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Breast meat characteristics of broilers fed fermented mixture of cassava pulp and *Moringa oleifera* leaf meal

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ABSTRAK

Penelitian dilakukan dengan tujuan mengkaji pengaruh pemberian fermentasi campuran onggok dan tepung daun kelor (Moringa oleifera) (FCPMO) terhadap karakteristik daging ayam broiler. Mulai hari ke-8 dan seterusnya, 400 ayam broiler stain Lohmann didistribusikan ke empat kelompok perlakuan meliputi CONT (ransum kontrol berbasis jagung dan kedelai tanpa aditif), BACI (ransum kontrol ditambah 0,1% antibiotik zinc bacitracin), FERM (ransum mengandung 20 % FCPMO) dan FERB (ransum mengandung 20% FCPMO dan 0,1% probiotik Bacillus subtilis). Pada hari ke-35, satu ayam pejantan dari setiap pen (10 ayam per kelompok perlakuan) disembelih. Sampel daging dada selanjutnya diambil untuk analisis laboratorium. Hasil penelitian menunjukkan bahwa FCPMO tidak berpengaruh (P>0,05) terhadap pH dan water holding capacity (WHC) daging ayam. Dibandingkan kelompok lain, daging dari kelompok FERB memiliki susut masak yang lebih tinggi (P<0,05). Daging FERM dan FERB memiliki kadar air lebih rendah (P<0,05) daripada daging CONT dan BACI. Pemberian FCPMO dengan atau tanpa B. subtilis meningkatkan (P<0,05) kandungan protein kasar pada daging ayam. Perlakuan FCPMO menghasilkan kadar lemak yang lebih rendah (P<0,05) dalam daging. Dibandingkan daging lainnya, daging BACI memiliki kadar abu yang lebih tinggi (P<0,05). Kandungan kolesterol cenderung lebih rendah (P=0,08) pada FERB daripada pada daging BACI dan FERM. Nilai IC_{50} 2,2-diphenylpicrylhydrazyl (DPPH) lebih tinggi (P<0,05) dalam daging CONT dibandingkan daging lain. Pemberian FCPMO menghasilkan asam lemak tak jenuh ganda (PUFA) lebih tinggi (P<0,05), asam lemak tak jenuh tunggal (MUFA) lebih rendah (P<0,05) dan tidak berpengaruh terhadap asam lemak jenuh (SFA) daging dada ayam broiler. Sebagai kesimpulan, pemberian FCPMO menghasilkan daging ayam yang lebih sehat tercermin dari tingginya kandungan protein, PUFA dan antioksidan, serta rendahnya lemak pada daging.

Kata kunci : asam lemak daging, ayam broiler, kualitas daging, pakan fermentasi

ABSTRACT

The present study aimed to investigate the effect of feeding fermented mixture of cassava pulp and *M. oleifera* leaf meal (FCPMO) on the breast meat characteristics of broiler chickens. From day 8 onward, 400 Lohmann broiler chicks were allotted to four dietary groups included CONT (maize-soybean-based feed with no additive), BACI (maize-soybean-based feed supplemented with 0.1% zinc bacitracin), FERM (feed containing 20% of FCPMO) and FERB (feed containing 20% of FCPMO and 0.1% probiotic *Bacillus subtilis*). At day 35, one male chick from each replicate (10 chicks per treatment group) was taken and slaughtered. After being eviscerated and de-feathered, sample of breast meat was

obtained for the assessment of meat characteristics. Results showed that feeding FCPMO had no effect (P>0.05) on pH and water holding capacity (WHC) of breast meats. Compared to other groups, FERB meat had higher (P<0.05) cooking loss. FERM and FERB meats had lower (P<0.05) moisture content than that of CONT and BACI meats. Feeding FCPMO with or without *B. subtilis* increased (P<0.05) crude protein content of breast meats. Feeding FCPMO resulted in lower (P<0.05) fat content in the breast meat. Compared to other meats, breast meats from BACI had higher (P<0.05) ash content. Cholesterol was lower (P=0.08) in FERB than in BACI and FERM meats. The 2,2-diphenylpicrylhydrazyl (DPPH) IC_{50} values were higher (P<0.05) in meat of CONT than that in other groups. Feeding FCPMO resulted in higher (P<0.05) polyunsaturated fatty acids (PUFA), lower (P<0.05) monounsaturated fatty acids (MUFA) and had no effect on saturated fatty acids (SFA) contents of broiler breast meats. In conclusion, feeding FCPMO produced healthy broiler meat as reflected by the higher contents of protein, PUFA and antioxidants, and lower fat in meats.

