



## **Effect of *Sauropus androgynus* Leaf Extract and Fish Oil Plus Vitamin E on Performance, Carcass Quality, and Meat Amino Acid Composition in Broiler Chickens**

**(Pengaruh ekstrak daun katuk, minyak ikan plus vitamin E terhadap performa, kualitas karkas, dan komposisi asam amino pada broiler)**

**Urip Santoso<sup>1\*</sup>, Yosi Fenita<sup>1</sup>, and Kususiya<sup>1</sup>**

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Bengkulu University, Bengkulu, Indonesia

**ABSTRACT.** This study aimed to evaluate the effect of *Sauropus androgynus* leaf extract (SALE), lemuru fish oil and vitamin E supplementation on performance, carcass quality, and amino acid composition of broiler meat. One hundred and ninety-five broilers aged 21 days were distributed into 13 groups as follows: broilers were fed diet with 0.5% commercial supplement feed (P0), 10 g/kg SALE plus 1% lemuru fish oil (LFO) (P1), 10 g SALE/kg and LFO 1% plus 60 mg vitamin E (P2), 10 g SALE/kg and LFO 2% (P3), 10 g SALE/kg and LFO 2% plus 60 mg vitamin E (P4), 10 g SALE/kg and LFO 3% (P5), 10 g SALE/kg and LFO 3% plus 60 mg vitamin E (P6), 18 g SALE/kg and LFO 1% (P7), 18 SALE g/kg and LFO 1 % plus 60 mg vitamin E (P8), 18 g SALE/kg and FLO 2% (P9), 18 SALE g/kg and FLO 2% plus vitamin E (P10), 18 g SALE/kg and LFO 3% (P11 ), and 18 g SALE/kg and LFO 3% plus 60 mg vitamin E. The variables measured were performance, carcass quality, organoleptic properties, protein and amino acid composition of meats. The experimental results showed that the treatment had no significant effect ( $P > 0.05$ ) on body weight, feed consumption, feed conversion ratio, carcass weight, meat color, meat odor, meat taste, and cooking losses. However, the treatment had a significant effect ( $P < 0.05$ ) on carcass color, spleen weight, and protein content. In addition, the treatment also changes the amino acid composition of the meat. In conclusion, supplementation of 18 g SALE/kg diet, 3% LFO plus vitamin E resulted in meat with the highest protein and amino acid content.

**Keywords:** Amino acid composition, carcass quality, fish oil, performance, *Sauropus androgynus*, vitamin E

**ABSTRAK.** Penelitian ini bertujuan untuk mengevaluasi pengaruh suplementasi ekstrak daun katuk (EDK), minyak ikan lemuru, dan vitamin E terhadap performa, kualitas karkas, dan komposisi asam amino daging broiler. Seratus sembilan puluh lima ekor broiler umur 21 hari didistribusikan ke dalam 13 kelompok sebagai berikut: broiler diberi pakan mengandung 0,5% pakan imbuhan komersial (P0), 10 g/kg EDK plus 1% minyak lemuru (MIL) (P1), EDK 10 g/kg dan MIL 1% plus 60 mg vitamin E (P2), EDK 10 g/kg dan MIL 2% (P3), EDK 10 g/kg dan MIL 2% plus 60 mg vitamin E (P4), EDK 10 g/kg dan MIL 3% (P5), EDK 10 g/kg dan MIL 3% plus 60 mg vitamin E (P6), EDK 18 g/kg dan MIL 1% (P7), EDK 18 g/kg dan MIL 1% plus 60 mg vitamin E (P8), EDK 18 g/kg dan MIL 2% (P9), EDK 18 g/kg dan MIL 2% plus vitamin E (P10), EDK 18 g/kg dan MIL 3% (P11), dan EDK 18 g/kg dan MIL 3% plus 60 mg vitamin E. Variabel yang diukur adalah performa, kualitas karkas, dan sifat organoleptik, protein dan komposisi asam amino daging. Hasil penelitian menunjukkan bahwa perlakuan berpengaruh tidak nyata ( $P > 0,05$ ) terhadap berat badan, konsumsi pakan, konversi pakan, berat karkas, warna daging, bau daging, rasa daging, dan susut masak. Akan tetapi perlakuan berpengaruh nyata ( $P < 0,05$ ) terhadap warna karkas, berat limfa, kadar protein. Selain itu, perlakuan juga mengubah komposisi asam amino daging. Kesimpulannya, suplementasi 18 g EDK/kg pakan, 3% MIL plus vitamin E menghasilkan daging dengan kandungan protein dan asam amino tertinggi.

**Kata kunci:** Komposisi asam amino daging, kualitas karkas, minyak ikan, performa, *Sauropus androgynus*, vitamin E

### **INTRODUCTION**

To support the achievement of the meat self-sufficiency target, the development of the broiler industry is very urgent. This is because broilers could provide meat relatively quickly, with a harvest age of 35-42 days. However, the broiler industry in Indonesia still faces several challenges in providing adequate and high quality meat. The first problem is the low efficiency of broiler production, which is caused by the high

price of broiler diets. The second problem is that consumers want broiler meat which is high protein and balanced amino acids. In addition, consumers also want good carcass and meat color, low fishy odor, and delicious meat taste. Although commercial feed additives have been able to produce efficient production, the quality of broiler meat produced still does not meet consumer demands.

