PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

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Pakistan Journal of Nutrition 14 (11): 799-807, 2015
ISSN 1680-5194

Effect of Fermented *Sauropus androgynus* Leaves on Meat Composition, Amino Acid and Fatty Acid Compositions in Broiler Chickens

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Abstract: The present study was conducted to evaluate the effect of fermented *Sauropus androgynus* leaves (SAL) on meat composition of broiler meats. One hundred and twelve broiler chicks aged 15 days were distributed to seven groups with four replicates of four broiler chicks each. One group was fed diet without fermented SAL as the control and other six groups were fed diets 2.5 or 5% *Neurospora crassa* fermented SAL; 2.5 or 5% EM4 fermented SAL and 2.5 or 5% *Saccharomyces cerevisiae* fermented SAL. It was shown that inclusion of fermented SAL significantly affected the contents of vitamin A, beta-carotene, iron, fat, cholesterol and protein in broiler meats ($p<0.01$). Inclusion of fermented SAL to diets significantly affected aspartic acid, glutamic acid, serine, glycine, histidine, arginine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine ($p<0.01$) but it had no effect on threonine. Inclusion of fermented SAL to diets significantly affected myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid ($p<0.01$). In conclusion, 5% *Saccharomyces cerevisiae* fermented SAL inclusion resulted in the best broiler meat quality as indicated by lower fat and cholesterol with higher vitamin A, beta-carotene, protein, iron contents with better amino acid and fatty acid balances.

Key words: Fermented *Sauropus androgynus* leaves, cholesterol, amino acid, fatty acid, broiler meat

INTRODUCTION

Experimental reports showed that medical herbs such as tu-chung (Santoso *et al*., 2000b) and *Sauropus androgynus* leaves (Santoso and Sartini, 2001) reduced fat deposition in chickens. *Sauropus androgynus* leaves also reduced cholesterol content in broiler carcass (Santoso and Sartini, 2001; Subekti, 2003) and its content in eggs (Santoso *et al*., 2005). However, feeding *Sauropus androgynus* leaves tended to lower body weight gain (Santoso and Sartini, 2001) which may reduce the profit of broiler industry because the price of broiler chickens in developing countries is still based on body weight rather than quality. *Sauropus androgynus* had several antinutrition such as alkaloid (Agrawal *et al*., 2014; Malik, 1997; Susanti *et al*., 2014) tannin (Agrawal *et al*., 2014; Malik, 1997; Susanti *et al*., 2014), saponin (Malik, 1997; Susanti *et al*., 2014), calsium oxalate (Sheela *et al*., 2004). Tannin was known to decrease body weight gain in broiler chickens (Yang *et al*., 2003), whereas saponin was also well known to be deleterious compounds (Francis *et al*., 2002). *Sauropus androgynus* leaves contained tannin (0.46 g/100 g DW) and saponin (2.84 g/100 g DW) (Singh *et al*., 2011, whereas Hermana (2013) reported that *Sauropus androgynus* leaves contained 88.68 mg tannin/100 g FW. In addition, the leaves of *Sauropus androgynus* contained oxalic acid (33.25 mg/100 g FW) (Sheela *et al*., 2004).

To overcome the above problems, the antinutritions in *Sauropus androgynus* leaves should be reduced to minimize their negative effects. One of method to reduce antinutrition in *Sauropus androgynus* was biofermentation. Fermentation has an important role in nutritional improvement of foods. Fermentation yields the bioactive peptides resulted from protein cleavage and therefore it increases in the biological properties of the feedstuff (Steinkraus, 2002). In addition, fermented feed products are a good source of peptides and amino acids (Rajapakse *et al*., 2005; Sathivel *et al*., 2003). Experimental results showed that fermentation increased nutritional values and feed utilization in poultry and protein and soluble protein and increase protein cleavage to peptide and amino acid (Susi, 2012; Lahay and Rinduwati, 2007), increased crude protein and fat digestibility (Sukaryana *et al*., 2011), improved amino acid balance (Ari *et al*., 2012; Susi, 2012), reduced crude fiber (Lahay and Rinduwati, 2007) and reduced antinutritions such as trypsin inhibitor (Ari *et al*., 2012; Ibrahim *et al*., 2002), oligosaccharide (Ibrahim *et al*., 2002), tannin (Olaniyi and Mehhizadeh, 2013) and phytic acid (Ari *et al*., 2012; Ibrahim *et al*., 2002; Olagunju and Ifesan, 2013), phenol, phytin phosphorus and oxalate (Olagunju and Ifesan, 2013), saponin (Olaniyi and Mehhizadeh, 2013), alkaloid (Shu *et al*., 2010).