Keywords: broiler, fatty acids, fermented feed, meat quality

INTRODUCTION

Broiler meat has been a primary protein source for the majority of people in Indonesia. Besides an affordable price, broiler meat contains high quality of nutrition, easy to prepare and has a delicious taste (Wahyono and Utami, 2018). Recently, broiler farmers have complained about the increasing price of feed that eventually increases broiler production cost. Among the feed ingredients, corn accounts for more than half of broiler rations. The increase in price of corn can therefore substantially reduce the cost-efficiency of broiler farming. Attempts have been conducted to partly reduce the proportion of corn in broiler diets. The use of agro-industrial by-products such as cassava pulp as the alternative energy source for broilers seems to be profitable, but the inclusion of the by-products into broiler diets is often limited by their high and low contents of fibre and protein, respectively (Khempaka *et al.*, 2009; Sugiharto, 2019).

Fermentation could be a conventional method to improve the nutritional properties and thus increase the incorporation levels of the agro-industrial by-products in broiler diets. Also, supplementation with protein source may compensate the low content of protein in the agro-industrial by-products (Sugiharto, 2019). In this current study, we mixed cassava pulp with *Moringa oleifera* leaf meal, which is rich in protein (Nkukwana *et al.*, 2015; Sugiharto *et al.*, 2019), prior to fermentation. The filamentous fungus *Chrysonilia crassa* was selected as a fermentation starter given its fibre-degrading and nutritional-improving activities (Sugiharto *et al.*, 2017; 2019).

Currently, consumers are increasingly aware of the nutritional values of broiler meat they consume. High protein and low fat contents as well as the high proportion of unsaturated fatty acids (PUFAs) are among the most popular demands from the consumers of broiler meats. Also, the yield and freshness of the meat are important factors affecting consumer preference (Lee *et al.*, 2017; Marcinčák *et al.*, 2018). Many studies revealed that the quality of broiler meats may substantially be affected by diets. With regard to the effect of fermented feed, Lee *et al*. (2017) reported that feeding fermented soybean hulls resulted in higher contents of protein, lower fat and improved water holding capacity (WHC) of broiler breast meats. Likewise, Marcinčák *et al*. (2018) noticed the improvements of oxidative status and the ratio of n-6/n-3 PUFAs in broiler meat with feeding fermented bioproduct. Formerly, we also documented that feeding cassava pulp fermented using the fungus *Acremonium charticola* increased the protein content of broiler breast meat (Sugiharto *et al.*, 2017). Aside from the effect of fermented feed, *M. oleifera* leaf meal has been reported to positively affect the characteristics of broiler meats. Nkukwana *et al*. (2015) documented that dietary inclusion of *M. oleifera* improved proximate composition and shelf-life of broiler breast meat. Likewise, Cui *et al*. (2018) noticed the improved PUFAs content and oxidative stability in broiler breast meat when feeding *M. oleifera* leaf meal.

The present study aimed to investigate the effect of feeding fermented mixture of cassava pulp and *M. oleifera* leaf meal (FCPMO) on the breast meat characteristics of broiler chickens. To best of our knowledge, this is the first study elucidating the effect of FCPMO on the meat quality of broiler chickens.

MATERIALS AND METHODS

Production of Fermentation Starter and FCPMO

The production of fermentation starter was commenced by rejuvenating the *C. crassa* isolate from the fungus stock culture (stored on potato dextrose agar [PDA] at 4^{o}C). The rejuvenated fungi were aerobically re-cultivated on the same agar medium for 48 h at 38^{o}C. To harvest the fungal spores, sterilized distilled water (10 mL) were poured onto the incubated plate, and the latter suspension was then used to inoculate the used rice (100 g) as the substrate for the production of fermentation starter. Prior to inoculation, the used rice (obtained from the local market in Semarang) was cleaned, soaked in tap water (for about 1 h), steamed (for 1 h) and spread out on tray until cool. The inoculated used rice was aerobically incubated at room temperature, and after 48 h the fermented used rice was sun-dried, ground and sieved. The latter product was subsequently used as fermentation starter (containing >1 × 10^{8} cfu/g of *C. crassa* colonies according to plate count method) to produce the FCPMO.