Thus, an alternative feed additive is needed to meet consumer demands. Ingredients that could produce meat with the quality expected by consumers include *Sauropus androgynus* leaves (Santoso *et al.*, 2015; Santoso *et al.*, 2017b), fish oil (Navidshad, 2009; Santoso *et al.*, 2017a), and

\*Email Korespondensi: santoso@unib.ac.id  
Diterima: 16 November 2020  
Direvisi: 23 Februari 2021  
Disetujui: 7 Mei 2021  
DOI: <https://doi.org/10.17969/agripet.v21i2.18633>

vitamin E (Cheng *et al.*, 2016). Very little research has been done on the use of a combination of *Sauropus androgynus* leaves, fish oil and vitamin E in broilers. This study is very important to evaluate the possibility of interactions between these materials. When herbs, vitamins and feed ingredients are combined, there will be synergistic or antagonistic interactions (Boer *et al.*, 2015). Santoso *et al.* (2020) stated that there was synergistic or antagonistic interaction when garlic and/or turmeric powder was added to *Sauropus androgynus*-bay leaves mixture supplemented diet. For this reason, this study aimed to evaluate the effect of *Sauropus androgynus* leaves, lemuru fish oil, and vitamin E on performance, carcass quality, protein, and amino acid composition of broiler chicken meat. It was hypothesized that the treatments will improve carcass quality, protein, and amino acid composition of meats without reducing the performance of broiler chickens.

## MATERIALS AND METHODS

### *Sauropus androgynus* Leaf Extraction

*Sauropus androgynus* leaves were air-dried for 5 days, and then ground into powder. The powder was then extracted using hot water (90°C) with ratio 1:5 for 20 minutes. The result of leaves extraction was then filtered, and the filtrate was then dried at  $\pm 50^\circ\text{C}$  for 36 hours.

### Animals and Diets

One hundred and ninety five broilers were distributed into 13 treatment groups of 15 broiler chickens reared in individual cages. The experimental design used was a completely randomized design. The 13 treatment groups were as follows: The control, diet containing 0.5% commercial feed additive (P0); diet containing 10 g *Sauropus androgynus* leaf extract (SALE)/kg diet plus 1% lemuru fish oil (LFO) (P1); diet containing 10 g SALE/kg and 1% LFO plus 60 mg vitamin E (P2); diet containing 10 g SALE/ kg and 2% LFO (P3); diet containing 10 g SALE/ kg and 2% LFO plus 60 mg vitamin E (P4); diet containing 10 g SALE/kg and 3% LFO (P5); diet containing 10 g SALE/ kg and 3% LFO plus 60 mg vitamin E (P6); diet containing 18 g SALE/ kg and 1% LFO (P7); diet containing 18 g SALE/ kg and 1% LFO plus 60 mg vitamin E (P8); diet containing 18 g SALE/ kg and 2% LFO (P9); diet containing 18 g SALE/ kg and 2% LFO plus vitamin E (P10); diet containing 18 g SALE/ kg and 3% LFO (P11); diet containing 18 g SALE/ kg and 3% LFO plus 60 mg vitamin E (P12).

The composition of the research diets used has been published by Santoso *et al.* (2010). Broiler chickens were given experimental diets for 21 days starting from 21 days of age. Diets and drinking water were given *ad libitum*.

### Sampling and Laboratory Analysis

At the end of the experiment, 4 female broilers for each treatment group were slaughtered and the thigh meats were taken, and then analyzed for protein content and amino acid composition. For organoleptic tests, twenty semi-trained sensory panelists were asked to compare the relative palatability of taste, odor, and color of meats. The organoleptic test was measured at the end of the study. The meat color was measured by comparing the breast meat color with the standard ID-DLO reference scale of 1-5. Panelists were asked to score the taste and odor of breast meat from grades 1 through 5. The odor of meat was judged on value 1 (very fishy), value 2 (fishy), value 3 (slightly fishy), value 4 (less fishy) and value 5 (not fishy). They were also asked to taste and assess the taste of the meat from bad (value 1) to very tasty (value 5). For taste test, the meat was cooked at 80°C for 20 minutes, cooled and tested.

Carcass weight was measured by reducing the weight of live broilers by weight of head, neck, feather, and blood, internal organs except lungs and kidneys, and shank. Breast and thigh weights, cooking loss, and carcass color was also measured. Carcass color was measured by comparing the color of breast skin with standard color according to DSM broiler fan. Cooking loss was measured by boiling breast meat at 80°C for 20 minutes. Drip loss was measured by storing meat in the freezer for 72 hours. The protein contents were analyzed using the AOAC method (1990), whereas amino acid compositions were determined using Nwosu *et al.* (2008).

## RESULTS AND DISCUSSION

### Growth Performance and Carcass Quality

The results showed that SALE, LFO and vitamin E supplementation had no significant effect on weight gain, feed intake and feed conversion ratio, carcass weight, meat color, cooking loss, meat odor, meat taste ( $P > 0.05$ ), but had a significant effect ( $P < 0.05$ ) on carcass color (Table 1). P1, P2 and P7 significantly have a yellowish carcass color ( $P < 0.05$ ) when compared to P0, P5, P8, P9, P10, P11 and P12.

