropus androgynus* (SA) is well known to be used as one of the local medicinal plants.

Supplementation of SA leaves extract or powder as one of medicinal plant has been introduced to broilers (Sulfiar *et al.*, 2020; Letis *et al.*, 2017), layers (Nurmeiliasari *et al.*, 2020; Santoso 2018), and another local chicken called Burgo chicken (Putranto *et al.*, 2012). According to Awaludin *et al.* (2020) and Hayati *et al.* (2016) there are 5 unsaturated fatty acid compounds in *S. androgynus* such as octadecanoic acids, 9-eicosyne, 5,8,11-heptadecatrienoic acid methyl ester, 9,12,15-octadecatrienoic acid ethyl ester, and 11,14,17-icosatrienoic acid methyl ester. Also, one steroid compound is androstan-17-one 3-ethyl-3-hydroxy-5 α (Awaludin *et*

al., 2020; Hayati *et al.*, 2016). Thus, androstan-17-one,3-ethyl-3-hydroxy-5 α represents 17-ketosteroid (precursor) which is an intermediate compound on steroid hormone biosynthesis (Hayati *et al.*, 2016).

Nurmeiliasari *et al.* (2020) and Santoso (2018) reported supplementation of SA extract in broilers improved the quality of chicken meat and increased egg quality and egg production in layers. As a comparison, in another Indonesia local chicken, Putranto *et al.* (2012) reported that supplementation of SA extract increased serum steroid hormone estradiol-17 β metabolite concentration and the number of follicles in Burgo hens. Concerning SA has a steroid compound mentioned earlier, utilization of this medicinal plant should contribute positive impacts on reproductive traits. However, there is no scientific report about supplementation of SA on kampung hens reproductive traits especially on serum steroid hormone metabolites, lately.

Utilization of SA to improve hens serum steroid hormone estradiol-17 β (E2) metabolite concentration and its effect on follicles of Burgo chicken had been reported by previous studies (Putranto *et al.*, 2012). Previous studies reported the presence and role function of steroid hormone E2 metabolite on reproduction is also closely related to the presence and function of steroid hormone progesterone (P4) metabolite, especially their similar effect on ovarium function to produce follicles (Navara 2013; Janczak *et al.*, 2009; Reddy *et al.*, 2006).

Therefore, the purpose of this study was to analyze SA leaves powder supplementation on kampung hens reproductive traits including serum steroid hormones metabolites concentration, follicles yield, and egg productions.

MATERIALS AND METHODS

The present study was conducted at Com-

Table 1. *Sauropus androgynus* Leaves Powder Content Analysis Result (%/100 g)

No	Contents	Percentage (%)
1	Water	9.15
2	Ash	10.2
3	Crude Protein	34.2
4	Crude Fat	2.9
5	Crude Fiber	13.2
6	Calcium	1.03
7	Phospor	0.62
8	Gross Energy	4,307.3

Gross Energy = unit in kcal/kg

mercial Zone and Animal Laboratory Department of Animal Science Faculty of Agriculture Universitas Bengkulu Indonesia. All procedures and protocols were officially approved for Ethical Clearance number 02/KEH-LPPM/EC/2020 and verified by the Animal Ethical Committee of Universitas Bengkulu.

Sauropus androgynus Leaves Powder Preparation

Fresh SA leaves were dried under a room temperature for 3 days and continued by drying under the sun for approximately 6 to 8 hours until leaves are completely dry. Dried leaves were mixed and ground using a feed grinder machine until they become ready-to-use powder. The SA leaves powder was homogenized by stirring it evenly and stored using an airtight plastic until it is supplemented in the ration. SA leaves analysis result released by the registered laboratory is presented in Table 1.

Animal, Treatments and Diets

Forty kampung hens age range from 4 to 5 months were used in this latest study. According to Trisiwi (2017), kampung hen reaches its sexual maturity at about 5 months of age. Based on this reason, we assumed all hens used in this study were classified as pullets in their very early sexual maturity phase. The average of body weight was $1,026.75 \pm 196.18$ g/hen, and all in good health condition.