To produce the FCPMO, the dried cassava pulp (with moisture content of about 12%; obtained from the local supplier in Boyolali regency, Central Java Province) was steamed for 1 h and allowed to cool afterwards. The mature *M. oleifera* leaves were collected from the gardens surrounding the university. The leaves were air dried at room temperature and then milled. Prior to fermentation, the 60 g of steamed cassava pulp was mixed thoroughly with 35 g of *M. oleifera* leaf meal. The mixture was then quickly inoculated with 5 g of the fermentation starter as described above. To conduct the solid state fermentation (with moisture content of about 40%), the prepared mixture was added with 100 mL autoclaved distilled water. Aerobic incubation at room temperature was subsequently applied to the mixture, and after 72 h the fermented mixture was sun dried. Sample of FCPMO was proximally analysed (AOAC, 1995) and the rest of the product was placed at room temperature until use. The FCPMO contained 8.75% water, 17.6% crude protein (CP), 4.41% lipid, 9.01% crude fibre (CF) and 10.1% crude ash, whereas cassava pulp contained 11.0% water, 2.24% CP, 0.91% lipid, 31.8% CF and 3.65% ash. *M. oleifera* leaf meal had 11.1% water, 29.9% CP, 5.38% lipid, 13.2% CF and 12.6% ash. These proximate values are presented in dry basis.

In vivo Experiment

A total of 400 Lohmann broiler chicks were reared in accordance to the commercial condition during the first week. From day 8 onward, the chicks were divided to four groups of dietary treatments, which were CONT (maize-soybean-based feed with no additive), BACI (maize-soybean-based feed supplemented with 0.1% antibiotic zinc bacitracin), FERM (feed containing 20% of FCPMO) and FERB (feed containing 20% of FCPMO and 0.1% probiotic *Bacillus subtilis*). Each dietary group consisted of 10 replicates/pens with 10 chicks in each. The feeds (in mash form; Table 1) were formulated as starter (days 8-21) and finisher feeds (days 22-35), and were provided *ad libitum* for the entire study period. The chicks were vaccinated at days 4 (with Newcastle disease-infectious bursal disease vaccines [ND-IBD] through ocular route), 14 (IBD vaccine through drinking water) and 19 (ND vaccine through drinking water). Throughout the experimentation, the chicks were raised in an opened-sided broiler house with rice husk as bedding materials. At day 35, one male chick from each replicate (10 chicks per treatment group) was taken and slaughtered. After being eviscerated and de-feathered, sample of breast meat was obtained and then immediately frozen (-20°C) until the assessment of meat characteristics. Prior to analysis, the frozen meat was firstly thawed for about 30 min at room temperature. To measure the pH value, 1 g of breast meat obtained from each sample was homogenized in 9 mL of distilled water, and pH of the resulting filtrate was then measured using the digital pH meter (Hanna Instruments, Woonsocket, Rhode Island). The press methods using filter paper (Grau and Hamm, 1953) was employed to determine the water holding capacity (WHC) of breast meat, while the standard proximate analysis (AOAC, 1995) was conducted to measure the chemical composition of broiler breast meat. To determine the cooking loss, the breast meat sample was placed in plastic bag and cooked in boiling water at 80^{o}C for 1 h. The meat was permitted to cool at room temperature and then weighed. The different weights between before and after cooking was considered as cooking loss. Cholesterol content in breast meat was measured according to a modified saponification process as described by Stewart *et al*. (1992). Enzymatic method (using

Table 1. Ingredients and Chemical Compositions of Experimental Diets

Items (%, unless otherwise noted)	Starter Phase				Finisher Phase			
	CONT	BACI	FERM	FERB	CONT	BACI	FERM	FERB
Yellow maize	54.8	54.8	38.5	38.5	58.5	58.5	42.4	42.4
SBM	35.7	35.7	32.3	32.3	32.7	32.7	28.8	28.8
MBM	4.70	4.70	4.25	4.25	2.35	2.35	2.25	2.25
Soybean oil	1.55	1.55	1.75	1.75	3.25	3.25	3.35	3.35
FCPMO	-	-	20.0	20.0	-	-	20.0	20.0
DL-methionine, 990 g	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine, 780 g	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DCP	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated compositions:								
ME[2] (kcal/kg)	2,900	2,900	2,900	2,900	3,060	3,060	3,060	3,060
CP	22.0	22.0	22.0	22.0	20.0	20.0	20.0	20.0
CF	5.60	5.60	6.30	6.30	5.60	5.60	6.30	6.30
Ca	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
P (available)	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Lysine	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Methionine	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60