The present study showed that the addition of SALE or SALE plus vitamin E tended to

increase body weight gain. Navidshad (2009) investigated the effect of fish oil at level of 2-4% and found that adding 2-4% fish oil tended to reduce weight gain. Based on the result of Navidshad (2009) it was assumed that the addition of SALE or SALE plus vitamin E could eliminate the negative effect of fish oil on body gain. Santoso *et al.* (2018b) examined the effect of fermented SALE to replace commercial feed additives and reported that fermented SALE

tended to increase body weight gain. Selvam *et al.* (2017) investigated the effect of vitamin E on the performance of broiler chickens reared in high stocking density plots. They reported that vitamin E addition tended to increase body weight gain. Pompeu *et al.* (2018) investigated the effect of vitamin E on the performance of broiler chickens using meta-analysis reported that adding vitamin E did not increase body weight in broilers.

Table 1. The effect of *Sauropus androgynus* leaf extract and lemuru oil plus vitamin E on broiler performance and carcass quality

Variables	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
BWG, g/bird	940	1,089	1,062	1,062	1,098	1,077	954	1,137	916	947	1,017	960	1,094 <sup>ns</sup>
Feed intake, g/bird	2,604	2,574	2,773	2,802	2,640	2,748	2,621	2,978	2,528	3,014	2,640	2,600	2,656 <sup>ns</sup>
F C R	2.77	2.36	2.61	2.64	2.40	2.55	2.75	2.62	2.76	3.18	2.60	2.71	2.43 <sup>ns</sup>
Carcass Wat., g	1,108	1,210	1,303	1,270	1,308	1,280	1,231	1,288	1,143	1,260	1,286	1,166	1,324 <sup>ns</sup>
Carcass wt., %	62.1	63.2	65.2	66.8	67.5	64.8	64.7	64.8	64.4	63.8	64.4	66.6	65.3 <sup>ns</sup>
Breast wt., %	8.01	7.74	9.48	8.66	9.89	8.68	8.18	10.32	8.80	8.98	8.76	9.38	8.54 <sup>ns</sup>
Thigh wt., %	19.64	21.30	22.08	21.74	20.02	20.44	20.76	20.04	20.62	20.36	23.24	20.88	21.54 <sup>ns</sup>
Cooking loss, %	17.6	17.0	15.7	14.9	15.0	19.2	15.3	19.4	17.9	16.1	21.9	17.6	17.2 <sup>ns</sup>
Carcass color	0.94 <sup>a</sup>	1.63 <sup>b</sup>	2.06 <sup>b</sup>	1.50 <sup>ab</sup>	1.56 <sup>ab</sup>	1.00 <sup>a</sup>	1.56 <sup>ab</sup>	2.00 <sup>b</sup>	1.31 <sup>a</sup>	1.06 <sup>a</sup>	1.06 <sup>a</sup>	1.19 <sup>a</sup>	1.25 <sup>*</sup>
Meat color	2.0	2.6	2.8	2.8	2.8	2.6	2.4	2.9	2.1	2.4	2.4	2.5	2.3 <sup>ns</sup>
Meat odor	2.5	3.0	2.63	3.0	2.75	3.0	3.0	2.75	2.25	2.75	2.75	2.25	2.25 <sup>ns</sup>
Meat taste	3.26	3.11	2.92	3.32	2.87	3.18	3.03	3.13	3.18	2.71	3.03	2.68	3.08 <sup>ns</sup>

BWG= body weight gain, <sup>ns</sup>= non significant, <sup>\*</sup>= P<0.05, <sup>\*\*</sup>=P<0,01; P0 = The control.; P1 = 10 g/kg of *Sauropus androgynus* leaf extract (SALE) plus 1% lemuru fish oil (LFO); P2 = 10 g/kg and 1% LFO plus 60 mg vitamin E; P3 = 10 g SALE/kg and 2% LFO; P4 = 10 g SALE/kg and 2% LFO plus 60 mg vitamin E; P5 = 10 g SALE/kg and 3% LFO; P6 = 10 g SALE/kg and 3% LFO plus 60 mg vitamin E; P7 = 18 g SALE/kg and 1% LFO; P8 = 18 g SALE/kg and 1% LFO plus 60 mg vitamin E; P9 = 18 g SALE/kg and 2% LFO; P10 = 18 g SALE/kg and 2% LFO plus vitamin E; P11 = 18 g SALE/kg and 3% LFO; P12 = 18 g SALE/kg and 3% LFO plus 60 mg vitamin E.

The color of the carcass was more yellow only in P1, P2, and P7 where these treatments were given SALE plus 1% LFO. The addition of LFO more than 1% tended to reduce the carcass color so that the carcass color was relatively the same as P0 (control). The yellow color of carcass is caused partly by  $\beta$ -carotene, while fish oil does not contain this pigment (Moffat and McGill, 1993). Therefore, increasing the use of fish oil will reduce  $\beta$ -carotene levels and thus reduce carcass color.

The meat color at P0 tended to be lower than other treatment groups. *Sauropus androgynus* leaves which are rich in irons, namely 7.84 mg/g leaves powder (Santoso *et al.*, 2015) are thought to contribute to redder meat in broilers. In addition, vitamin E inclusion might also contribute to redder meat color (Zhang *et al.*, 2013; Cheng *et al.*, 2016).