Hens were placed in individual postal-litter system cage equipped with individual feeding

and drinking devices. Prior the research, they were managed for a week of adaptation to intensive rearing system, individual cage, feeding, drinking, and other daily management and research activities such as data collection.

Kampung hens were given experimental diets for 8 weeks. The composition of these diets is presented in Table 2. Diets and drinking water were given *ad libitum*.

Experimental Procedure and Sample Collection

Each kampung hen was housed in an individual postal-litter system cage. The size of these cages was 100 x 100 x 100 cm facilitated with feeding and drinking water tools. The cage and the tools were sprayed by disinfectans a week prior the adaptation phase of study.

A completely randomized design (CRD) was used as a research design with 4 treatments of ten replications. The 4 treatments were as follows :

AK0 : kampung hens were given a diet without SA leaves powder (SALP).

AK1 : kampung hens were given a diet with 4% SALP.

AK2 : kampung hens were given a diet with 8% SALP.

AK3 : kampung hens were given a diet with 12% SALP.

The variables measured in this study were the concentration of serum steroid hormone estradiol-17 β (E2) metabolite, serum steroid hormone progesteron (P4) metabolite, number and

Table 2. Composition of Diets (%/kg)

No	Feed Contents (%)	AK0	AK1	AK2	AK3
1	Scrumble corn	42	41	38	36
2	Layer concentrate	29	29	29	29
3	Rice bran	26.5	24	23	21
4	Palm oil	1	1	1	1
5	SA leaves powder	0	4	8	12
6	Mineral mix	1	1	1	1
7	Commercial feed additive	0.5	0	0	0
Total Percentage (%)		100	100	100	100
Nutrition Composition					
Energy (kcal/kg)		2,758.4	2,794.5	2,791.3	2,788.2
Protein (%)		17.3	17.7	18.02	17.9
Calcium (%)		3.84	3.9	4.04	4.03
Phospor (%)		0.6	0.57	0.57	0.55

AK0 = kampung hens were given a diet without *S. androgynus* leaves powder (SALP); AK1 = kampung hens were given a diet with 4% SALP; AK2 = kampung hens were given a diet with 8% SALP; AK3 = kampung hens were given a diet with 12% SALP

weight of large follicle yield (LFY), medium follicle yield (MFY), small follicle yield (SFY), and the number of egg production. At 56 days of age, 5 ml of blood samples were collected from randomly 5 hens of each treatment group for serum steroid hormone metabolites measurement. Those hens were then slaughtered and ovary and connective tissue were collected for LFY, MFY and SFY counts. Egg productions were collected daily during 8 weeks of the treatment period.

Serum Steroid Hormones Metabolites Analysis

Serum steroid hormones E2 metabolites and serum steroid hormone P4 metabolites were determined by a combination of Sandwich Enzyme

Immuno Assay (EIA) procedure and Enzim Linked Immonoflorescent Assay (ELFA) procedure conducted in Kimia Farma Laboratory, Jakarta Indonesia. At 07:00 in the morning, the amount of 5 ml blood was taken from an artery in the inner part of a wing, saved in a non EDTA tube, kept in a cooler, and sent to the 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. Then, P4 antiserum cross-reacts mainly with P4, 5 α -pregnanedione, pregnenolone, 11-deoxycorticosterone, 17 α -hydroxyprogesterone, and 11 α -hydroxyprogesterone at 100, 62.2, 6.3, 3.9, 2.3, and 1.2%, respectively (Putranto *et al.*, 2007;