[1]Premix contained (per kg of diet) of vitamin A 7,750 IU, vitamin D3 1,550 IU, vitamin E 1.88 mg, vitamin B1 1.25 mg, vitamin B2 3.13 mg, vitamin B6 1.88 mg, vitamin B12 0.01 mg, vitamin C 25 mg, folic acid 1.50 mg, Ca-d-pantothenate 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, BHT 25 mg, Co 0.20 mg, Cu 4.35 mg, Fe 54 mg, I 0.45 mg, Mn 130 mg, Zn 86.5 mg, Se 0.25 mg, L-lysine 80 mg, Choline chloride 500 mg, DL-methionine 900 mg, $CaCO_3$ 641.5 mg, Dicalcium phosphate 1500 mg

[2]Metabolizable energy was calculated on the basis of formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}
CONT: maize-soybean-based feed with no additive, BACI: maize-soybean-based feed supplemented with 0.1% zinc bacitracin, FERM: feed containing 20% of FCPMO, FERB: feed containing 20% of FCPMO and 0.1% *B. subtilis*, SBM: soybean meal, MBM: meat bone meal, FCPMO: fermented mixture of cassava pulp and *M. oleifera* leaf meal, DCP: Dicalcium phosphate, ME: metabolisable energy, CP: crude protein, CF: crude fibre

enzymatic kit from Merck Diagnostica, Darmstadt, Germany) was then employed to determine the cholesterol content in meats following saponification (Bragagnolo and Rodriguez-Amaya, 2001). For the measurement of the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging action, the breast meat sample was firstly extracted with ethanol. Later, the resulting filtrate was examined by DPPH free radical scavenging test as described by Wu *et al.* (2009). The absorbance was evaluated at 517 nm. The fatty acid concentrations in breast meat was determined based on gas chromatography technique. Fatty acids were recognized based on the comparison between the retention times and the standard retention times of each sample.

Quantification of fatty acids were conducted by normalizing and transforming of the area percentage to mg per 100 g of edible portion, using a lipid conversion factor (Holland *et al.*, 1998).

Data obtained from the present study were statistically analysed using ANOVA (SAS Inst. Inc., Cary, NC, USA). When the notable ($P<0.05$) difference existed across the treatment groups, the Duncan's multiple-range test was then carried out.

RESULTS AND DISCUSSION

Data on the physical and chemical traits of broiler breast meats are presented in Table 2. It was shown in this study that feeding FCPMO had no effect ($P>0.05$) on the pH values of breast meats. In general, the meat pH was acceptable for the Indonesian consumers as the regulation states that the fresh chicken meat should have the pH values of 6-7 (Indonesian National Standard, 2009). In line with the latter reference, Abdulla *et al*. (2017) reported that the pH of fresh broiler meat ranged from 6.34 to 6.47. No effect ($P>0.05$) of treatments was found on the WHC of breast meats in this current work. Indeed, Mir *et al*. (2017) suggested that there is a positive correlation between WHC and pH values, and the absent effect of treatments on the WHC of broiler meats in the current study could therefore be understood. Compared to other groups, FERB meat had higher ($P<0.05$) cooking loss in this current study. There was no exact explanation for such condition, but perhaps *M. oleifera* leaf meal in the rations resulted in higher drip loss (Nkukwana *et al.*, 2015) and cooking loss (Cui *et al*., 2018) than that of control. Yet, this inference should be taken with caution as there was no significant difference in cooking loss between meats from FERM and CONT and BACI groups in this study. With regard to probiotic effect, it was difficult to relate the higher cooking loss in FERB and the effect of probiotic *B. subtilis*, as in most cases the probiotic supplementation decreased (Zhou *et al.*, 2015; Bai *et al.*, 2016) or had no impact (Alfaig *et al*., 2014; Abdulla *et al*., 2017) on the cooking loss of broiler meats. In this present work, we also noticed that meat from BACI had greater ($P<0.05$) cooking loss than that from CONT group. In line with the latter condition, Hamid *et al*. (2019) formerly found the increased cooking loss in breast and thigh meats following the administration of antibiotic growth promoters (AGP; enduracidin, colistin sulfate, bacitracin zinc, chlortetracycline and virginiamycin) in diets. In most conditions, cooking loss is linearly correlated to drip loss, which is loss of moisture from the fresh meats (Mir *et al*., 2017). With regard to the use of AGP, Nkukwana *et al*. (2015) revealed that administrations of salinomycin and zinc bacitracin in diets resulted in increased cumulative drip loss