The supplementation of vitamin E at 60 mg/kg in the current study did not reduce cooking loss. This result agrees with the observation of

Cheng *et al.* (2016) who reported that giving of vitamin E at 20 IU/kg or 13.34 mg/kg did not reduce cooking loss. However, the result of the current study did not agree with the observation of Zhang *et al.* (2013) who reported that giving at 200 mg vitamin E/kg diet could reduce cooking loss.

The taste of meat is influenced predominantly by glutamic acid, IMP, K<sup>+</sup>, and arachidonic acid (Takahashi *et al.*, 2012). In this study, glutamic acid at P5, P11, and P12 was higher than other treatments. However, the increase of glutamic acid in these groups did not improve the taste of meat. The other active compounds such as IMP, K<sup>+</sup>, and arachidonic acid might not increase so that the taste of meat did not improve.

Fish oil has been known to increase the fishy odor of broiler meat. Rymer and Givens (2010) reported that the addition of fish oil increased fishy odor of broiler meat, and the addition of 100-200 mg vitamin E/kg reduced

fishy odor. In addition, SALE is rich with antioxidant such as vitamin C, vitamin E,  $\beta$ -carotene, flavonoids, phenolic compounds, and other compounds (Samad *et al.*, 2014; Mustarichie *et al.*, 2019). Thus, no increase in the fishy odor of meat in broilers that were given lemuru fish oil due to, among other things, the addition of SALE and/or vitamin E.

**Protein and Amino Acid Content of Meat**

The results showed that SALE, LFO and vitamin E supplementation significantly affected levels of protein ( $P < 0.05$ ), aspartic acid, glutamic acid, serine, glycine, histidine, proline, tyrosine, valine, methionine, cystine, isoleucine, leucine, phenylalanine, and lysine ( $P < 0.05$ ) in broiler meat (Table 2).

Table 2. The effect of *Sauropus androgynus* leaf extract and lemuru oil plus vitamin E on protein and amino acid composition of thigh meat