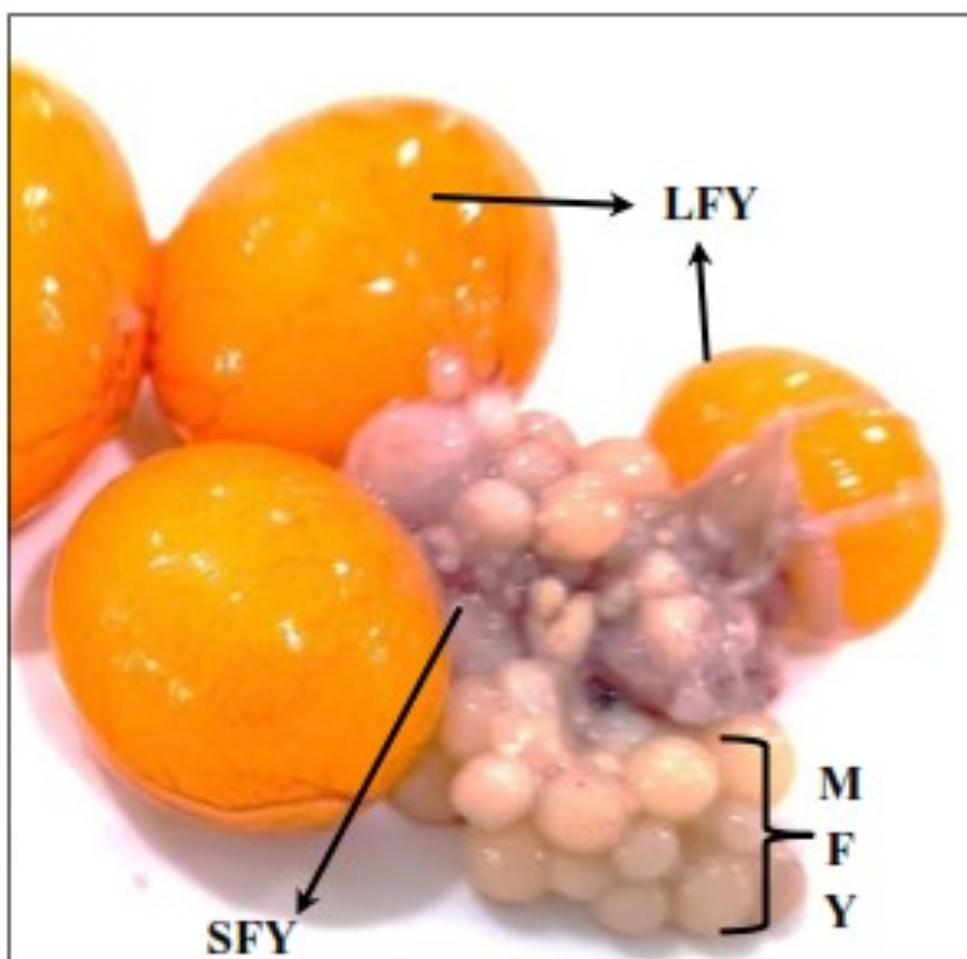


Figure1. Profile of Indonesia Kampung Hen's Follicles (LFY, MFY, and SFY) Who Supplemented by *S. androgynus* Leaves Powder. LFY = Large Follicle Yield, MFY = Medium Follicle Yield, SFY = Small Follicle Yied.

Putranto *et al.*, 2012).

Follicles Measurement

At the end of the study, follicles found in the ovary were collected and divided into 3 groups based on their diameter. 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 (Putranto *et al.*, 2012). Follicles were counted assisted with a hand counter for the number of follicles/hen unit, whereas their weights were measured by a digital balance.

Statistical Analysis

All data were analyzed using analysis of variance, and the significant results were further tested using Duncan Multiple Range Test (DMRT).

