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The effect of *Sauropus androgynus* leaves extracted at different methods on performance and carcass quality in broiler chickens

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ABSTRACT: The objective of this study was to analyze the effect of *Sauropus androgynus* leaves extracted at different methods on performance and carcass quality in broiler chickens. One hundred and eighty broilers aged 20 days were divided into six groups as follows: Broilers were fed a diet containing 5 g commercial feed supplement /kg as the control (P0); Broilers were fed a diet containing 2.5 g commercial feed supplement /kg plus 2.5 g *Sauropus androgynus* leaf (SAL) extracted at a 90ºC/kg (P1); Broilers were fed diet containing 5 g SAL extracted at a 5°C/kg (P2); Broilers were fed diet containing 5 g SAL extracted at a 30°C/kg (P3); Broilers were fed diet containing 5 g SAL extracted at a 60ºC/kg (P4); Broilers were fed diet containing 5 g SAL extracted at a 90°C/kg (P5). *Sauropus androgynus* leaves extracted at different methods significantly ($P<0.05$) affected body weight, and cholesterol, protein, vitamin E, and β-carotene contents of meats ($p<0.05$), but it did not significantly affect feed intake, feed conversion ratio, carcass weight, carcass color cooking loss, meat taste and odor, and meat fat contents ($P>0.05$). In conclusion, supplementation of *Sauropus androgynus* extracted at 30ºC could replace 100% commercial feed supplement. Supplementation of *Sauropus androgynus* leaf extracted at 60ºC or 90°C reduced meat cholesterol. In addition, this extract inclusion resulted in higher meat protein content as compared with the control.

Keywords: *Sauropus androgynus*; Carcass Quality; Performance; Broilers

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INTRODUCTION

Recently, the poultry industry has been faced with the problem to produce low cholesterol broiler meats, but rich in protein, β-carotene, and vitamins. Feed supplements that are sold commercially only contain several vitamins, micro minerals, and antioxidants, but no β-carotene, flavor enhancers, fishy odor, and cholesterol-lowering compounds. In general, commercial feed supplements, which are prepared from synthetic compounds such as antibiotics and other sybthetic compounds have been proven to have high side effects (Friedman, Temkin, and Carmeli, 2016) such as causing drug resistance (Mund *et al*., 2017). Thus, it is necessary in replacing the commercial feed supplement with a natural feed supplement which might have a more beneficial effect. This feed supplement should contain active compounds which provide a smaller side effect and has the potential to be used as a natural feed supplement for producing modified meats. The potential natural feed supplements include medicinal plants (Madhupriya *et al*., 2018). One of the herbs that meet the above criteria is *Sauropus androgynus* (Santoso, Fenita and Sulistyowati, 2017; Santoso, Fenita and Kususiyah, 2017).

Sauropus androgynus leaves are rich in vitamin C, vitamin E, β-carotene, flavonoids, phenolic compounds, and glutamic acid (Bose *et al.*, 2018; Samad *et al.*, 2014; Yunita, Rantam, and Yuwono, 2019). These compounds are well known to have anti-lipid and antioxidant (Bose *et al.*, 2018) and antimicrobial properties (Baba and Malik, 2015). Soeparno (2011) stated that glutamic acid and other amino acids play an important role in protein synthesis. Monomethyl succinate and cis-2-methyl cyclopentanol acetate could be converted into succinate and acetate in which these compounds play a role in the Krebs cycle to produce ATP larger. Phenyl malonate acid can be converted into malonyl-CoA, which plays a role in the metabolism of fatty acids (Santoso, 2018). Therefore, the *Sauropus androgynus* leaves may be able to modify the fatty acids, cholesterol, and protein of meats.

Sauropus androgynus leaf extract reduced the fat and cholesterol contents of broiler meats (Santoso, Fenita, and Kususiyah, 2017, but increased egg vitamin E (Santoso, Fenita, and Sulistyowati, 2017). These authors extracted *Sauropus androgynus* leaves using hot water (90^{o}C). However, the hot water extraction will damage some active compounds in the *Sauropus androgynus* leaves. Mukhriani (2014) found that although the extraction with hot water (temperature 90°C) produces a high yield although some important compounds are damaged. Boiled food had lower levels of phenol (Aisyah, Rasdiansyah, and Muhaimin, 2014), vitamin C (Tincheva, 2019), total carotenoids, protein, crude fiber, essential amino acid, and ash contents (Danhassan, Salihu, Inuwa, 2018).

