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Research Article
Effect of Fermented *Sauropus androgynus* Leaf Extract on the Chemical Composition of Broiler Meat

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Abstract
Objective: The present study was conducted to evaluate the effect of fermented *Sauropus androgynus* leaf extract on the chemical composition of broiler meat. **Methodology:** One hundred broilers aged 15 days were distributed into five treatment groups. Each treatment group was represented by four replicates of five broiler chickens each. The five treatment groups were as follows: (1) Broiler chickens were fed diet without fermented *Sauropus androgynus* leaf extract (FSALE) as the control (P0), (2) Broiler chickens were fed a diet supplemented with 4.5 g FSALE kg^{-1} diet (P1), (3) Broiler chickens were fed a diet supplemented with 9 g FSALE kg^{-1} diet (P2), (4) Broiler chickens were fed a diet supplemented with 13.5 g FSALE kg^{-1} diet (P3) and (5) Broiler chickens were fed a diet supplemented with 18 g FSALE kg^{-1} diet (P4). **Results:** The extract contained 25.46% protein, 1.34% fat, 3,642.6 µg β-carotene g^{-1}, 8.17 mg iron g^{-1} and 0.17 mg sterol/100 mg. The extract was rich in palmitic acid (29.96%) and glutamic acid (2.221%). The results showed that FSALE inclusion significantly increased the contents of protein ($p<0.01$), β-carotene ($p<0.001$) and iron ($p<0.001$) of broiler meats, but it significantly reduced the contents of fat ($p<0.05$) and cholesterol ($p<0.05$) of broiler meats. The FSALE inclusion significantly reduced oleic acid ($p<0.05$) and increased docosahexaenoic acid ($p<0.001$). In addition, FSALE significantly reduced aspartic acid, serine, glycine, histidine, arginine, threonine and cystine ($p<0.01$), but FSALE significantly increased glutamic acid, alanine, proline, tyrosine, valine, methionine, phenylalanine and lysine ($p<0.001$). **Conclusion:** The FSALE inclusion at level of 4.5-18 g kg^{-1} in the diet increased the contents of iron, protein and β-carotene but it reduced the content of cholesterol in broiler meat. In addition, FSALE inclusion changed the composition of amino acids and fatty acids.

Key words: Fermented *Sauropus androgynus* leaf extract, protein, β-carotene, amino acid, fatty acid, cholesterol

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The presence of a positive correlation between the concentration of fats such as cholesterol in the blood and the risk of atherosclerosis, coronary heart disease, stroke and other metabolic diseases[1,2] encourages the broiler industry to produce low-fat meat. In addition, the reduction of fat deposition in depots is very important for the industry, because fat depots have lower prices than carcasses.

The total depot fat in broiler carcasses is 6%, which will decrease the industry profits as much as 4-6%. *Sauropus androgynus* leaves and their extracts have been shown to decrease fat deposition in broiler and layer chickens[3,4]. However, a decrease in fat deposition by *Sauropus androgynus* leaves and the extract is still low, thus it may be not effective when it is applied on a commercial scale[5].

The tendency of weight loss in broiler chickens by *Sauropus androgynus* leaves[6] will reduce the economic profit of the livestock industry. *Sauropus androgynus* leaves have some antinutrients such as oxalate, saponins, tannin and oligosaccharides[7] and they can cause lung disorders[8]. Therefore, a method to improve nutrient contents and to reduce the antinutrient substances of the leaves is required. It has been established that fermentation improves feed nutrients and breaks down protein into simpler compounds such as peptides and amino acids, lowering crude fiber[9-12] and reducing the contents of antinutrients such as tannins, oligosaccharides, phytic acid, phenol, saponins, oxalate, phytin phosphorus[11,13-16], phenolic compounds and alkaloids[14].