Table 2. Physical and Chemical Characteristics of Broiler Breast Meats

Items	Dietary treatments				SE	P value
	CONT	BACI	FERM	FERB		
pH	6.46	6.43	6.48	6.47	0.02	0.12
WHC (%)	39.2	39.1	39.8	39.0	0.49	0.70
Cooking loss (%)	23.6^{c}	25.8^{b}	24.5^{bc}	27.8^{a}	0.54	<0.01
Moisture (%)	75.2^{b}	76.1^{a}	74.3^{c}	74.4^{c}	0.09	<0.01
Crude protein (%)	22.3^{c}	20.0^{d}	23.5^{a}	23.0^{b}	0.15	<0.01
Crude fat (%)	0.70^{ab}	0.77^{a}	0.63^{bc}	0.58^{c}	0.03	<0.01
Ash (%)	0.66^{b}	0.78^{a}	0.60^{b}	0.63^{b}	0.02	<0.01

[a,b,c]Means with different letters within the same row reflect significant difference

CONT: maize-soybean-based feed with no additive, BACI: maize-soybean-based feed supplemented with 0.1% zinc bacitracin, FERM: feed containing 20% of FCPMO, FERB: feed containing 20% of FCPMO and 0.1% *B. subtilis*, SE: standard error

in broiler breast meats. The latter workers suggested that the higher fat and lower protein contents in the breast meats (due to AGP administration) may be attributed to the higher water loss from meats. Note that fat possess lower water affinity than protein.

It was noted in this study that breast meats from FERM and FERB had lower (P<0.05) moisture content than that from CONT and BACI groups. This finding was in agreement with Marcinčák *et al*. (2018) reporting that feeding fermented bioproduct resulted in lower moisture content of broiler breast meat as compared to control meat. Unlike the latter study, Sugiharto *et al*. (2017) did not find any noticeable effect of feeding cassava pulp fermented with *A. charticola* on the moisture content of broiler breast meat. Differences in the nature of fermented feeds, dietary compositions and conditions during the trial may exert divergent responses by broilers that in turn affect the moisture content in broiler meats. In this study, treatment with AGP elevated moisture content of broiler breast meats. This finding was different from the most published studies showing no effect of salinomycin and zinc bacitracin (Nkukwana *et al*., 2015) as well as neomycin (Sugiharto *et al*., 2017) on the moisture content of broiler breast meats. There is no definite explanation for these conflicting data above so far, but possibly different types of AGP, broiler strains, feed and experimental conditions may account to the variation in moisture content of broiler meats. It was clearly shown in this study that feeding FCPMO with or without probiotic *B. subtilis* increased (P<0.05) the content of crude protein in broiler breast meats. This present finding was in agreement with Sugiharto *et al*. (2017) at which feeding *A. charticola*-fermented cassava pulp increased crude protein content of broiler breast meat. Likewise, feeding fermented cottonseed meal (Nie *et al*., 2015) and fermented bioproduct (Marcinčák *et al*., 2018) increased the crude protein level in breast meats of broilers. It was most likely that probiotic properties of *C. crassa* used as fermentation starter improved the protein utilization (protein efficiency ratio) and thereby increased protein production in the musculature (intramuscular protein anabolism) of broiler chickens (Khaksefidi and Rahimi, 2005; Hossain *et al*., 2012; Nie *et al*., 2015). Moreover, *M. oleifera* leaf meal seemed also to contribute to the increased protein content in breast meat in this current study. Formerly, Rehman *et al*. (2018) suggested that feeding *M. oleifera* leaf powder elevated the tissue protein deposition as reflected by the increase in muscle fibre diameter of broiler chickens. Interesting result was seen in this current work, in which dietary inclusion of FCPMO resulted in lower (P<0.05) fat content in the breast meat of broilers. In accordance, earlier study by Marcinčák *et al*. (2018) documented that feeding fermented bioproduct resulted in lower fat content in broiler breast meat. Also, Nie *et al*. (2015) documented that feeding fermented cottonseed meal decreased the fat content in broiler breast meats. It was most likely that the fermented feed could lower the intramuscular fat synthesis in broiler chickens (Nie *et al*., 2015). Moreover, the fermented product may increase the breakdown of tissue triglycerides and β-oxidation of fatty acid resulting in decreased fat deposition in the muscle of broilers (Sugiharto and Ranjitkar, 2019). In this work, the combination of FCPMO and probiotic *B. subtilis* resulted in the lowest (P<0.05) crude fat content in broiler breast meat. The synergistic effect of fermented feed and probiotic (Sugiharto and Ranjitkar, 2019) seemed to play a remarkable role in lowering the fat content in broiler meat. Compared to other meats, the breast meats from BACI had higher (P<0.05) ash content in the present study. This result did differ from other reports showing no effect of antibiotic treatment on the ash content of broiler meats (Abdulla *et al*., 2017; Sugiharto *et al*., 2017). The reason for such higher ash content in BACI meat group was not exactly known, but it was possible that antibiotic treatment may increase the mineral digestibility and thus deposition in the muscle tissue of broilers (Wang *et al*., 2016).