Based on the description above, it is assumed that extraction at a lower temperature will produce a higher yield of active compounds. Higher levels of active compounds will result in better carcass performance and quality. Therefore, the objective of the present study was to analyze the effect of *Sauropus androgynus* leaf extracted at different methods on performance and carcass quality in broiler chickens. It was hypothesized that extraction at lower water temperatures might result in better performance and carcass quality of broiler.

MATERIALS AND METHODS

Sauropus androgynus leaves extraction

Method 1. *Sauropus androgynus* leaf powder was extracted in cold water (5^{o}C) for 20 minutes and blended until it becomes juice. The juice obtained was then filtered. The filtrate obtained was dried at 50°C for 36 hours.

Method 2. *Sauropus androgynus* leaf powder was extracted in warm water (30^{o}C) for 20 minutes and then blended and filtered. The filtrate was then dried at 50°C for 36 hours.

Method 3. *Sauropus androgynus* leaf powder was extracted in hot water (60^{o}C) for 20 minutes and then filtered. The filtrate was then dried at 50°C for 36 hours.

Method 4. *Sauropus androgynus* leaf powder was extracted in hot water (90^{o}C) for 20 minutes and then filtered. The filtrate was then dried at 50°C for 36 hours.

The experimental treatments

A completely randomized design was used in this investigation. One hundred and eighty broilers (strain Lohman) aged 20 days were grouped into 6 groups as follows:

1. Broilers were given a diet containing 5 g commercial feed supplement/kg diet (P0);
2. Broilers were given a diet containing 2.5 g commercial feed supplement /kg diet plus 2.5 g *Sauropus androgynus* leaves (SAL) extracted at 90^{o}C/kg diet (P1);
3. Broilers were given a diet containing 5 g SAL extracted at 5°C/kg diet (P2);
4. Broilers were given a diet containing 5 g SAL extracted at 30°C/kg diet;
5. Broilers were given a diet containing 5 g SAL extracted at 60^{o}C/kg diet, and;
6. Broilers were given a diet containing 5 g SAL extracted at 90°C/kg diet.

Each treatment consisted of 5 replicates of 6 broilers. The composition of the diet is presented in Table 1. The diet contained 19% crude protein and 3200 kcal ME/kg. Broilers were reared in cages up to the age of 42 days. Diets and drinking water were given *ad libitum*. Feed intake, feed conversion ratio, and body weight were measured weekly.

Sampling and laboratory analysis

At 42 days of age, 4 female broilers for each treatment group were slaughtered, and the liver was removed. Thigh meats were collected for analysis of cholesterol, vitamin E, protein, and fat contents. The method of AOAC (2012) was used to measure the protein and fat contents of meats. Cholesterol content was determined by the method of Laila and Putra (2019). β-carotene and vitamin E were determined by the methods of Starek et al. (2015).

Meat-bone ratio was measured as follows. Carcass weight was measured by the following equation.

$$\text{Carcass weight (\%)} = \text{carcass weight/live bodyweight} \times 100\%$$

Meat and bone of breast and leg were separated, and the meat and bone were then weighed. The ratio of meat and bone was calculated. Carcass color (breast skin color) was measured by comparing the color of the carcass to the yolk color fan. The color of the meat was assessed by comparing the color of breast meat to the color standard of the ID-DLO scale of 1-5. Breast meat (10 g) was cooked in a sealed plastic bag at 80^{o}C for 20 minutes. The cooled meat was then weighted. The cooking loss (CL) was calculated using the following equation.

$$\text{CL (\%)} = \frac{\text{(the breast weight before cooking – the breast weight after cooking)}}{\text{The breast weight before cooking}} \times 100\%$$

Ten trained sensory panelists were asked to evaluate the odor and taste of meat. The odor of meat was assessed based on the value of 1 (very fishy) to the value of 5 (not fishy). For the taste test, trained panelists previously tasted the breast meat broth obtained by boiling the meat at various concentrations. After the panelists could distinguish the taste of meat, they were then asked to taste and judge the taste of the meat from a value of 1 (not tasty) to a value of 5 (very good). Meats were boiled at 80°C for 20 minutes, cooled, and then tested.

Data analysis

All data were analyzed by ANOVA and then were tested using Duncan's Multiple Range Test when significant results were obtained.

RESULT AND DISCUSSION

Broiler Performance

Table 2 shows the effect of different extraction methods of *Sauropus androgynus* leaves (SAL) on the performances of broiler chickens. Results of the analysis of variance showed that the SAL extract significantly affected (P<0.05) body weight, but did not significantly influence feed intake and feed conversion ratio. DMRT test showed that broilers in P0 (the control) were significantly (P<0.05) heavier than P2, P4, and P5, but not significantly different from P1 and P3.