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Fermentation by *Lactobacillus* or *Saccharomyces cerevisiae* (Dordevic *et al*., 2010) and *Bacillus subtilis* (Juan and Chou, 2010) also increased antioxidant properties. Experimental results also showed that fermentation increased mineral availability. It was shown that fermented products (Santoso *et al*., 2000a; Santoso *et al*., 2001a,b) reduced fat deposition in animals.

The present study was conducted to evaluate effect of fermented *Sauropus androgynus* leaves on contents of protein, fat, iron, beta-carotene, vitamin A, cholesterol and amino acid and fatty acid compositions in broiler meats.

MATERIALS AND METHODS

Animals: Commercial broiler chicks aged one day old were procured from a commercial hatchery. All chicks were provided sugar containing drinking water they immediately arrived to the house to minimize stress. All the chicks were first brooded together on deep litter system of housing for two week to acclimatize. They were maintained in a house under continuous lighting. All the broiler chicks were given routine vaccinations against ND at 4 and 21 days of age. Vitamins were administered to all the chicks in their drinking water for 1 day only after vaccination.

Feeding treatment: The all chicks were fed broiler starter mash containing 23% CP and 3200 kcal ME from 1-14 days of age, followed experimental diets containing 19% CP and 3150 kcal ME (Table 1) from 15-35 days of age. Feed and water were provided *ad libitum*.

At 15 days of age, all chicks were weighed and selected on weight basis and then one hundred and twelve broiler chicks were distributed to 7 treatment groups. Each group had 4 replications of 4 chicks kept in separate bamboo pens with 100 x 65 cm dimension. The treatment groups were as follows: (1) Broiler fed diets without *Sauropus androgynus* leaves as the control; (2) Broilers fed diets with 2.5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; (3) Broilers fed diets with 5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; (4) Broilers fed diets with 2.5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp. (EM4); (5) Broilers fed diets with 5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp. (EM4); (6) Broilers fed diets with 2.5% Sauropus androgynus leaves fermented by *Saccharomyces cerevisiae*; (7) Broilers fed diets with 5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*. Broiler chickens were weighed individually on a weekly basis and feed consumption was recorded daily.

Sampling and laboratorium analysis: At the end of experiment (35 days of age), 5 broiler chickens in each treatment groups were selected and slaughtered. In order to avoid variations in the cutting procedures, the same operator was employed (Zhang *et al*., 2013). Thigh meats were then collected to analysis of cholesterol, protein, fat, fatty acid composition, amino acid composition, beta-carotene, vitamin A and Fe. Fat and protein were measured by AOAC methods (AOAC, 1980). Cholesterol content was determined spectrophotometrically at wave lengths of 490 nm by the method of Searcy *et al*. (1960) as modified by Bohac *et al*. (1988), while saponification was done by adding of 3% of pyrogallol (Zivkovic *et al*., 2002). Amino acid composition was measured by the method as described by Morel *et al*. (2003). The lipids were extracted according to Folch *et al*. (1957) with a chloroform-methanol mixture and methylized by 20% *boron trifluoride methanol complex* in methanol solution (Morrison and Smith, 1964). Fatty acid composition was then determined by gas chromatography. Beta-carotene, iron and vitamin A were measured by the method of Slamet *et al*. (1990) and Subekti (2003).

Statistical analysis: All data were subjected to analysis of variance and if it was significantly different it was further tested by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Nutritional composition of *Sauropus androgynus*: It was noted that *Sauropus androgynus* leaves meal had low crude protein and crude fat contents and their contents were not changed by fermentation (Table 2). Othman *et al*. (2015) reported that *Sauropus androgynus* leaves contained total carotenoid (mg/g DW) = 190.30, neoxanthin = 142.40, violaxanthin = 28.06, letein = 15.57, beta-cryptoxanthin = 0.07, alpha-carotene = 1.36, beta = carotene = 2.84, zeaxanthin = nd, whereas Azis and Muktiningsih (2006) reported that these leaves contained carotene 10, 020 µg/100 g. The present study showed that *Sauropus androgynus* leaves fermented by *Neurospora crassa* had the highest beta-carotene and iron contents (Table 2). *Neurospora crassa* is carotenogenic, namely producing beta-carotene (Nuraini, 2006; Nuraini *et al*., 2009). In addition they found that feedstuffs fermented by *Neurospora crassa* also showed higher beta-carotene content.