RESULTS

Serum Steroid Hormone Metabolites Concentration

Table 3 shows the effect of SA leaves on parameters of serum steroid hormone E2 metabolite concentration, serum steroid hormone P4 metabolite concentration, number and diameter of follicles, and egg production. SA leaves supplementation had a significant effect on serum steroid E2 hormone metabolite concentration ($P < 0.01$). It was shown that AK0 had significantly lower serum steroid hormone E2 metabolite concentration than AK1 and AK2. Then AK1 had significantly higher serum steroid hormone E2 metabolite concentration compared to AK2 and AK3 ($P < 0.01$). Thus, the supplementation of 4% SA leaves powder resulted in the highest concentration of serum steroid hormone E2 metabolite (3094 pg/hen), followed by the level of 8 and 12% which were 1789 and 606 pg/hen, respectively. In comparison to the control group, the concentration of serum steroid hormone E2 metabolite concentration of AK1 was 5.5-fold higher. Then, AK2 and AK3 serum steroid hormone E2 metabolite concentration were 1.8-fold and 1.1-fold higher, respectively, than AK0 as the control.

SA leaves powder supplementation had a significant effect on serum steroid hormone progesterone (P4) metabolite concentration ($P < 0.05$). This was shown that AK0 had a significantly lower serum steroid hormone P4 metabolite concentration than AK1. Then AK1 had a

significantly higher serum steroid hormone P4 metabolite concentration compared to AK2 and AK3 ($P < 0.05$). Thus, the supplementation of 4% SA leaves powder resulted in the highest concentration of serum steroid hormone P4 metabolite (9.6 ng/hen), followed by the level of 8 and 12% which were 2.5 and 1.5 ng/hen respectively. In comparison to the control group, the concentration of AK1 was 14.5-fold higher. Although non significantly different, AK2 and AK3 serum steroid hormone P4 metabolite concentration were 3.7-fold and 2.2-fold higher, respectively, than AK0 as the control.

Follicles Yield

SA leaves powder supplementation had a significant effect on the number of medium follicle yield (MFY) ($P < 0.01$), but it had no effect on large follicle yield (LFY) and small follicle yield (SMF). Figure 1 shows profile of kampung hen's follicles (LFY, MFY, and SFY) found in this study. It was shown that AK0 had a lower number of LFY compared to AK1, AK2, and AK3. Then AK1 had a higher number of LFY compared to AK2 and AK3. Thus, the supplementation of 4% SA leaves powder resulted in the highest number of LFY (23 follicles), followed by the level of 8 and 12% which were 19 and 17 follicles respectively. In comparison to the control group, LFY number of AK1 was 8 follicles higher. Then, AK2 and AK3 LFY numbers were 4 and 2 follicles higher, respectively, compared to AK0 as the control. Data in Table 3 was shown that AK0 had significantly lower number of MFY compared to AK2. AK2 had a significantly higher number of MFY compared to AK3 ($P < 0.01$). Thus, the supplementation of 8% SA leaves powder resulted in the highest number of MFY (50 follicles), followed by the level of 4 and 12% which were 43 and 20 follicles, respectively. In comparison to the control group, MFY numbers of AK2 was 33 follicles higher. Although non significantly different, AK1 and AK3 MFY number were 26 and 3 follicles higher, respectively, compared to AK0 as the control. It was shown in Table 3, although statistically non-significant AK0 had higher number of SFY compared to AK1, AK2, and AK3. Then AK1 had a higher number of SFY compared to AK2 and AK3. No supplementation of SA leaves powder (0%) tended to be resulted in the highest number of SFY (674 follicles) compared of all supplementation levels. SFY number of AK0 was 295 follicles tended to be

higher than AK1, 305 and 353 follicles tended to be higher than AK2 and AK3, respectively. Most populated follicles found in form of SMF which was 674 follicles/hen and based on its diameter they were categorized as primary follicles (primordial).

SA leaves powder supplementation had no significant effect on the weight of follicles in kampung hens ($P > 0.05$). Although non significantly different, AK0 tended to be lighter compared to AK1. However, AK0 was tended to be heavier than AK2 and AK3 LFY. Then AK1 tended to be heavier compared to AK2 and AK3. Thus, the supplementation of 4% SA leaves powder tended to have resulted in the highest

weight of LFY (29 g/hen), followed by the level of 8 and 12% which were 14 g/hen. In comparison to the control group, LFY weight of AK1 was 13.4 g heavier. However, LFY weight of AK2 and AK3 was 1.62 and 1.89 g tended to be lighter compared to AK0. The heaviest follicle weight was found in large LFY was 29 g and based on its diameter they were categorized as tertiary or preovulatory follicles.