The results of previous studies[17-19] have shown that the supplementation of *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves to the diet is effective in reducing the content of fat or cholesterol and increasing the content of protein, iron, vitamin A and β-carotene of broiler meat. In addition, the compositions of amino acids and fatty acids of broiler meat are more balanced. Moreover, this product also improves the carcass quality without reducing body weight[18] and the blood profile of broiler chickens [19]. The effectiveness of this product may be enhanced if the product is extracted because the extraction will better release the active compound. Therefore, the present study was designed to evaluate the effect of supplementation of fermented *Sauropus androgynus* leaf extract on amino acid and fatty acid compositions, cholesterol, protein, fat, iron and β-carotene of broiler meat.

MATERIALS AND METHODS

Extraction of fermented *Sauropus androgynus* leaves: *Sauropus androgynus* leaves were fermented by *Saccharomyces cerevisiae*[17] and then they were extracted with hot water at 90°C for 20 min as described by Santoso *et al.*[3]. The contents of amino acids, fatty acids, protein, fat, iron, β-carotene and sterol were then measured.

Animals and experimental design: Two hundred broilers were purchased (strain Arbor Acres) from commercial hatcheries. On arrival, the broilers were placed in a single pen surrounded by a zinc ring and maintained on rice husks at a depth of approximately 5 cm. From 1-14 days of age, supplemental heat was provided using coal as fuel. The temperature was maintained at 32-34°C in the first week and gradually decreased in the second week. The broiler chickens were maintained on the floor in a house under continuous lighting. To avoid stress, the broiler chickens were immediately provided drinking water containing sugar and antistress. They were fed a commercial starter diet for 14 days.

At 15 days of age, the broiler chickens were weighed and selected based on body weight. A completely randomized design was used in the present study. The relative humidity and temperature of the house ranged from 65-75% and 23-32°C, respectively. A fan was turned on when the house temperature exceeded 29°C to prevent overheating. The experimental diets contained 19% crude protein and 3200 kcal ME kg^{-1}. One hundred broiler chickens aged 15 days were distributed into five treatment groups. Each treatment group was represented by four replicates of five broiler chickens each. The 5 treatments were as follows: (1) Broiler chickens were fed a diet without fermented *Sauropus androgynus* leaf extract (FSALE) as the control (P0), (2) Broiler chickens were fed a diet supplemented with 4.5 g FSALE kg^{-1} diet (P1) (3) Broiler chickens were fed a diet supplemented with 9 g FSALE kg^{-1} diet (P2) (4) Broiler chickens were fed a diet supplemented with 13.5 g FSALE kg^{-1} diet (P3) and (5) Broiler chickens were fed a diet supplemented with 18 g FSALE kg^{-1} diet (P4). All broiler chickens were provided their diet and drinking water *ad libitum*.

Sampling and laboratory analysis: At the end of the study (aged 35 days), four broiler chickens for each treatment group were selected and slaughtered. The leg meat from each treatment was collected and analyzed for cholesterol, protein, fat, fatty acids, amino acids, iron and β-carotene. Fat and protein were determined by the method of AOAC[20], whereas meat cholesterol was determined by the method described by Dinh *et al.*[21,22]. Amino acid composition was measured by the method as described by Henderson and Brooks[23] and β-carotene was determined by the method of Grzelinska *et al.*[24]. Fatty acid composition of the meat was determined by the method described by De Almeida *et al.*[25].

Data analysis: The experimental results were subjected to analysis of variance. Significant differences among the treatment groups were determined by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Composition of FSALE: The components of fermented *Sauropus androgynus* leaf extract are presented in Table 1-3. The extract contained 25.46% protein, 1.34% fat, 3,642.6 μg β-carotene g^{-1}, 8.17 mg iron g^{-1} and 0.17 mg sterol/100 mg. The extract was rich in palmitic acid (29.96%), glutamic acid (2.221%) and valine (0.523%). The contents of fat, iron and β-carotene of the extract were relatively similar to the contents of these nutrients in the leaf powder[16]. This means that the extraction did not increase the contents of these nutrients. The present study showed that FSALE contained a high sterol content.

Nutritional composition of broiler meat: The nutrient contents of FSALE are presented in Table 4. The results showed that FSALE inclusion significantly increased the contents of protein ($p<0.01$), β-carotene ($p<0.001$) and iron ($p<0.001$) of broiler meat, but it significantly reduced the contents of fat ($p<0.05$) and cholesterol ($p<0.05$) of broiler meat. The DMRT test showed that P0 had lower protein content than the other groups, whereas P0 had a higher fat content than P4, but it did not significantly differ from the other groups. Furthermore, P0 had lower β-carotene and iron contents than the other groups.