Low protein content of these leaves was disagreement with the observation of Nahak and Sahu (2010) who found that *Sauropus androgynus* leaves had high protein content. Nahak and Sahu (2010) found that the contents of protein and fat in fresh *Sauropus androgynus* leaves were 5.24 and 0.13%, respectively. Oboh (2006) reported that fermenting cassava peel meal by *Saccaromyces cerviseae* and *Lactobacillus* spp increased in protein content and decreased in cyanide and phytate.

Among three fermentors used in the present study, *Neurospora crassa* was more effective to increase iron

Table 1: Composition of experimental diets

Feedstuff (%)	0% fermented SAL	2.5% fermented SAL	5% fermented SAL
Corn	57.0	56.0	54.5
Rice bran	5.0	5.0	5.0
Broiler concentrate	34.2	33.2	32.2
Mineral mixture	1.7	1.7	1.7
Salt	0.1	0.1	0.1
Top mix	0.5	0	0
Fermented Sauropus androgynus leaves	0	2.5	5.0
Vegetable oil	1.5	1.5	1.5
Protein (%)	19.4	19.4	19.4
Metabolizable Energy (kkal/kg)	3155.6	3155.6	3155.6
Ca (%)	1.51	1.51	1.51
P (%)	0.86	0.86	0.86

SAL: *Sauropus androgynus* leaves

Table 2: Composition of *Sauropus androgynus* leaves powder

	USA	NCFSA	EM4FSA	SCFSA
Protein (%)	4.91	5.08	5.12	4.86
Crude fat (%)	1.14	1.68	1.28	1.19
Beta-carotene (μg/g)	3,510.3	3,716.9	3,476.9	3,510.4
Fe (mg/g)	7.84	9.99	8.14	7.43

USA: Unfermented *Sauropus androgynus* leaves powder; NCFSA: *Neurospora crassa* fermented *Sauropus androgynus* leaves powder; EM4FSA: EM4 fermented *Sauropus androgynus* leaves powder; SCFSA: *Saccaromyces cereviseae* fermented *Sauropus androgynus* leaves powder

content. Mohite *et al.* (2013) found that fermentation increased the amount of iron because of lowering effect on phytate.

The present study showed that *Sauropus androgynus* leaves meal was rich in glutamic acid (Table 3) and palmitic acid (Table 4). This study was in contrary with the observation of Samad *et al.* (2014) who found that *Sauropus androgynus* leaves was rich in linolenic acid rather than palmitic acid. *Saccharomyces cervisiae* culture was rich in linoleic acid (Burder and Reinink, 1974; Gutierrez and Da-Silva, 1993) which contributed to higher in linoleic acid. Similar pattern might be occurred for *Neurospora crassa* and EM4. Fermentation changed amino acid and fatty acid composition of *Sauropus androgynus* leaves meal (Table 3-4). It was shown that fermentation increased unsaturated fatty acid but it reduced saturated fatty acid and therefore resulted in lower saturated-unsaturated fatty acids ratio. However, fermentation tended to reduce total amino acid contents. Cao *et al.* (2012) reported that *Ginkgo biloba* leaves fermented by *Aspergillus niger* increased total amino acid. This differences might in part be caused by the differences of fermentor used, substrate or both.