Table 1. The composition of experimental diets

Feedstuff (%)	P0	P1	P2	P3	P4	P5
Yellow corn	55.6	55.6	55.6	55.6	55.6	55.6
Palm oil	6.53	6.53	6.53	6.53	6.53	6.53
Soybean meal	29.6	29.6	29.6	29.6	29.6	29.6
Fish meal	4.7	4.7	4.7	4.7	4.7	4.7
Calcium carbonate	1.32	1.32	1.32	1.32	1.32	1.32
Mineral mixture	1.35	1.35	1.35	1.35	1.35	1.35
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Commercial feed supplement	0.5	0.25	0	0	0	0
SAL	0	0.25	0.5	0.5	0.5	0.5
Calculated composition						
Protein, %	20.6	20.7	20.8	20.8	20.8	20.8
ME, kcal/kg	3,260	3,265	3,270	3,270	3,270	3,270

SAL= *Sauropus androgynus* leaves; P0= Broilers were fed diet supplemented to commercial feed supplement; P1= Broilers were fed diet supplemented to 0.25% commercial feed supplement and 0.25% SAL extracted at 90^{o}C; P2= Broilers were fed diet supplemented to 0.5% SAL extracted at 5^{o}C; P3= Broilers were fed diet supplemented to 0.5% SAL extracted at 30^{o}C; P4= Broilers were fed diet supplemented to 0.5% SAL extracted at 60^{o}C; P5= Broilers were fed diet supplemented to 0.5% SAL extracted at 90^{o}C.

Table 2. The effect of different extraction methods of *Sauropus androgynus* leaves on the performance of broiler chickens

Variables	P0	P1	P2	P3	P4	P5	SD
Bodyweight (g/bird)	2,417 b	2,336 ab	2,291a	2,345ab	2,255a	2,279a	58.3
Feed intake (g/bird)	3,158	3,108	3,083	3,097	3,117	3,075ns	29.7
FCR	2.18	2.26	2.34	2.26	2.41	2.35ns	0.08

P0= Broilers were fed diet supplemented to commercial feed supplement; P1= Broilers were fed diet supplemented to 0.25% commercial feed supplement and 0.25% *Sauropus androgynus* leaves (SAL) extracted at 90^{o}C; P2= Broilers were fed diet supplemented to 0.5% SAL extracted at 5^{o}C; P3= Broilers were fed diet supplemented to 0.5% SAL extracted at 30^{o}C; P4= Broilers were fed diet supplemented to 0.5% SAL extracted at 60^{o}C; P5= Broilers were fed diet supplemented to 0.5% SAL extracted at 90^{o}C.

This means that the inclusion of 50% SAL extracted at 90^{o}C plus 50% commercial feed supplement (P1) or SAL extracted at 30^{o}C (P3) could replace a commercial feed supplement. Thus, *Sauropus androgynus* leaves extracted at 90^{o}C could only be given to the broiler as much as 2.5 g to produce a bodyweight that is relatively the same as the control. Based on body weight data, it could be concluded that the *Sauropus androgynus* leaves should be extracted at 30^{o}C. The extraction of the leaves at 5^{o}C, 60^{o}C, or 90^{o}C might impair active compounds and/or inappropriate conditions to extract the leaves resulting in lower body weight. Mukhriani (2014) found that although the

extraction with hot water produces a high yield, however, some important compounds were damaged. Hot water extraction is not suitable for heat-sensitive compounds, and the method might be better for essential oil extraction (Azmin *et al.*, 2016).

Carcass Quality

The effect of different extraction methods of *Sauropus androgynus* leaves (SAL) on carcass and meat quality is presented in Table 3. The results showed that the SAL extract did not significantly affect (P>0.05) carcass percentage, carcass color, meat taste and meat color, but significantly affected (P<0.05) meat odor, and cooking loss. The meat odor of P0 and P1 had a lower score than P2, P3, P4, and P5, whereas the cooking loss of P5 was lower than P0, P1, P3, and P4.

The present study agrees with the observation of Santoso, Kususiyah, and Suharyanto (2015) who found that the supplementation of *Sauropus androgynus* leaves extract did not improve relative carcass weight. Percentage carcass in broilers included in the normal category, which ranges from 60 to 70%.