An increase in protein content of broiler meat indicated that there was improvement in the availability of protein for broilers because of better protein digestibilities. Chen *et al.*[26] reported that soybean fermented by a *Aspergillus* and *Lactobacillus* mixture increased *in vitro* and *in vivo* protein digestibility. Adam *et al.*[27] also found that fermentation increased *in vitro* protein digestibility.

As shown in Table 1, FSALE is rich in β-carotene and iron. Thus, FSALE might partly contribute to an increase in the β-carotene and iron contents of broiler meat. In addition, fermentation might improve the availability of β-carotene and iron for broilers. This result agreed with the observation of Santoso *et al.*[17].

The compounds that play a role in lowering fat contents might be alkaloids and non alkaloids[28], 3-O-β-D-glucosyl-(1→6)-β-D-glucosyl-kaempferol[29], flavonoids[30], tannins[31] and polyphenol[32].

Sterol found in FSALE (Table 1) may partly contribute to the lower cholesterol content as reported by Subekti[4]. The other compounds that play a role in lowering cholesterol might be alkaloids and non-alkaloids[28], saponins[33], polyphenol[32,34] and flavonoids[35]. Patil *et al.*[36] reported that the reduction of cholesterol and triglycerides by alkaloid were in part caused by the reduction of lipogenic enzyme activities and increased bile acid excretion in feces.

Table 1: Contents of protein, fat, β-carotene, iron and sterol of fermented *Sauropus androgynus* leaf extract

Variables	Values
Protein (%)	25.46
Fat (%)	1.34
β-carotene (μg g^{-1})	3,642.60
Iron (mg g^{-1})	8.17
Sterol (mg/100 mg)	0.17

Table 2: Composition of fatty acids of fermented *Sauropus androgynus* leaf extract

Fatty acids (g/100 g of fat)	Values
Myristic acid	7.98
Palmitic acid	29.96
Stearic acid	4.58
Oleic acid	5.96
Linoleic acid	5.06
Linolenic acid	0.057

Table 3: Composition of amino acids of fermented *Sauropus androgynus* leaf extract

Amino acid (%)	Values
Aspartic acid	0.754
Glutamic acid	2.221
Serine	0.258
Glycine	0.216
Histidine	0.243
Arginine	0.258
Threonine	0.221
Alanine	0.408
Proline	0.227
Tyrosine	0.393
Valine	0.523
Methionine	0.281
Cystine	0.226
Isoleucine	0.245
Leucine	0.185
Phenylalanine	0.360
Lysine	0.275

Composition of fatty acids of broiler meat: The composition of fatty acids of broiler meat is presented in Table 5. The FSALE inclusion significantly reduced oleic acid ($p<0.05$) and increased docosahexaenoic acid ($p<0.001$) but had no effect on lauric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid and eicosapentaenoic acid ($p>0.05$). It was shown that P0 had lower docosahexaenoic acid than P2, P3 and P4 but was not significantly different from P1. The P0 had higher oleic acid than P1, P2 and P3 but was not significantly different from P4.

Docosahexaenoic acid is synthesized from a-linolenic acid[37]. The inclusion of linolenic acid from the extract may not be adequate to increase the synthesis of docosahexaenoic acid. Fermentation may enhance the digestion and availability of linolenic acid of FSALE resulting in higher linolenic acid as a substrate for docosahexaenoic acid synthesis. Table 5 shows that the linolenic acid contents of P1, P2 and P3 were lower than that of P0, suggesting that there is a conversion of linolenic acid to docosahexaenoic acid. Combining *Lactobacillus* sp. with flaxseed demonstrated the efficacy of conversion of α-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid[38]. It was established that the inclusion of *Sauropus androgynus* leaf extract increased the number of *Lactobacillus* sp.[39,40]. Thus, an increase in *Lactobacillus* sp. may contribute to enhanced efficiency of linolenic acid conversion to docosahexaenoic acid.