Amino acids, %	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Aspartic acid	1.747 <sup>b</sup>	1.654 <sup>b</sup>	1.765 <sup>b</sup>	1.612 <sup>b</sup>	1.689 <sup>b</sup>	1.104 <sup>a</sup>	1.572 <sup>b</sup>	0.92 <sup>a</sup>	1.581 <sup>b</sup>	0.958 <sup>a</sup>	1.065 <sup>a</sup>	1.798 <sup>b</sup>	1.678 <sup>b*</sup>
Glutamic acid	1.474 <sup>a</sup>	1.506 <sup>a</sup>	1.814 <sup>b</sup>	1.522 <sup>a</sup>	1.686 <sup>ab</sup>	2.455 <sup>c</sup>	1.408 <sup>a</sup>	1.706 <sup>b</sup>	1.589 <sup>a</sup>	1.867 <sup>b</sup>	1.952 <sup>b</sup>	2.511 <sup>c</sup>	3.558 <sup>d*</sup>
Serine	0.897 <sup>a</sup>	1.262 <sup>b</sup>	1.211 <sup>b</sup>	1.285 <sup>b</sup>	1.327 <sup>b</sup>	1.367 <sup>b</sup>	1.309 <sup>b</sup>	1.145 <sup>b</sup>	1.231 <sup>b</sup>	1.375 <sup>b</sup>	1.361 <sup>b</sup>	1.353 <sup>b</sup>	1.383 <sup>b*</sup>
Glycine	2.166 <sup>a</sup>	2.115 <sup>a</sup>	2.266 <sup>a</sup>	1.94 <sup>a</sup>	2.174 <sup>a</sup>	2.223 <sup>a</sup>	2.161 <sup>a</sup>	1.819 <sup>a</sup>	2.124 <sup>a</sup>	2.177 <sup>a</sup>	2.189 <sup>a</sup>	3.028 <sup>b</sup>	2.373 <sup>a*</sup>
Histidine	1.035 <sup>b</sup>	0.829 <sup>a</sup>	1.062 <sup>b</sup>	1.015 <sup>b</sup>	0.896 <sup>a</sup>	1.538 <sup>c</sup>	0.787 <sup>a</sup>	1.403 <sup>c</sup>	0.808 <sup>a</sup>	1.438 <sup>c</sup>	0.869 <sup>a</sup>	1.222 <sup>b</sup>	1.031 <sup>a*</sup>
Arginine	3.109	3.494	3.541	3.629	3.068	2.566	3.616	2.785	3.499	3.488	3.791	3.781	3.927 <sup>ns</sup>
Threonine	1.15	1.126	1.157	1.147	1.251	1.254	1.203	1.217	1.149	1.278	1.33	1.192	1.258 <sup>ns</sup>
Alanine	1.079	1.060	1.079	1.031	1.092	1.178	1.106	0.958	0.076	1.117	1.151	1.139	1.12 <sup>ns</sup>
Proline	0.181 <sup>b</sup>	0.172 <sup>b</sup>	0.216 <sup>c</sup>	0.195 <sup>b</sup>	0.334 <sup>d</sup>	0.33 <sup>d</sup>	0.254 <sup>c</sup>	0.275 <sup>c</sup>	0.177 <sup>b</sup>	0.249 <sup>c</sup>	0.263 <sup>c</sup>	0.262 <sup>c</sup>	0.142 <sup>a*</sup>
Tyrosine	0.156 <sup>a</sup>	0.169 <sup>a</sup>	0.199 <sup>b</sup>	0.204 <sup>b</sup>	0.22 <sup>b</sup>	0.257 <sup>c</sup>	0.202 <sup>b</sup>	0.219 <sup>b</sup>	0.198 <sup>b</sup>	0.437 <sup>d</sup>	0.306 <sup>c</sup>	0.282 <sup>b</sup>	0.162 <sup>a</sup>
Valine	0.836 <sup>a</sup>	0.775 <sup>a</sup>	0.99 <sup>b</sup>	0.944 <sup>ab</sup>	1.032 <sup>a</sup>	1.528 <sup>c</sup>	0.898 <sup>a</sup>	1.068 <sup>b</sup>	0.804 <sup>a</sup>	1.1 <sup>b</sup>	1.063 <sup>b</sup>	1.066 <sup>b</sup>	0.828 <sup>a*</sup>
Methionine	0.953 <sup>c</sup>	0.497 <sup>a</sup>	0.77 <sup>b</sup>	0.748 <sup>b</sup>	0.702 <sup>b</sup>	1.046 <sup>c</sup>	0.703 <sup>b</sup>	1.035 <sup>c</sup>	0.626 <sup>b</sup>	1.101 <sup>c</sup>	0.853 <sup>b</sup>	0.898 <sup>b</sup>	0.667 <sup>b*</sup>
Cystine	0.121 <sup>b</sup>	0.07 <sup>a</sup>	0.097 <sup>b</sup>	0.103 <sup>b</sup>	0.163 <sup>c</sup>	0.209 <sup>d</sup>	0.073 <sup>a</sup>	0.133 <sup>ab</sup>	0.079 <sup>a</sup>	0.183 <sup>c</sup>	0.112 <sup>b</sup>	0.11 <sup>b</sup>	0.103 <sup>b*</sup>
Isoleucine	0.114 <sup>a</sup>	0.133 <sup>ab</sup>	0.168 <sup>b</sup>	0.145 <sup>b</sup>	0.287 <sup>c</sup>	0.219 <sup>c</sup>	0.091 <sup>a</sup>	0.156 <sup>b</sup>	0.111 <sup>a</sup>	0.249 <sup>c</sup>	0.162 <sup>b</sup>	0.167 <sup>b</sup>	0.177 <sup>b</sup>
Leucine	0.762 <sup>a</sup>	0.686 <sup>a</sup>	0.94 <sup>b</sup>	1.179 <sup>c</sup>	1.797 <sup>e</sup>	1.488 <sup>d</sup>	1.814 <sup>e</sup>	1.017 <sup>b</sup>	1.698 <sup>d</sup>	1.166 <sup>c</sup>	1.903 <sup>e</sup>	1.497 <sup>d</sup>	1.647 <sup>d*</sup>
Phenylalanine	0.513 <sup>bc</sup>	0.364 <sup>a</sup>	0.593 <sup>bc</sup>	0.587 <sup>b</sup>	0.602 <sup>c</sup>	0.843 <sup>d</sup>	0.673 <sup>c</sup>	0.708 <sup>c</sup>	0.46 <sup>b</sup>	0.91 <sup>d</sup>	0.645 <sup>c</sup>	0.711 <sup>c</sup>	0.39 <sup>a*</sup>
Lysine	1.345 <sup>a</sup>	1.277 <sup>a</sup>	1.377 <sup>a</sup>	2.184 <sup>c</sup>	1.33 <sup>a</sup>	1.529 <sup>b</sup>	1.347 <sup>a</sup>	1.535 <sup>b</sup>	1.208 <sup>a</sup>	1.634 <sup>b</sup>	2.276 <sup>c</sup>	2.303 <sup>c</sup>	2.245 <sup>c*</sup>
Protein (%)	17.97 <sup>a</sup>	18.02 <sup>a</sup>	18.00 <sup>a</sup>	19.39 <sup>b</sup>	18.75 <sup>b</sup>	19.59 <sup>b</sup>	19.89 <sup>b</sup>	19.48 <sup>b</sup>	19.26 <sup>b</sup>	20.77 <sup>b</sup>	20.27 <sup>b</sup>	20.24 <sup>b</sup>	23.03 <sup>c*</sup>

<sup>ns</sup>= non significant, \*= $P < 0.05$ , \*\*= $P < 0.01$ ; P0 = The control.; P1 = 10 g/kg of *Sauropus androgynus* leaf extract (SALE) plus 1% lemuru fish oil (LFO); P2 = 10 g/kg and 1% LFO plus 60 mg vitamin E; P3 = 10 g SALE/kg and 2% LFO; P4 = 10 g SALE/kg and 2% LFO plus 60 mg vitamin E; P5 = 10 g SALE/kg and 3% LFO; P6 = 10 g SALE/kg and 3% LFO plus 60 mg vitamin E; P7 = 18 g SALE/kg and 1% LFO; P8 = 18 g SALE/kg and 1% LFO plus 60 mg vitamin E; P9 = 18 g SALE/kg and 2% LFO; P10 = 18 g SALE/kg and 2% LFO plus vitamin E; P11 = 18 g SALE/kg and 3% LFO; P12 = 18 g SALE/kg and 3% LFO plus 60 mg vitamin E. P6, P7, P8, P10 and P11. Lysine of P3, P5, P7, P9,, P10, P11 and P12 was higher than P0, P1, P2, P4, P6, P8. P3, P10, P11 and P12 had the highest lysine.