There was a tendency (P=0.08) that the level of cholesterol was lower in the breast meat of FERB than that particularly in BACI and FERM birds (Figure 1). It was most likely that probiotic *B. subtilis* played a remarkable role in lowering the content of cholesterol in broiler meats. In agreement with our latter inference, previous study showed the efficacy of probiotic *B. subtilis* in lowering the levels of cholesterol in carcass of broilers (Santoso *et al*., 1995) and plasma of laying hens (Fathi *et al*., 2018). Santoso *et al*. (1995) pointed out that *B. subtilis* may reduce the synthesis of cholesterol in the liver by lowering the activity of hepatic 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase. In addition, *B. subtilis* may modify the intestinal microflora, which eventually alter the lipid metabolism and reduced cholesterol levels in broiler meats (Fathi

et al., 2018).

It was shown in this present study that the DPPH IC_{50} values were higher ($P<0.05$) in meat of CONT than that of other groups (Figure 2). In respect to zinc bacitracin, previous study showed the antioxidative activity of zinc bacitracin on broilers chickens (Ismail *et al*., 2013). For this reason, zinc bacitracin seemed to improve the antioxidant status of broiler meats in this present study. Earlier study by Cao *et al*. (2012) documented that feeding Ginkgo biloba leaves fermented with *Aspergillus niger* resulted in improved antioxidative capacity of broiler meats. Likewise, Hu *et al*. (2016) reported that feeding fermented rapeseed meal improved the serum total antioxidative capacity of broiler chickens. In accordance with these former studies, our current findings showed that feeding FERM or FERB was

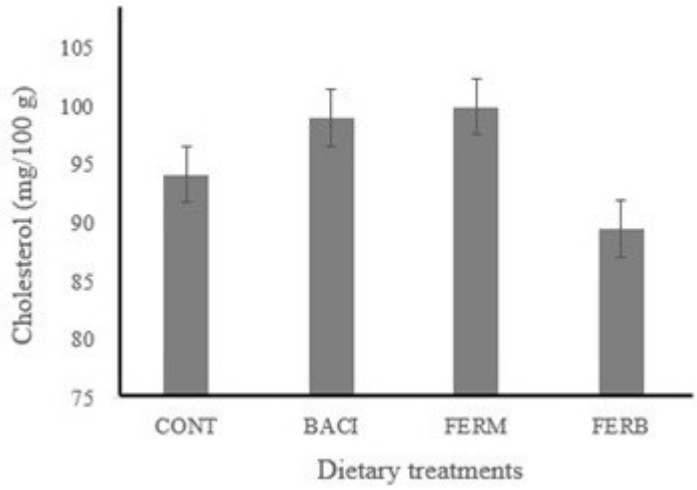

Figure 1. Cholesterol contents of broiler breast meats. CONT: maize-soybean-based feed with no additive, BACI: maize-soybean-based feed supplemented with 0.1% zinc bacitracin, FERM: feed containing 20% of FCPMO, FERB: feed containing 20% of FCPMO and 0.1% *B. subtilis*