Egg Production

Results in Table 3 show that SA leaves powder supplementation had no significant effect on total egg production in kampung hens ($P > 0.05$). Although non-significantly different,

Table 3. Effect of *S. androgynus* Leaves Powder Supplementation on Indonesia Kampung Hens Reproductive Trait Parameters

Parameters	Level of Supplementation			
	AK0 ± STD	AK1 ± STD	AK2 ± STD	AK3 ± STD
Serum Steroid Hormones Metabolites Concentration				
E2 (ng/hen)	564.040** ± 9.49	3 093.650** ± 167.00	1 789.370** ± 37.40	606.450** ± 22.04
P4 (pg/hen)	0.663* ± 0.03	9.583* ± 1.70	2.484* ± 0.06	1.484* ± 0.05
Number of Follicles (follicles/hen)				
LFY	15 ± 3.00	23 ± 0.89	19 ± 1.92	17 ± 3.21
MFY	17** ± 2.41	43** ± 2.51	50** ± 6.63	20** ± 4.53
SFY	674 ± 61.11	379 ± 24.46	369 ± 49.62	321 ± 47.01
Weight of Follicles (g/hen)				
LFY	15.490 ± 3.08	28.893 ± 0.40	13.875 ± 1.85	13.600 ± 2.52
MFY	0.533 ± 0.07	0.917 ± 0.10	0.517 ± 0.01	0.367 ± 0.07
SFY	10.730 ± 0.83	11.950 ± 0.38	11.370 ± 0.77	9.680 ± 0.99
Egg Production (egg)				
Total	93 ± 7.57	89 ± 7.46	65 ± 7.35	23 ± 4.06
Individual	21	19	17	12

Significant at *($P < 0.05$), **($P < 0.01$); AK0 = kampung hens were given a diet without *S. androgynus* leaves powder (SALP); AK1 = kampung hens were given a diet with 4% SALP; AK2 = kampung hens were given a diet with 8% SALP; AK3 = kampung hens were given a diet with 12% SALP; E2 = hormone estradiol-17 β in ng/hen unit; P4 = hormone progesterone in pg/hen unit; Number LFY = large follicle yield in follicles/hen unit; Number MFY = medium follicle yield in follicles/hen unit; Number SFY = small follicle yield in follicles/hen unit; Weight LFY = large follicle yield in g/hen unit; Weight MFY = medium follicle yield in g/hen unit; Weight SFY = small follicle yield in g/hen unit; Total = total egg production in egg unit; Individual highest individual egg production in egg unit

AK0 as control group tended had highest total egg production (93 egg/hen) compared to all supplemented groups (AK1, AK2, and AK3). Among supplemented kampung hen groups, AK1 tended had higher total egg production (89 eggs/hen) compared to AK2 and AK3 which were 65 and 23 eggs/hen, respectively. Thus, the supplementation of 4% SA leaves powder tended to be resulted in the highest total egg production/hen compared to the level of 8 and 12% supplementation. Total egg production of AK0 was 4 eggs higher than AK1, 28 and 70 eggs higher than AK2 and AK3, respectively.

It was shown that AK0 as control tended had highest individual egg production (21 egg) compared to all supplemented groups (AK1, AK2, and AK3). Among supplemented kampung hen groups, AK1 tended had higher individual egg production (19 eggs) compared to AK2 and AK3 which were 17 and 12 eggs, respectively. The supplementation of 4% SA leaves powder tended to be resulted in the highest individual egg production compared to the level of 8 and 12% supplementation. Individual egg production of AK0 was 3 eggs higher than AK1, 4 and 9 eggs higher than AK2 and AK3, respectively. It seems that high individual egg production in this latest study of Indonesia kampung hens correlates with total egg production.