Protein levels of P0, P1, P2 were significantly ( $P < 0.05$ ) lower than P3, P4, P5, P6, P7, P8, P9, P10, P11, and P12. P12 has the highest meat protein content when compared to other treatments

Aspartic acid at P5, P7, P9, and P10 was lower than other treatments ( $P < 0.05$ ). Glutamic acid at P0, P1, P3, P6, and P8 was lower than other treatments. Glutamic acid at P5, P11, and P12 was higher than other treatments. Serine at P0 was lower than other treatments. The glycine at P12 was higher than other treatments. Histidine at P1, P4, P6, P8, P10, and P12 was lower than other treatments. Meat proline at P12 was the lowest. P0, P1, P3, P8 had lower meat proline than P2, P4, P5, P6, P7, P9, P10, P11. P4 had the highest meat proline. Meat tyrosine at P0, P1, and P12 had lower meat tyrosine than other treatments. P5 and P9 and P10 had higher tyrosine than P2, P3, P4, P5, P6, P7, and P11. P9 had the highest tyrosine.

Meat methionine at P0, P5, P7 and P9 were higher than other treatments, while P4, P5, and P9 had higher cystine than other treatments. P5 has the highest levels of cystine. Isoleucine at P0, P6, P8 was lower than P2, P3, P4, P5, P7, P9, P10, P11, and P12. Leucine at P0 and P1 were lower than the other treatments. P4, P6, and P10 had the highest leucine. Phenylalanine of P1 and P12 were the lowest, while P5, P9 had the highest phenylalanine. P0 and P2 had phenylalanine relatively the same as P3, P4, P6, P7, P8, P10, and P11. Lysine of P3, P5, P7, P9,, P10, P11, and P12 was higher than P0, P1, P2, P4, P6, P8. P3, P10, P11, and P12 had the highest lysine.

The SALE administration at 10 g/kg diet plus 1% LFO did not increase protein, but when the level of fish oil was increased to 2-3%, the meat protein increased. It is suspected that there is a more efficient positive interaction between the active compounds in *Sauropus androgynus* leaf

extract and 2%-3% LFO which can increase protein availability for broilers. It has been known that fish oil is rich in n-3 fatty acids. Girolamo *et al.* (2014) reported that the supplementation of n-3 fatty acid improved protein metabolism. Furthermore, the anabolic stimuli from substrates (e.g. amino acids or proteins), hormones (e.g. insulin) and/or physical activity in skeletal muscle can be enhanced by long-term fish oil administration. Smith *et al.* (2011) reported that omega-3 fatty acid supplementation stimulate protein synthesis in older adults. Furthermore, Santoso *et al.* (2010) using the same treatment as the present study reported that the addition of SALE, LFO and vitamin E decreased TBA, which means decreased fatty acid oxidation. It is suspected that protein oxidation also decreases with the addition of SALE and vitamin E. The decrease in protein oxidation might also contribute to an increase in protein content.

Giving a combination of 18 g SALE/kg, 3% LFO plus 60 mg vitamin E produced the highest meat protein. It is suspected that the maximum positive interaction occurs between the active compounds in SALE, LFO, and vitamin E which could increase protein availability. Zdanowska-Sasiadek *et al.* (2016) reported that vitamin E supplementation increased protein content in chickens. SALE is rich in antioxidants such as vitamin C,  $\beta$ -carotene, flavonoids, phenols, and other compounds, while vitamin E is also an antioxidant. It is suspected that these antioxidants interact positively so that their antioxidant properties increase. Increasing this antioxidant activity is thought to reduce meat protein oxidation, so that meat protein levels are maintained.

The addition of SALE, LFO and vitamin E changed the amino acid composition. McGlory *et al.* (2019) stated that omega-3 fatty acids changed the composition of amino acids in meat. In addition, Santoso *et al.* (2018c) reported that SALE inclusion significantly changed meat amino acid composition. Furthermore, Santoso *et al.* (2017b) reported that *Sauropus androgynus* leaves is rich in glutamic acid, which may partly contribute to an increase in meat glutamic acid. They also reported that fermented SALE inclusion significantly changed meat amino acid composition.

The addition of SALE, LFO and vitamin E changed the amino acid composition. McGlory *et al.* (2019) stated that omega-3 fatty acids changed the composition of amino acids in meat. In addition, Santoso *et al.* (2018c) reported that

SALE inclusion significantly changed meat amino acid composition. Furthermore, Santoso *et al.* (2017b) reported that *Sauropus androgynus* leaves is rich in glutamic acid, which may partly contribute to an increase in meat glutamic acid. They also reported that fermented SALE inclusion significantly changed meat amino acid composition.

SALE increased the number of *Bacillus subtilis* and *Lactobacillus sp.* of gastrointestinal tract (Santoso *et al.*, 2001). *Lactobacillus* species could enhance health effects in the gastrointestinal tract through many mechanisms, which include maintaining normal intestinal microflora, changing metabolism by increasing the activity of digestive enzymes, decreasing the activity of bacterial enzyme and ammonia production, stimulating the immune system, and improving digestion (Kabir, 2009). Diets with 0.4% *Bacillus subtilis* enhanced the absorption of protein, stimulated hormone secretion, suppressed harmful microflora, and improved the duodenal structure and immune functions of Muscovy ducks (Sheng-Qiu *et al.*, 2013). Al-Fataftah *et al.* (2013) reported that an increase in *Lactobacillus sp* enhanced the contents of lysine in broiler meats. They also reported that the microbial strains had potential for enhancing biosynthesis of lysine. Thus, the change in microorganism balance of gastrointestinal tract may influence amino acid metabolism in poultry. There was a considerable synthesis of serine from the  $\alpha$ -carbon of glycine in the liver of poultry (Vohra *et al.*, 1956).