Meat composition: Table 5 showed effect of fermented *Sauropus androgynus* leaves on meat composition. It was shown that inclusion of fermented *Sauropus androgynus* leaves significantly affected the contents of vitamin A, beta-carotene, iron, fat, cholesterol and protein in broiler meats ($p<0.01$). It was shown that P0 had higher fat content than P2, P2, P5 and P6. In contrary, P0 had lower protein content than P1, P3, P4 and P5. P0 had lower Vitamin A than P1, P5 and P6. P0 had lower beta-carotene than P1, P3, P5 and P6. P0 had lower iron content than P1, P5 and P6. P6 had lower cholesterol content than P0, whereas P1 and P5 were higher. An increase in Vitamin A and beta-carotene might in part be contributed from *Sauropus androgynus* leaves (Table 2). In addition, fermentation might improve the availability of beta-carotene for broilers. Olson and Hayaishi (1965) suggested that there are two mechanism of beta-carotene conversion to vitamin A, namely (1) carotenes cleaved at the central 15-15' double bond to yield two molecules of Vitamin A and (2) carotene is cleaved peripherally to yield one molecule of vitamin A via a series of 13-apo-carotenals. Fermenting *Sauropus androgynus* leaves by *Saccaromyces cereviseae* resulted in the highest Vitamin A in broiler meats. In addition, this fermentor was also effective to increase iron content of broiler meats. An increase in iron content of meat might partly be caused by an increase the availability of iron for broiler chickens.

Compounds that play a role in lowering fat contents might be alkaloid and non alkaloid (Santoso *et al.*, 2010b), 3-O-b-D-glucosyl-(1→6)-b-D-glucosyl-kaempferol (Yu *et al.*, 2006), flavonoid (Zarrouki *et al.*, 2010), tannin (Aiura and de Carvalho, 2007), polyphenol (Zang *et al.*, 2006). An increase in fat soluble substrate such as vitamin E (Santoso *et al.*, 2010a), vitamin A and beta-carotene (the present study) might also contribute to the lower net fat content.

Although *Sauropus androgynus* leaves had low protein content and fermentation of these leaves did not increase protein content, protein content in some

Table 3: Amino acid composition of fermented *Sauropus androgynus* leaves powder

Amino acid (%)	USA	NCFSA	EM4FSA	SCFSA
Aspartic acid	0.39	0.30	0.29	0.30
Glutamic acid	0.92	0.88	0.84	0.83
Serine	0.09	0.07	0.09	0.09
Glycine	0.02	0.01	0.02	0.02
Histidine	0.01	0.02	0.01	0.02
Arginine	0.09	0.08	0.09	0.09
Threonine	0.05	0.03	0.05	0.09
Alanine	0.11	0.09	0.09	0.10
Proline	0.04	0.04	0.06	0.04
Tyrosine	0.03	0.02	0.03	0.03
Valine	0.08	0.05	0.06	0.07
Methionine	0.10	0.06	0.09	0.08
Cysteine	0.07	0.04	0.07	0.05
Isoleucine	0.02	0.01	0.01	0.02
Leucine	0.03	0.03	0.04	0.04
Phenylalanine	0.07	0.05	0.06	0.06
Lysine	0.06	0.04	0.06	0.03
Total	2.18	1.82	1.96	1.96

USA: Unfermented *Sauropus androgynus* leaves powder; NCFSA: *Neurospora crassa* fermented *Sauropus androgynus* leaves powder; EM4FSA: EM4 fermented *Sauropus androgynus* leaves powder; SCFSA: *Saccaromyces cereviseae* fermented *Sauropus androgynus* leaves powder

Table 4: Fatty acid composition of fermented *Sauropus androgynus* leaves meal

Fatty acid (%)	USA	NCFSA	EM4FSA	SCFSA
Lauric acid	nd	nd	Nd	Nd
Myristic acid	8.81	6.72	5.63	10.76
Palmitic acid	48.73	45.36	42.20	45.81
Stearic acid	3.08	3.70	4.72	2.82
Oleic acid	6.72	6.84	15.88	7.24
Linoleic acid	5.11	9.07	7.65	6.49
Linolenic acid	nd	nd	Nd	Nd
Saturated	60.62	55.78	52.55	59.39
Unsaturated	11.83	15.91	23.53	13.73
Saturated-unsaturated ratio	5.12	3.51	2.23	4.33

USA: Unfermented *Sauropus androgynus* leaves powder; NCFSA: *Neurospora crassa* fermented *Sauropus androgynus* leaves powder; EM4FSA: EM4 fermented *Sauropus androgynus* leaves powder; SCFSA: *Saccaromyces cereviseae* fermented *Sauropus androgynus* leaves powder. nd: not detected

treatment groups was higher than the control. This phenomenon indicated that there was improvement in the availability of protein for broilers because of better protein digestibilities. Chen *et al.* (2010) reported that soybean fermented by *Aspergillus* and *Lactobacillus* mixture increased *in vitro* and *in vivo* protein digestibility. Adam *et al.* (2013) also found that fermentation increased *in vitro* protein digestibility.