DISCUSSION

Serum steroid hormone metabolites concentration

The result showed that the supplementation of SA leaves dosed 4 and 8 % for 56 days of duration increased serum steroid hormone E2 metabolite concentration. The increase of serum steroid hormone E2 metabolite concentration in supplemented kampung hens could reach from 1 until 5-fold higher compared to control group. This similar increase pattern agrees with the observation of Putranto *et al.* (2012), who found that SA leaves extract supplementation increased serum steroid hormone E2 concentration in Burgo chicken.

The average concentration of serum steroid hormone E2 metabolite of the control group was low. Putranto *et al.* (2012) stated that female chicken only synthesized steroid hormone by using their natural cholesterol in the cortex adrenal and liver. In contrast, the average concentration of serum steroid hormone E2 metabolite with supplemented SA leaves was higher than

control. SA leaves supplementation might involve increasing process of the concentration of serum steroid hormone E2 metabolite in Indonesia kampung hens. According to Awaludin *et al.* (2020) and Hayati *et al.* (2016), the SA plant contains benzoic acid. Benzoic acid in the body can be converted into estradiol benzoate. Estradiol benzoate and other compounds such as androstan-17-one,3-ethyl-3-hydroxy-5 alpha represent 17-ketosteroid (Hayati *et al.*, 2016) play a role in improving reproductive function such as estrogen steroid hormone development and stimulating follicle growth.

The higher concentration of serum steroid hormone E2 metabolite in this study reflected that Indonesia kampung hen produces more estrogen steroid hormone not only by using their cholesterol in the body. Ovary of aves species begins to develop and cause secretion of steroid hormones such as estradiol when the individual reached sexual maturity (Jamieson, 2007). Furthermore, the serum steroid hormone E2 metabolite will increase as the follicles developed. The previous study (Putranto *et al.*, 2012) reported that the majority of estrogen is formed as estrone, E2, and estradiol-17 α .

Supplementation of SA leaves powder treatment resulted from a similar profile on serum steroid hormone P4 metabolite concentration to serum steroid hormone E2 metabolite concentration in kampung hens. Serum steroid hormone P4 metabolite concentration in non-supplemented kampung hens is tended to be lower than supplemented hens as the control. The increase of serum steroid hormone P4 metabolite concentration in supplemented kampung hens could reach from 2 until 14-fold higher compared to the control group. Hormone P4 is produced in the ovary of females (Jamieson, 2007). Its concentration is dynamically changes due to reproductive cycles of females such as during estrus and pregnancy in mammals (Putranto *et al.*, 2007). Similar to serum steroid hormone E2 metabolite concentration mentioned earlier (Awaludin *et al.*, 2020; Hayati *et al.*, 2016), SA leaves supplementation may involve in the increasing process of the concentration of serum steroid hormone P4 metabolite. Cholesterol in the body as a basic component and benzoic acid content in SA plant as precursor to steroid hormone production (Putranto *et al.*, 2012) contributed to their role function to develop more volume of serum steroid hormone P4 and also took the main role in ovulation stage.

It could be assumed that there is another cause for this increase pattern of hormone P4 in this study (Putranto, unpublished data). Jamieson (2007) reported, amount of hormone P4 produced not only by the ovary of hens, however it is also caused by an additional amount of hormone P4 produced by preovulatory follicles which are ready to be ovulated. The higher concentration of serum steroid hormone P4 metabolite in this study is showing us that there is a connection to the number of LFY of sexually mature hens. Jamieson (2007) and Johnson (1990) reported that hormone P4 can be also produced by a corpus luteum of large size follicles. As we can see the number of large-size follicles of SA leaves supplemented kampung hens are more populated than non-supplemented hens.