Some of the essential amino acids in human nutrition are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Thus, the increase in lysine, phenylalanine, leucine, isoleucine, valine, and histidine of meat in several treatments when compared to the control give benefit for human health. However, there was a decrease in methionine in broilers when broilers were fed diet with the addition of SALE, LFO, and vitamin E when compared to the controls. In fact, Santoso *et al.* (2018a) reported that feeding 5% *Sauropus androgynus* leaf powder increased the methionine content of meat. Santoso *et al.* (2015) also reported that giving fermented *Sauropus androgynus* leaves as much as 2.5% or 5% also increased meat methionine. However, Santoso and Fenita (2016) reported that the addition of SALE tended to decrease egg methionine. Thus, the addition of SALE is one of the factors causing the decrease in meat methionine.

An increase in total meat amino acid contents is also benefit for improving the immune responses. Li *et al.* (2007) reported that there was an important role of amino acids in immune responses by regulating: (1) the activation of T lymphocytes, B lymphocytes, natural killer cells and macrophages; (2) cellular redox state, gene expression and lymphocyte proliferation; and (3) the production of antibodies, cytokines and other cytotoxic substances. Furthermore, they reported that dietary supplementation of specific amino acids in animals and humans with malnutrition and infectious disease enhanced the immune status, thereby reducing morbidity and mortality.

### CONCLUSION

SALE, LFO and/or vitamin E supplementation resulted in meat with higher levels of protein and amino acids than controls. The supplementation of 18 g SALE/kg diet, 3% LFO plus vitamin E produced meat with the highest protein and amino acid content.

### ACKNOWLEDGMENT

The authors are grateful to Institute for Research and Community Service Bengkulu University for its administrative service and Laboratory of Animal Science, Faculty of Agriculture, Bengkulu University for analysis service and other facilities.

### REFERENCES

- Al-Fataftah, A.A., Herzallah, S.M., Alshwabkeh, K., Ibrahim, S.A., 2013. Administration of lactic acid bacteria to enhance synthesis of vitamin B12 and B6 and lower cholesterol levels in poultry meat. *J. Food Agric. Environ.* 11: 604-609.
- AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemist. Inc., Arlington, Virginia.
- Boer, de A., Van Hunsel, F., Bast, A., 2015. Adverse food-drug interactions. *Regulatory Toxicol. Pharma.* 73: 859-865.
- Cheng, K., Niu, Y., Zheng, X.C., Zhang, H., Chen, Y.P., Zhang, M., Huang, X.X., Zhang, L.L., Zhou, Y.M., Wang, T., 2016. A comparison of natural (D- $\alpha$ -tocopherol) and synthetic (DL- $\alpha$ -tocopherol acetate) vitamin E supplementation on the growth performance, meat quality and oxidative status of broilers. *Asian-Australasian. J. Anim. Sci.* 2(5): 681-688.
- Girolamo, F.G.D., Situlin, R., Mazzucco, S., Valentini, R., Toigo, G., Biolo, G., 2014. Omega-3 fatty acids and protein metabolism: enhancement of anabolic interventions for sarcopenia. *Curr. Opin. Clin. Nutr. Metab. Care.* 17(2): 145-150.
- Kabir, S.M.L., 2009. The role of probiotics in the poultry industry. *Int. J. Mol. Sci.* 10: 3531-3546.
- Li, P., Yin, Y.L., Li, D., Kim, S.W., Wu, G., 2007. Amino acids and immune function. *Br. J. Nutr.* 98: 237-252.
- McGlory C., Calder, P.C., Nunes E.A., 2019. The Influence of omega-3 fatty acids on skeletal muscle protein turnover in health, disuse, and disease. *Front. Nutr.* 6: 144.
- Moffat, C.F., McGill, A.S., 1993. Variability of the composition of fish oil: significance for the diet. *Proc. Nutr. Soc.* 52: 441-456.
- Mustarichie, R., Salsabila, T., Iskandar, Y., 2019. Determination of the major component of water fraction of katuk (*Sauropus androgynus* L. Merr) leaves by liquid chromatography-mass spectrometry. *J. Pharm. Bio allied Sci.* 11(4): 611-618.
- Navidshad, B., 2009. The Effects of fish oil on growth performance and carcass characteristics of broiler chicks fed a low-protein diet. *Int. J. Agric. Biol.* 11: 635-638.
- Nwosu, J. N., Ubbaonu, C.N. , Banigo, E.O.I., Uzomah, A., 2008. The effect of processing on the amino acid profile of oze (*Bosqueia angolensis*) seed flour. *Life Sci. J.* 5(4): 69-74.
- Pompeu, M.A., Cavalcantib, L.F.L., Toral, F.L.B., 2018. Effect of vitamin E supplementation on growth performance, meat quality, and immune response of male broiler chickens: A meta-analysis. *Livest. Sci.* 208: 5-13.
- Rymer, C., Givens, D.L., 2010. Effect of vitamin E and fish oil inclusion in broiler diets on meat fatty acid composition and on the flavour of a composite sample of breast meat. *J. Sci. Food Agric.* 90(10): 1628-1633.
- Samad, A.P.A., Santoso, U., Lee, M.C., Nan, F.H., 2014. Effects of dietary katuk