Table 3. The effect of different extraction methods of *Sauropus androgynus* leaves on broiler carcass and meat quality

Variable	P0	P1	P2	P3	P4	P5	SD
Carcass weight (%)	66.6	65.2	66.6	64.5	63.5	66.7ns	1.33
Carcass color	1.5	1.2	1.6	1.5	1.7	1.5ns	0.17
Meat taste	3.1	2.7	2.7	2.8	2.7	2.9ns	0.16
Meat odor	3.4a	3.6a	3.7b	3.7b	3.8b	3.8b*	0.15
Cooking loss (%)	17b	17b	15ab	18b	17b	13a*	1.83
Meat color	2.0	2.3	1.5	2.5	2.0	2.3ns	0.35

P0= Broilers were fed diet supplemented to commercial feed supplement; P1= Broilers were fed diet supplemented to 0.25% commercial feed supplement and 0.25% *Sauropus androgynus* leaves (SAL) extracted at 90^{o}C; P2= Broilers were fed diet supplemented to 0.5% SAL extracted at 5^{o}C; P3= Broilers were fed diet supplemented to 0.5% SAL extracted at 30^{o}C; P4= Broilers were fed diet supplemented to 0.5% SAL extracted at 60^{o}C; P5= Broilers were fed diet supplemented to 0.5% SAL extracted at 90^{o}C.

Carcass color is produced from pigments, among others, β-carotene. The unchanged carcass color was in line with the unchanged β-carotene content of meat in this study. *Sauropus androgynus* leaves contain β-carotene as much as 3642.60 µg/g leaves (Santoso, Fenita, and Kususiyah, 2017). It is suspected that the extraction with water is less able to extract the pigment. This pigment is insoluble in water but soluble in fat solvents.

The iron content of *Sauropus androgynus* leaves was 8.17 mg/g (Santoso, Fenita, and Kususiyah, 2017). The iron is needed for myoglobin synthesis, a pigment for meat color. However, the present study showed meat color was unchanged by SAL extract inclusion. Myoglobin is a type of protein. In this study, meat protein was increased by *Sauropus androgynus* leaves extract supplementation (see Table 4), however, the meat color did not improve. From this phenomenon, it could be assumed that the increased protein in meat is a protein other than myoglobin.

Meat odor score was lower in P0 and P1 which means that they had more odor than other treatments. The lower fishy odor is thought to be caused by lowering the oxidation of unsaturated fatty acids in the meat. *Sauropus androgynus* leaves are rich in antioxidant compounds such as flavonoids, tannin, phenols, vitamin C, and vitamin E. These leaves contain vitamin C of 314.5 mg/100 g fresh leaves and vitamin E of 17.8 mg/100 g (Platel and Srinivasan, 2017). Petrus (2013) reviewed that *Sauropus anrogynus* leaves contained 785 mg flavonoids/100 g, tannin 88.68 mg/100 g, vitamin C247 IU, total carotenoids 5.15

mg/100 fresh leaves, and vitamin E 0.43 IU. The present study shows that extraction using water with various temperatures resulted in a similar odor of meats. Thus, it is suspected that *Sauropus androgynus* leaves extraction at various water temperatures has less effect on the levels of compounds that act as antioxidants.

No change in meat taste in SAL extract groups in the present study might be related to similar content of meat glutamic acid among the treatment groups. Active compounds of broiler meats are glutamic acid, K^+, and IMP. The glutamic acid contents of broiler meats in the present study for P0, P1, P2, P3, P4 and P5 were 1,2%, 1,3%, 1,2%, 1,2%, 1,6% and 1,5%, respectively. This result showed that meat glutamic acid contents were relatively similar among the treatment groups. This could partly explain the unchanged meat taste when broiler chickens were given a diet supplemented with SAL extract.

Sauropus androgynus leaves extracted at 90^{o}C (P5) produced a lower cooking loss. One factor influencing cooking loss is protein. However, the present study showed that all *Sauropus androgynus* leaves extract treatment groups had comparable protein content. Meat fat content in the P5 treatment tends to be lower. Meat with low-fat content has a low cooking loss (Keklik, Bozkurt, and Tekin, 2018). In addition, it is suspected that P5 contains more myofibrillar protein (actin and myosin) which has a higher water-binding ability. These proteins are the major proteins responsible for binding hydration water and immobilizing free water (Maurer and Oostenbrink, 2019). The myofibrils could hold water because of the three-dimensional network of the filaments. The lower cooking loss of meat indicates that the meat protein is undamaged. Protein deterioration may be caused by lipid oxidation. Lipid oxidation products such as aldehydes can easily react with proteins and cause damage to meat proteins (Bhattacharya, Kandupon and Vishnurej, 2016; Guyon, Meynier and Lamballerie, 2019).