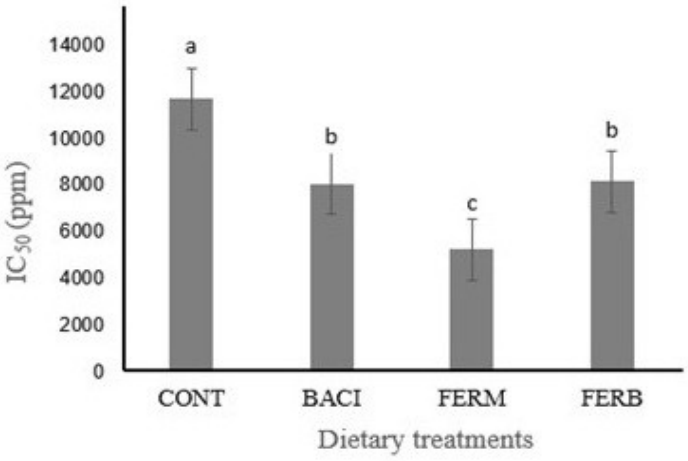

Figure 2. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of broiler breast meats. [1]IC_{50} value is the effective concentration of the sample needed to scavenge 50% of DPPH radical. CONT: maize-soybean-based feed with no additive, BACI: maize-soybean-based feed supplemented with 0.1% zinc bacitracin, FERM: feed containing 20% of FCPMO, FERB: feed containing 20% of FCPMO and 0.1% *B. subtilis*

attributed to the lower DPPH IC_{50} values as compared to feeding the control diet. It was most likely that fermented feed increased the production of antioxidant peptides, lactic acids, amino acids and antioxidant vitamins, which may work as a part of antioxidant system, resulting in improved antioxidative status of broiler chickens and thus meats produced (Cao *et al.*, 2012; Hu *et al*., 2016). The contribution of *M. oleifera* leaf in improving the antioxidative status of broiler meats may also be acknowledged in this current study. Recently, Mahfuz and Piao (2019) have documented that *M. oleifera*-rich in antioxidants could modify and improve the antioxidant status of broiler meats.

Data on the profile of fatty acids of broiler breast meats are shown in Table 3. Interesting result was found in this present study, at which feeding FCPMO with or without probiotic *B. subtilis* resulted in higher ($P<0.05$) proportion of total PUFA in breast meats when compared with that in CONT and BACI meats. The increased PUFA contents in meats was mainly contributed by the increase in C18:3n6, C20:2, C20:3n6, C22:2 and C22:6. In general, PUFAs have been attributed to the health improvement in humans (Minihane and Lovegrove, 2006). This present finding may therefore suggested that feeding FCPMO with or without *B. subtilis* was essential to produce the healthy meats for human consumption. In line with our finding, Cao *et al*. (2012) formerly reported that feeding *Ginkgo biloba* leaves fermented using *Aspergillus niger* increased the proportion of total PUFA in breast meats of broilers slaughtered at 42 days of age. It was most likely that fermented feed increased the activity of peroxide-scavenging enzyme that can alleviate the PUFA oxidation (Cao *et al*., 2012). In addition to the effect of fermented feed, *M. oliefera*, which is rich in polyphenols, may also serve as antioxidative agents that can protect PUFA in meats from oxidation (Cao *et al*., 2012). In agreement, Cui *et al*. (2018) noticed that *M. oliefera* leaf meal increased PUFA concentration and improve oxidative stability of broiler meats.

With regard to MUFA, our study demonstrated that feeding FCPMO produced breast meats with lower ($P<0.05$) MUFA contents. The decreased MUFA content was contributed mainly by the decrease in C15:1, C16:1 and C19:1 in broiler meats. In accordance with our data, Kim *et al*. (2017) showed the decreased MUFA concentrations in broiler breast meats with feeding fermented *Ginkgo biloba* and *Citrus junos*. However, our present findings were different from most published papers showing no significant effect of feeding fermented feed on the MUFA content of broiler breast meats. For instance, Chao *et al*. (2012) and Chung and Choi *et al*. (2016) did not notice any impact of feeding *Aspergillus niger*-fermented *Ginkgo biloba* leaves and fermented ginseng marc with red koji, respectively, on the MUFA content of broiler breast meats. The differences in the nature of fermented feed, dietary compositions/ingredients and experimental conditions may be responsible for the discrepant results above. With regard to *M. oliefera* leaf, the inclusion of such plant seemed not to affect the concentration of MUFA in broiler meats, as Cui *et al*. (2018) and Sebola *et al*. (2018) did not find any effect of feeding *M. oliefera* leaf meal on the MUFA level of broiler breast meats in their earlier studies.