The concentration of serum steroid hormone P4 metabolite in Indonesia kampung hens showed a positive correlation between the availability of additional hormone P4 produced by follicles that are ready to ovulate known as de Graff's follicles (Jamieson, 2007). The number of follicles that develop into de Graff's follicles is directly proportional to the concentration of serum steroid hormone P4 metabolite secreted by the ovum. Jamieson (2007) stated that the production of hormone P4 increases drastically controlled by luteinizing hormone (LH). Furthermore LH is induced by the form of cAMP because follicle formation approaches the time of ovulation.

It has been reported that there is a correlation between spontaneous and preovulatory ovulation due to the surge in hormone P4 during the ovulatory cycle in Japanese quail, ducks, laying-type chickens and turkeys (Liu *et al.*, 2004). Spontaneous ovulation and oviposition in birds are caused by the role of the hormone system. In turkeys who received implanted hormone P4, egg production tended to be decreased (Liu *et al.*, 2004), and quails can be stopped laying eggs for a short time (Tell *et al.*, 1999).

Follicles Yield

The previous studies reported the average population of the follicle in kampung hens varied between 14 to 23 follicles per hen (Putri *et al.*, 2013), and our study resulted in a population ranged between 15 to 23 follicles (Table 3). The population of follicles counted was large size follicles only (LFY). The growth and development of follicles is strongly influenced by many factors including lighting, stress, and nutrition

(Jamieson, 2007).

Most of follicles development activities in aves are occurred in the inner cortex of ovary and it is different from mammals (Jamieson, 2007). The post primordial phase of chicken follicles is easy to observe and distinguish from stroma ovary tissue, a bonded grapes-like. In this study, the follicle counts are based on follicle size classification by previous reports (Putranto *et al.*, 2012; Johnson, 1990). 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.

Even though supplementation of SA leaves powder in Indonesia kampung hens initiate a higher average number of large and medium-size follicles compared to non-supplemented hens, it's significantly affected on medium-size follicles only. On contrary, the average number of small-size follicles in supplemented kampung hens is tended to be lower than non-supplemented hens as the control group. In the case of small-size follicles, a previous report by Putranto *et al.* (2012) assumed SA leaves powder tends to reduce the digestibility of crude fat. Presumably, there is an inhibitory effect contained in SA leaves on the synthesis of liquid in bile which forces a secretion of liquid to increase. The higher secretion of bile as a result of decreased crude fat digestibility indicates a decreasing absorption of fat and other fat components and fat derivatives such as decreased cholesterol, LDL, HDL, and triglycerides. We assumed that the decreasing of acid in bile caused a decreasing crude fat digestibility and causes a low-fat absorption.

The effect of cholesterol absorption inhibition leads to an obstruction of ovaries to synthesis estrogen and this will inhibit the follicle formation process. The ovaries can produce the hormone estrogen which has an important role during the egg formation process (Johnson, 1990), and the concentration of hormone estrogen increases along with the development of ovarian follicles. Therefore, this caused an inhibitory effect on small-size follicles development especially from the pre-eararchal follicles phase to the preovulatory and ovulatory follicles phase. It is also reflected in the number of medium-size and large-size follicles of supplemented kampung hens compared to non-supplemented hens in this

study.

The weight of follicles in this study is assumed has a correlation with follicle size, follicle numbers and SA leaves powder supplementation level. In total, the average weight of large-size and medium-size follicles in supplemented kampung hens is higher than non-supplemented hens. It is correlated to the number or follicle population. A higher population of large and medium-size follicles contribute to a heavier total weight of follicles. However in small-size follicles data, follicle population does not contribute to a heavier weight. The weight of non-supplemented kampung hens follicle is lower than supplemented hens. We assumed that small-size follicles number of non-supplemented kampung hens is more populated by smaller size follicles compared to supplemented hens. It caused a higher number of follicles with lighter weight.