Meat Composition

Table 4 presents the effect of different extraction methods of *Sauropus androgynus* leaves (SAL) on meat composition. The experimental results showed that the treatments did not affect the contents of fat in broiler meat ($P<0.05$), but they affected the meat contents of cholesterol, protein, β-carotene, and vitamin E. The content of cholesterol in P4 and P5 was significantly lower ($P<0.05$) than that of P0, P1, and P2, whereas the content of β-carotene in P2 was significantly lower than that of P0, P3, P4, and P5.

The meat protein contents were significantly higher in SAL extract treatment groups as compared with the control (P0). The vitamin E contents of P2 and P5 were significantly higher than that of the P0 (control).

Table 4. The effect of different extraction methods of *Sauropus androgynus* leaves on the nutritional composition of meat

Variable	P0	P1	P2	P3	P4	P5	SD
Protein (%)	17.35^{a}	18.31^{b}	18.39^{b}	18.77^{b}	19.09^{bc}	18.42^{b*}	0.61
Fat (%)	26.00	25.24	25.05	24.94	24.40	23.95^{ns}	0.69
Cholesterol (mg%)	1.12^{b}	1.05^{b}	0.98^{b}	0.95^{ab}	0.92^{a}	0.87^{a}	0.08
ß-carotene (µg/100g)	295.67^{b}	248.17^{ab}	185.12^{a}	261.72^{b}	268.96^{b}	287.08^{b}	39.51
Vit. E (mg/100 g)	0.23^{a}	0.25^{ab}	0.27^{b}	0.25^{ab}	0.22^{a}	0.27^{b*}	0.02

P0= Broilers were fed diet supplemented to commercial feed supplement; P1= Broilers were fed diet supplemented to 0.25% commercial feed supplement and 0.25% *Sauropus androgynus* leaves (SAL) extracted at 90^{o}C; P2= Broilers were fed diet supplemented to 0.5% SAL extracted at 5^{o}C; P3= Broilers were fed diet supplemented to 0.5% SAL extracted at 30^{o}C; P4= Broilers were fed diet supplemented to 0.5% SAL extracted at 60^{o}C; P5= Broilers were fed diet supplemented to 0.5% SAL extracted at 90^{o}C.

The extraction of *Sauropus androgynus* leaf at 60ºC or 90ºC may be a good condition to extract active components relate to anticholesterolemic properties as indicated by the lower cholesterol content of broiler meats in P4 and P5. Santoso, Fenita, and Kususiyah (2017) reported that the inclusion of fermented *Sauropus androgynus* leaves extracts at 4.5 g/kg reduced meat fat and cholesterol contents.

Meat protein was higher in broilers given SAL extract. *Sauropus androgynus* leaves contain high protein, namely 25.46% (Santoso, Fenita, and Kususiyah, 2017). The protein contribution from the leaves is thought not to be sufficient to increase meat protein. Another factor that may contribute is the increase in protein synthesis by the leaves addition. *Sauropus androgynus* leaves contain glutamic acid. Glutamic acid and other amino acids play a role in protein synthesis either as a substrate or as a modulator (Liu *et al.*, 2002). Santoso, Fenita, and Kususiyah (2017) found that fermented leaves extract increased broiler meat protein. The present study shows that different methods of extraction resulted in similar meat protein content.

The extraction of *Sauropus androgynus* leaf at cold conditions (5ºC) may produce the extract with a low β-carotene as indicated by the lower β-carotene content of broiler meats as compared with the control. Other SALE groups had similar meat β-carotene content to the control. This study disagrees with the observation of Santoso, Fenita, and Kususiyah (2017) who found that 4.5 g fermented SALE/kg inclusion increased the β-carotene content of meats. Fermentation of *Sauropus androgynus* leaves might increase the availability of β-carotene for broilers, thus causing an increase in meat β-carotene contents in Santoso, Fenita, and Kususiyah (2017) study.

Samad *et al.* (2014) reported that *Sauropus androgynus* leaves are rich in vitamin E. The vitamin E content of *Sauropus androgynus* leaves is 0.43 IU per 100 g (Petrus, 2013). This may contribute to the higher meat vitamin E in P2 and P5. Thus, the extraction of *Sauropus androgunus* leaves with water at 5ºC or 90ºC might extract more vitamin E.

CONCLUSIONS

In conclusion, supplementation of *Sauropus androgynus* extracted at 30ºC could replace 100% commercial feed supplement. Supplementation of *Sauropus androgynus* leaf extracted at 60ºC or 90°C reduced meat cholesterol. In addition, this extract inclusion increased meat protein content.

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CONFLICT OF INTEREST

We declare there is no conflict of interest.

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