Compounds that play a role in lowering cholesterol in broiler fed 5% *Saccharomyces cerevisiae* fermented *Sauropus androgynus* might be alkaloids and non-alkaloids (Santoso *et al.*, 2010), saponins (Son *et al.*, 2007), polyphenol (Ngamukote *et al.*, 2011; Zang *et al.*, 2006), flavonoid (Shrime *et al.*, 2011), phytosterol (Subekti, 2003). Patil *et al.* (2010) reported that the reduction of cholesterol and triglycerides by alkaloids were in part caused by the reduction of lipogenic enzymes activities and increased bile acid excretion in feces. It is unknown why *Sauropus androgynus* leaves fermented by *Neurospora crassa* or EM4 failed to reduce cholesterol content. Syahruddin *et al.* (2013) reported that inclusion of *Sauropus androgynus* leaves fermented by *Trichoderma harzianum* at level of 2-14% reduced carcass cholesterol contents.

Amino acid composition of broiler meat: Table 6 showed effect of fermented *Sauropus androgynus* leaves on amino acid composition of broiler meats. Inclusion of fermented *Sauropus androgynus* leaves to diets significantly affected aspartic acid, glutamic acid, serine, glycine, histidine, arginine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine ($p<0.01$) but it had no effect on threonine. Experimental results showed that P2 and P3 had higher aspartic acid; whereas P1, P2, P3, P4 and P6 had higher glutamic acid than P0. Furthermore, P1, P2, P3, P4 and P5 had higher histidine and P2 and P6 had higher lysine than P0. However, P0 had higher serine than the treatment groups; higher glycine than P4 and P6 and higher methionine than P4 and P6. Total amino acid was higher in P1, P2 and P3 as compared to P0. These results showed that inclusion of fermented

Table 5: Effect of fermented *Sauropus androgynus* leaves on meat composition in broiler chickens

	P0	P1	P2	P3	P4	P5	P6	SD
Cholesterol, mg/100mg	1.50^{b}	1.75^{a}	1.52^{b}	1.56^{b}	1.75^{a}	1.42^{bc}	1.28^{c}	0.17**
Crude fat (%)	25.32^{a}	25.37^{a}	24.69^{c}	24.98^{b}	25.30^{a}	24.96^{b}	24.01^{d}	0.47**
Crude protein (%)	18.68^{c}	19.05^{b}	18.53^{c}	19.03^{b}	19.47^{a}	18.94^{b}	18.67^{c}	0.31**
Beta-carotene (μ/g)	2.22^{c}	3.06^{a}	2.28^{c}	2.97^{a}	2.38^{c}	3.03^{a}	2.07^{b}	0.36**
Vitamin A (μ/g)	877.06^{c}	902.28^{b}	881.26^{c}	861.18^{d}	823.55^{e}	917.47^{a}	918.33^{a}	32.43**
Fe (mg/g)	2.79^{cd}	3.03^{bc}	2.80^{cd}	2.65^{cd}	2.64^{d}	3.26^{b}	3.67^{a}	0.38**

P0: Control; P1: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P2: Diets with 5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P3: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P4: Diets with 5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P5: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*; P6: Diets with 5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*

Table 6: Effect of fermented *Sauropus androgynus* leaves on amino acid composition of broiler meats