The mechanism of lower oleic acid by the supplementation of FSALE is unknown. In poultry, linoleic acid and α-linolenic acid cannot be synthesized and tissue concentrations respond rapidly to dietary changes, whereas saturated and monounsaturated fatty acids, such as oleic acid, are synthesized and their concentrations are less readily influenced by diet[41]. However, Gallardo *et al.*[42] reported that the oleic acid content of broiler meat was changed by diet.

Composition of amino acids of broiler meat: The composition of amino acids of broiler meat is presented in Table 6. The FSALE significantly reduced aspartic acid, serine, glycine, histidine, arginine, threonine and cystine ($p<0.01$), but FSALE significantly increased glutamic acid, alanine, proline, tyrosine, valine, methionine, phenylalanine and lysine ($p<0.001$).

It was shown that P0 had higher aspartic acid than P1 and P3. P3 had higher glutamic acid than the other groups. P1 and P3 had lower serine, glycine, histidine, threonine and cysteine than P0, P2 and P4. P1 had lower arginine than the others and P3 had lower arginine than P0, P2 and P4. The P1 and P3 had higher alanine, proline, tyrosine, valine, methionine, phenylalanine and lysine than the other groups. The extract had no effect on isoleucine and leucine.

The mechanism of an increase in lysine in P1 and P3 is still unknown. It has been established that lysine is an essential amino acid in animals, thus it is not synthesized in the body. Most bacteria synthesize lysine from aspartic acid. *Sauropus androgynus* leaf extract increased the number of *Bacillus subtilis*[40] and *Lactobacillus* sp.[39,40]. Thus, it is assumed that these bacteria may synthesize lysine from aspartic acid.

The low glutamic acid in P1 might cause lower arginine synthesis by bacteria and therefore resulting in lower arginine content in P1. Hood and Lyman[43] and Xu *et al.*[44] reported that glutamic acid has an important role in arginine synthesis.

Al-Fataftah *et al.*[45] reported that an increase in *Lactobacillus* sp. enhanced the contents of lysine, methionine and cystine, as well as vitamin B12 and vitamin B6 in broiler

Table 4: Effect of fermented *Sauropus androgynus* leaf extract on the nutritional composition of broiler leg meat

Variables	P0	P1	P2	P3	P4	SD
Protein (%)	15.31^a	15.83^b	15.95^b	16.07^b	16.14^b	0.37**
Fat (%)	4.46^b	4.17^{ab}	3.97^{ab}	4.07^{ab}	3.81^a	0.40*
Cholesterol (mg g^{-1})	2.61^b	1.95^a	1.79^a	2.11^a	2.13^a	0.37*
β-carotene (μg g^{-1})	2.17^a	2.62^b	2.93^b	3.33^c	2.88^b	0.45***
Iron (mg g^{-1})	2.36^a	3.07^b	3.46^c	3.69^c	3.51^c	0.54***

P0: Broiler chickens were fed a diet without supplementation of fermented *Sauropus androgynus* leaf extract (FSALE) as the control, P1: Broiler chickens were fed a diet supplemented with 4.5 g FSALE kg^{-1}, P2: Broiler chickens were fed a diet supplemented with 9 g FSALE kg^{-1}, P3: Broiler chickens were fed a diet supplemented with 13.5 g FSALE kg^{-1}, P4: Broiler chickens were fed a diet supplemented with 18.0 g FSALE kg^{-1}. Values in the same row with different superscripts are significantly different. *p<0.05, **p<0.01, ***p<0.001

Table 5: Effect of fermented *Sauropus androgynus* leaf extract on the fatty acids composition of broiler leg meat