- (*Sauropus androgynus* L. Merr.) on growth, non-specific immune and diseases resistance against *Vibrio alginolyticus* infection in grouper *Epinephelus coioides*. *Fish Shellfish Immunol.* 36: 582-589.
- Santoso, U., Suharyanto, Handayani, E, 2001. Effects of *Sauropus androgynus* (katuk) leaf extract on growth, fat accumulation and fecal microorganisms in broiler chickens. *JITV.* 6: 220-226.
- Santoso, U., Kususiya, Fenita, Y., 2010. The effect of *Sauropus androgynus* extract, lemuru oil on fat deposition and fatty acid composition of meat in broiler chickens. *J. Indones. Trop. Anim. Agric.* 35(1): 48-54.
- Santoso, U., Fenita, Y., Kususiya, Bidura, I.G.N.G., 2015. Effect of fermented *Sauropus androgynus* leaves on meat composition, amino acid and fatty acid compositions in broiler chickens. *Pak. J. Nutr.* 14: 799-807.
- Santoso, U., Fenita, Y., 2016. The effect of *Sauropus androgynus* leaf extract on performance, egg quality and chemical composition of eggs. *J. Indones. Trop. Anim. Agric.* 41(3): 125-134.
- Santoso, U., Fenita, Y., Sulistyowati, E., 2017a. The effect of *Sauropus androgynus* leaf extract, fish oil and vitamin E on performance, egg quality and composition in laying hens. *J. Indones. Trop. Anim. Agric.* 42(2): 88-98.
- Santoso, U., Fenita, Y., Kususiya, 2017b. Effect of fermented *Sauropus androgynus* leaf extract on the chemical composition of broiler meat. *Pak. J. Nutr.* 16(5): 306-313.
- Santoso, U., Fenita, Y., Kususiya, Widianoro, O., Kadarsih, S, 2018a. The effect of medicinal herb on fat deposition, meat composition, amino acid and fatty acid composition of broiler meats. *J. Indones. Trop. Anim. Agric.* 43(1): 54-65.
- Santoso, U., Fenita, Y., Kususiya, Fauzi, M.I., Banes, S.S., Apriyanti, N., 2018b. The Effectiveness of Fermented *Sauropus androgynus* Leaf Extract for Replacing Commercial Feed Additive in Broiler Chickens. In: *Suplemen Pakan Ternak*, Plantaxia, Yogyakarta. pp.193-207.
- Santoso, U., Fenita, Y., Piliang, W.G., 2018c. The effect of *Sauropus androgynus* (katuk) leaf extracted by different methods on carcass quality, fat deposition and amino acid and fatty acid compositions in broiler chickens. In: *Suplemen Pakan Ternak*. Plantaxia, Yogyakarta. pp. 209-225.
- Santoso, U., Fenita, Y., Kususiya, K., Agustian, A., 2020. Effect of turmeric and garlic supplementation to fermented *Sauropus androgynus*-bay leaves containing diet on fat deposition and broiler meat composition. *J. Indones. Trop. Anim. Agric.* 45(2): 91-102.
- Selvam, R., Saravanakumar, M., Suresh, S., Sureshbabu, G., Sasikumar, M., Prashanth, D., 2017. Effect of vitamin E supplementation and high stocking density on the performance and stress parameters of broilers. *Bra. J. Poultry Sci.* 19(4): 587-594.
- Sheng-Qiu, T., Xiao-Ying, D., Chun-Mei, J., Jing-Jing, P., L. Shan-Shan L., Jin-Ding, C., 2013. Effect of *Bacillus subtilis* natto on growth performance in Muscovy ducks. *Brazilian J. Poultry Sci.* 15: 191-197.
- Smith, G.I., Atherton, P., Reeds, D.N., Mohammed, B.S., Rankin, D., Rennie, M.J., Mittendorfer, B., 2011. Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am. J. Clin. Nutr.* 93(2): 402-412.
- Takahashi, H., Rikimaru, K., Kiyohara, R., Yamaguchi, S., 2012. Effect of arachidonic acid-enriched oil diet supplementation on the taste of broiler meat. *Asian-Australas. J. Anim. Sci.* 25(6): 845-851.
- Vohra, P., Lantz, F.H., Kratzer, F.H., 1956. The effect of folic acid and vitamin B12 on the synthesis of serine and choline from glycine in the liver of young turkey poults. *J. Biol. Chem.* 211(1): 501-507.
- Zdanowska-Sasiadek, Z., Michalczyk, M., Damaziak, K., Niemiec, J., Polawska, E., Gozdowski, D., Rozanska, E., 2016. Effect of vitamin E supplementation on growth performance and chicken meat quality. *Europ. Poultry Sci.* 80: 1-14.

Zhang, Y., Shan, A., Jiang, W., Bi, C., Li, Z., 2013. The effect of vitamin E on growth performance and meat quality in broilers given diets containing distillers' dried grain with solubles (DDGS). *Bri. Poultry Sci.* 54(1): 138-143.