Amino acid (%)	P0	P1	P2	P3	P4	P5	P6	SD
Aspartatic acid	1.09^{b}	1.20^{ab}	1.35^{a}	1.36^{a}	1.25^{ab}	1.18^{ab}	1.24^{ab}	0.12**
Glutamic acid	1.80^{c}	2.01^{a}	1.93^{a}	1.97^{a}	1.98^{a}	1.82^{bc}	1.91^{ab}	0.15**
Serine	0.46^{a}	0.37^{c}	0.41^{b}	0.43^{b}	0.37^{c}	0.42^{b}	0.32^{d}	0.05**
Glycine	0.61^{ab}	0.64^{a}	0.62^{ab}	0.63^{a}	0.57^{c}	0.59^{bc}	0.61^{ab}	0.03**
Histidine	0.27^{e}	0.30^{cd}	0.38^{ab}	0.41^{a}	0.36^{b}	0.32^{c}	0.29^{de}	0.05**
Arginine	0.79^{b}	0.85^{a}	0.83^{ab}	0.81^{ab}	0.73^{c}	0.78^{b}	0.80^{ab}	0.04**
Threonine	0.51	0.59	0.55	0.57	0.55	0.54	0.49	0.06^{ns}
Alanine	0.72^{c}	0.81^{a}	0.82^{a}	0.81^{a}	0.72^{c}	0.78^{b}	0.80^{ab}	0.04**
Proline	0.44^{bc}	0.46^{b}	0.49^{a}	0.42^{c}	0.42^{c}	0.44^{bc}	0.43^{c}	0.03**
Tyrosine	0.30^{b}	0.31^{b}	0.36^{a}	0.37^{a}	0.32^{b}	0.29^{b}	0.25^{c}	0.04**
Valine	0.51^{d}	0.57^{c}	0.63^{a}	0.61^{ab}	0.59^{bc}	0.60^{b}	0.52^{d}	0.04**
Methionine	0.28^{ab}	0.31^{a}	0.30^{a}	0.27^{bc}	0.26^{c}	0.29^{ab}	0.22^{d}	0.03**
Cysteine	0.12^{bcd}	0.13^{bc}	0.15^{a}	0.13^{b}	0.11^{d}	0.11^{d}	0.11^{d}	0.02**
Isoleucine	0.40^{a}	0.43^{a}	0.42^{a}	0.37^{b}	0.35^{b}	0.42^{a}	0.37^{b}	0.03**
Leucine	0.56^{e}	1.04^{a}	0.93^{bc}	0.84^{d}	0.95^{abc}	0.97^{abc}	0.90^{cd}	0.23**
Phenylalanine	0.47^{b}	0.47^{b}	0.51^{a}	0.51^{a}	0.44^{c}	0.46^{bc}	0.52^{a}	0.03**
Lysine	0.92^{c}	0.95^{c}	1.03^{a}	0.98^{abc}	0.92^{c}	0.92^{c}	0.99^{ab}	0.05**
Total	10.24^{d}	11.22^{bc}	11.69^{a}	11.46^{ab}	10.87^{c}	10.91^{c}	10.74^{cd}	0.52**

P0: Control; P1: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P2: Diets with 5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P3: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P4: Diets with 5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P5: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*; P6: Diets with 5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*

Table 7: Effect of fermented *Sauropus androgynus* leaves on fatty acid composition of broiler meats

Fatty acid (%)	P0	P1	P2	P3	P4	P5	P6	SD
Myristic acid	0.70^{b}	0.68^{b}	0.62^{c}	0.31^{e}	0.41^{d}	0.76^{a}	0.63^{c}	0.16**
Palmitic acid	24.32^{d}	26.09^{b}	26.25^{b}	25.14^{c}	26.45^{b}	27.10^{a}	22.55^{e}	4.96**
Stearic acid	6.07^{b}	5.73^{c}	6.41^{a}	5.95^{bc}	6.14^{ab}	4.17^{d}	5.66^{c}	0.70**
Oleic acid	43.29^{b}	43.10^{bc}	42.56^{cd}	44.07^{a}	43.02^{bc}	42.39^{d}	39.25^{e}	1.48**
Linoleic acid	15.25^{a}	12.32^{c}	12.46^{c}	14.33^{b}	12.73^{c}	13.99^{b}	11.04^{d}	1.37**
Linolenic acid	0.51^{de}	0.48^{de}	0.56^{c}	0.62^{b}	0.53^{cd}	0.73^{cd}	0.46^{e}	0.09**
Saturated	31.09^{d}	32.49^{bc}	33.27^{a}	31.37^{d}	32.99^{ab}	32.03^{c}	28.84^{e}	5.23**
Unsaturated	59.05^{a}	55.91^{c}	55.57^{c}	59.02^{a}	56.27^{bc}	57.10^{b}	50.75^{d}	2.67**
Total	90.14^{ab}	88.40^{c}	88.84^{c}	90.41^{a}	89.27^{abc}	89.12^{bc}	79.58^{d}	5.99**
Ratio saturated: unsaturated	0.53^{e}	0.58^{bc}	0.60^{c}	0.53^{e}	0.59^{ab}	0.56^{d}	0.57^{cd}	0.09**

P0: Control; P1: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P2: Diets with 5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P3: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P4: Diets with 5% *Sauropus androgynus* leaves fermented by *Lactobacillus* sp (EM4); P5: Diets with 2.5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*; P6: Diets with 5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*

Sauropus androgynus leaves changed amino acid composition of broiler meats.