Naturally, a sexually matured hen has approximately 3 000 to 4 000 follicles in its ovary (Jamieson, 2007). The total number of follicles in this study was approximately only 11 to 17% of normally populated follicles in the mature hen. However, this study result is higher than populated follicles in Burgo chicken (Putranto *et al.*, 2012). It seems that chicken body weight and body size are related to the number of follicles produced. It agrees with Soenarsih and Hoda (2019) and Mariandayani *et al.* (2013) study reports which stated that body weight and body size of kampung chicken became the main cause of this problem. Burgo hen's body size is smaller than Indonesia kampung hen in common and bigger than the wild red forest chicken (Putranto *et al.*, 2012). Furthermore, we assumed many primary follicles (primordial) and post-ovulatory follicles in this study probably were in nano size and unseen by a usual visual count.

Eggs Production

The total egg production of kampung hens in this study varied between 23 to 93 eggs/group or averaged between 2,3 to 9,3 egg/hen during 56 days, specifically about 2.3 to 8.9 eggs/hen for kampung hens with supplemented SA leaves powder. When we assumed its production in a year, probably maximum of 53 to 56 eggs can be produced by kampung hens in this study. Probably the individual sexual maturity stage or status has become the main cause of egg production (Trisiwi 2017; Mariandayani *et al.*, 2013).

Later, Indonesia kampung hen's total egg production in this study is lower than some re-

cent reports. A recent study (Trisiwi 2017) reported that Indonesia kampung hens reared traditionally or under non-intensive management in a rural area could produce about 40 to 45 eggs/hen/year. According to Mariandayani *et al.* (2013), the total egg production of kampung hens reared intensively could reach about 150 eggs/hen/year. This difference in total egg production is mainly caused by during egg formation process, a sufficient volume of hormones is required to stimulate ovary to develop follicles (Putranto *et al.*, 2012). There is a possibility of serum steroid hormone P4 metabolite concentration affected by this lower egg production. Research reports showed that implanted hormone P4 caused decreasing egg production in turkey and the occurrence of stopping laying eggs in quails (Tell *et al.*, 1999). In addition, Etches (1996) stated that hypothalamus secretes gonadotropin hormones, identified as FSH and LH which play a role as a regulator in ovarian function including follicular growth until its ready to be ovulated.

Data in Table 3 shown us about Indonesia kampung hens total egg production and individual egg production. In the control group hens, its average of total egg production and individual egg production is higher compared to supplemented kampung hens. It seems that SA leaves powder supplementation did not affect the average of total egg production and individual egg production. Agrees to Trisiwi (2017) report, the researcher believed the individual sexual maturity stage or status has become a main cause of this matter.

We assumed some of kampung hens in the control group reach an earlier pullet stage or early preovulatory stage and more ready to ovulate compared to other hens in this study. Based on the study results, the control group kampung hens has a lower population of secondary and tertiary follicles. Probably it is because hens in the control group have already ovulated more follicles than other hens, hereafter they delivered more eggs earlier than other hens.

Awaludin *et al.* (2020) and Hayati *et al.* (2016) stated benzoic acid compound in SA plant when its digested in the body, those benzoic acid will be converted into estradiol-17 β benzoate which can improve the reproduction function and stimulates follicles growth. This mechanism can lead chickens potentially able to produce higher total egg production, individual egg production, and more efficient.

Another consideration is the body size of

the sub-species. Body weight was measured to predict the relationship between body weight and the number of follicles and egg production. It seems that the body size is related to the number of follicles and egg produced. Added by Putranto *et al.* (2012), Indonesia kampung chicken has a smaller body size compared to broiler and layer. With its small size and compact body, it may cause the number of egg production of Indonesia kampung hen is less populated than a layer.

CONCLUSION

The 4% level supplementation of SA leaves in Indonesia kampung hen feed increased serum steroid hormone E2 metabolite and serum steroid hormone P4 metabolite concentration, number and weight of follicles of medium size follicles, and it showed the best results. The role of precursor contained in SA leaves powder detected on Indonesia kampung hen's reproductive traits.

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