Fatty acid (g/100 g fat)	P0	P1	P2	P3	P4	SD
Lauric acid	0.004	0.008	0.00	0.00	0.006	0.009^{ns}
Myristic acid	0.386	0.140	0.148	0.262	0.141	0.205^{ns}
Palmitic acid	2.315	2.663	2.332	2.366	2.571	0.440^{ns}
Stearic acid	10.927	9.137	15.584	12.744	12.116	3.659^{ns}
Oleic acid	48.176^b	34.188^a	32.786^a	29.601^a	39.296^{ab}	8.897*
Linoleic acid	9.570	10.280	13.514	14.111	12.082	3.514^{ns}
Linolenic acid	0.468	0.383	0.316	0.351	0.486	0.142^{ns}
Docosahexaenoic acid (mg/100 g fat)	3.319^a	3.653^{ab}	3.837^{bc}	4.366^d	4.207^{cd}	0.466***
Eicosapentaenoic acid (mg/100 g fat)	2.165	3.067	2.006	2.777	2.923	1.049^{ns}

P0: Broiler chickens were fed a diet without supplementation of fermented *Sauropus androgynus* leaf extract (FSALE) as the control, P1: Broiler chickens were fed a diet supplemented with 4.5 g FSALE kg^{-1}, P2: Broiler chickens were fed a diet supplemented with 9 g FSALE kg^{-1}, P3: Broiler chickens were fed a diet supplemented with 13.5 g FSALE kg, P4: Broiler chickens were fed a diet supplemented with 18.0 g FSALE kg^{-1}. Values in the same row with different superscripts are significantly different. *p<0.05, ***p<0.001, ns: Non significant

Table 6: Effect of fermented *Sauropus androgynus* leaf extract on the composition of amino acids of broiler leg meat

Amino acid (%)	P0	P1	P2	P3	P4	SD
Aspartic acid	0.854^{c}	0.722^{a}	0.864^{c}	0.798^{b}	0.856^{c}	0.062***
Glutamic acid	3.096^{b}	2.214^{a}	3.125^{b}	3.374^{c}	3.097^{b}	0.427***
Serine	0.397^{b}	0.257^{a}	0.408^{b}	0.260^{a}	0.391^{b}	0.075***
Glycine	0.350^{b}	0.215^{a}	0.299^{b}	0.225^{a}	0.349^{b}	0.072**
Histidine	0.270^{b}	0.230^{a}	0.271^{b}	0.235^{a}	0.270^{b}	0.022***
Arginine	0.588^{c}	0.248^{a}	0.584^{c}	0.546^{b}	0.589^{c}	0.137***
Threonine	0.464^{b}	0.225^{a}	0.465^{b}	0.225^{a}	0.465^{b}	0.122***
Alanine	0.287^{a}	0.387^{b}	0.288^{a}	0.389^{b}	0.287^{a}	0.058***
Proline	0.166^{a}	0.221^{b}	0.164^{a}	0.221^{b}	0.164^{a}	0.032***
Tyrosine	0.256^{a}	0.377^{b}	0.255^{a}	0.378^{b}	0.255^{a}	0.063***
Valine	0.335^{a}	0.505^{b}	0.335^{a}	0.505^{b}	0.335^{a}	0.088***
Methionine	0.141^{a}	0.268^{b}	0.140^{a}	0.271^{b}	0.140^{a}	0.068***
Cystine	0.364^{b}	0.206^{a}	0.368^{b}	0.204^{a}	0.368^{b}	0.085***
Isoleucine	0.235	0.224	0.234	0.227	0.234	0.019^{ns}
Leucine	0.181	0.167	0.180	0.167	0.181	0.015^{ns}
Phenylalanine	0.269^{a}	0.370^{b}	0.275^{a}	0.370^{b}	0.274^{a}	0.051***
Lysine	0.198^{a}	0.260^{b}	0.200^{a}	0.259^{b}	0.200^{a}	0.034***

P0: Broiler chickens were fed a diet without supplementation of fermented *Sauropus androgynus* leaf extract (FSALE) as the control, P1: Broiler chickens were fed a diet supplemented with 4.5 g FSALE kg^{-1}, P2: Broiler chickens were fed a diet supplemented with 9 g FSALE kg^{-1}, P3: Broiler chickens were fed a diet supplemented with 13.5 g FSALE kg^{-1}, P4: Broiler chickens were fed a diet supplemented with 18.0 g FSALE kg^{-1}. Values in the same row with different superscripts are significantly different. **$p<0.01$, ***$p<0.001$, ns: Non significant

meat. They also reported that the microbial strains had the potential for enhancing biosynthesis of vitamin B12, vitamin B6, lysine, methionine and cystine.