Fujiwara *et al*. (2008) found that supplementation of fermented product such as soybean increased glutamic acid, protein and peptide contents in thigh chicken meat. Histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine, tryptophan and valine are essential amino acid in human nutrition (FAO, 2013). Therefore, an increase in histidine, valine, isoleucine, leucine, phenylalanine and lysine contents in broiler meats in the present study would be benefit for human. However, the reduction of methionine content in broilers fed EM4 fermented or *Saccaromyces cereviseae* fermented *Sauropus androgynus* leaves at level of 5% should be considered. In addition, lower tyrosine in broilers fed 5% Saccaromyces cereviseae fermented *Sauropus androgynus* leaves should also be considered. Chen *et al.* (2010) reported that fermenting soybean with *Aspergillus* and *Lactobacillus* mixture increased lysine availability.

Fatty acid composition of meat: Table 7 presented effect of fermented *Sauropus androgynus* leaves on fatty acid composition of broiler meats. Inclusion of fermented *Sauropus androgynus* leaves to diets significantly affected myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid ($p<0.01$). The present study showed that P2, P3, P4 and P6 had lower myristic acid, whereas P5 had higher myristic acid as compared to P0. Palmitic acid of P6 was lower than other treatments. Furthermore, stearic acid was lower in P1, P5 and P6, whereas oleic acid in P2, P5 and P6 were lower than P0. In addition, Linoleic acid was lower in treatment groups, whereas linolenic acid was higher in P2, P3 and P5. In overall, saturated fatty acids was lower in P6 but other treatment groups were higher as compared to P0. In contrary, unsaturated fatty acids were lower in treatment groups except for P3 as compared to P0. It was interested that total fatty acids were lower in P1, P2, P5 and P6 as compared to P0 with higher saturated-unsaturated fatty acids ratio in treatment groups except for P3. Unfortunately, the ratio of saturated-unsaturated fatty acids was higher in treatment groups except for P3.

Monounsaturated fatty acids could be synthesized from saturated fatty acids and monounsaturated fatty acid was then converted to oleic acid. Poultry did not synthesize linoleic acid and linolenic acid, so that these two fatty acids should be included from diets. Thus, an increase in linoleic acid of meat in P5 might be from an increase in efficiency of digestion and absorption of this fatty acid derived from diets.

Except for P6, the present study was disagreement with the observation of Cao *et al.* (2012) who found that the inclusion of fermented product to broiler diets tended to increase unsaturated fatty acid and to reduce saturated fatty acid in breast meat of broiler chickens. It was benefit for broilers fed 5% *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves, because it produced meats with lower saturated fatty acids, cholesterol and fat contents.

For other treatment groups although they had similar fat content and higher saturated-unsaturated fatty acids ratio to the control group, an increase in vitamin A and beta-carotene (the present study) and vitamin E (Santoso *et al.*, 2010) in broiler meat caused by fermented *Sauropus androgynus* leaves inclusion might prevent oxidation of meat fatty acids. Vitamin A, beta-carotene and vitamin E were natural antioxidants. It was known that antioxidants had an important role in inhibiting and scavenging free radicals. Therefore, it protected humans against infections and degenerative diseases. In addition, *Sauropus androgynus* leaves were also rich in flavonoid and vitamin C (Andarwulan, 2012) and other phenolic compounds (Nahak and Sahu, 2010) which also act as natural antioxidant.

Conclusion: In conclusion, 5% *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves inclusion resulted in the best broiler meat quality as indicated by lower fat, cholesterol and total fatty acids with higher vitamin A, beta-carotene and iron contents in broiler meats. This benefit might be increased if this fermented product was extracted.

ACKNOWLEDGMENTS

The present study was supported by Directorate General Higher Education, Department of National Education, Indonesia.

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