An increase in *Lactobacillus* sp. in the gastrointestinal tract by *Sauropus androgynus* leaves[39,40] might stimulate methionine synthesis and therefore the increased methionine content of broiler meat in P1 and P3. Al-Fataftah *et al*.[45] reported that an increase in *Lactobacillus* sp. enhanced the contents of methionine. Homoserine is the common precursor for isoleucine, threonine and methionine. It was assumed that because of inadequate homoserine as substrate for methionine and threonine synthesis, poultry tend to synthesize methionine rather than threonine resulting in a lower threonine content. A lower glycine content might be partly caused by the conversion of glycine to serine[46].

Creek[47] reported that phenylalanine could be converted to tyrosine in poultry, whereas methionine could be converted to cystine. It appears that the conversion of methionine to cystine was inhibited resulting in higher methionine and lower cystine in P1 and P3. In addition, the higher conversion of phenylalanine to tyrosine may partly explain the higher tyrosine. Another possible mechanism of higher tyrosine is the lower conversion of tyrosine into dopamine, norepinephrine and epinephrine.

The contribution of glutamic acid from FSALE may not fully explain the higher glutamic acid in P3 because other treatment groups did not show this. Glutamic acid is synthesized from ammonia and α-ketoglutarate by the action of glutamate dehydrogenase. In contrast, α-ketoglutarate can be produced by oxidative decarboxylation of glutamic acid by glutamate dehydrogenase.

Glutamic acid can synthesize aspartic acid, alanine, proline and arginine. Thus, it appears that the glutamic acid in P1 and P3 may be inadequate for synthesis of aspartic acid resulting in lower aspartic acid in these groups compared with the control. The body may focus on synthesis of alanine and proline from glutamic acid resulting in higher levels of alanine and proline in P1 and P3.

Phenylalanine is produced by most microorganisms from prephenate[48] in which this compound is decarboxylated with the loss of the hydroxyl group to produce phenylpyruvate. This compound is then transaminated using glutamate as the nitrogen source to produce phenylalanine and α-ketoglutarate. Amin and Onodera[49] stated that phenylalanine is also synthesized from phenylacetate by microorganisms. *Sauropus androgynus* contains cis-2-methyl cyclopentanol acetate[50], which may be converted to acetate and may be further converted to phenylacetate in the gastrointestinal tract by microorganisms.

The contribution of valine from *Sauropus androgynus* may partly explain higher valine contents in P1 and P3. A lower histidine content in meat may reduce the formation of carnosine, which may increase the lipid oxidation of meat. However, since alanine also has an important role in the synthesis of carnosine, the increase in lipid oxidation may be prevented. Kralik *et al*.[51] reported that histidine and alanine inclusion in the chicken's diet reduced lipid oxidation of chicken meat.

CONCLUSION

Fermented *Sauropus androgynus* leaf extract (FSALE) contained 25.46% protein, 1.34% fat, 3,642.6 μg β-carotene g^{-1}, 8.17 mg iron g^{-1} and 0.17 mg sterol/100 mg. The extract was rich in palmitic acid (29.96%), glutamic acid (2.221%) and valine (0.523%). The FSALE inclusion at a level of 4.5-18 g kg^{-1} in the diet increased the contents of iron, protein and β-carotene but it reduced the content of cholesterol of broiler meat. In addition, FSALE inclusion improved the compositions of amino acids and fatty acids.

SIGNIFICANT STATEMENT

This study discovered the possible uses of fermented *Sauropus androgynus* leaf extract, which can be beneficial for enriching nutrients such as protein, iron and β-carotene with lower fat and cholesterol in broiler meat. This study will help to uncover the critical area of high fat deposition but low protein, iron and β-carotene deposition in broiler meat that many researchers were not able to explore previously. Thus, a new theory may be created based on the usefulness of fermented *Sauropus androgynus* extracts on lowering fat deposition and enriching protein, iron and β-carotene in broiler meat.

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