Data in our current work revealed no substantial effect ($P>0.05$) of dietary treatment on the SFA content of broiler breast meats. With regard to the effect of fermented feed, Marcinčák *et al*. (2018) also did not find any effect of fermented bio-product on the on the SFA concentration of broiler breast meats. The absent effect of fermented feed on the SFA content of meats was also reported by Kim *et al*. (2017) when feeding fermented *Citrus junos* to broiler chickens. Likewise, Chung and Choi (2016) noticed no effect of feeding 1% fermented ginseng marc with red koji on the SFA contents of broiler breast meats. In contrast to the above mentioned studies, feeding fermented *Ginkgo biloba* leaves resulted in decreased contents of SFA in broiler breast meats in the study of Cao *et al*. (2012). The variations in the nature of fermented feed, dietary compositions and experimental conditions may be responsible for the divergent results above. In respect to the effect of *M. oliefera* leaf, dietary incorporation of *M. oliefera* leaf meal did not alter SFA content of breast meats in the studies of Cui *et al*. (2018) and Sebola *et al*. (2018). For this reason, it was therefore difficult to assume that *M. oliefera* leaf meal exert an effect on the SFA content in broiler meats.

CONCLUSION

Feeding FCPMO produced healthy broiler meat as reflected by the higher contents of protein, PUFA and antioxidants, and lower fat in meats.

Table 3. Fatty Acids Profile of Broiler Breast Meats

Fatty acids (g/100 g)	Dietary treatments				SE	P value
	CONT	BACI	FERM	FERB		
C11:0	0.04	0.03	0.00	0.13	0.07	0.55
C12:0	0.30	0.50	0.01	2.31	1.16	0.49
C13:0	0.37	0.33	0.00	0.40	0.17	0.34
C14:0	0.90^{b}	1.80^{a}	0.00^{c}	0.19^{bc}	0.25	<0.01
C14:1	0.52	0.70	0.00	2.65	1.27	0.48
C15:0	0.64^{a}	0.78^{a}	0.04^{b}	0.57^{ab}	0.19	0.04
C15:1	19.5^{a}	23.0^{a}	7.57^{b}	9.14^{b}	1.80	<0.01
C16:0	3.46	0.54	1.07	1.27	1.18	0.33
C16:1	5.27^{a}	4.58^{a}	0.22^{b}	0.93^{b}	0.62	<0.01
C17:0	0.88^{a}	0.56^{b}	0.22^{c}	0.19^{c}	0.09	<0.01
C17:1	0.61^{b}	0.38^{b}	4.45^{a}	3.77^{a}	0.48	<0.01
C18:0	3.97	4.55	1.51	3.16	1.31	0.39
C19:1	30.8^{a}	33.1^{a}	34.8^{a}	23.3^{b}	2.19	<0.01
C18:1	0.12	0.08	0.14	0.75	0.22	0.11
C18:2n6	26.1	24.2	25.5	21.4	1.32	0.07
C18:2	0.15	0.21	0.80	0.75	0.21	0.06
C20:0	0.38^{b}	0.06^{b}	0.13^{b}	1.84^{a}	0.23	<0.01
C18:3n6	1.53^{ab}	1.51^{ab}	2.25^{a}	1.02^{b}	0.27	0.02
C20:1	0.43	0.31	1.04	1.08	0.27	0.10
C18:3n3	0.46	0.31	0.74	1.13	0.22	0.70
C21:0	0.26^{b}	0.24^{b}	2.24^{a}	1.80^{a}	0.50	<0.01
C20:2	0.17^{b}	0.06^{b}	0.76^{b}	2.87^{a}	0.38	<0.01
C22:0	0.22	0.12	0.92	0.43	0.25	0.12
C20:3n6	0.34^{b}	0.40^{b}	1.99^{a}	1.96^{a}	0.35	<0.01
C22:1	0.08	0.02	0.22	0.24	0.11	0.42
C20:3n3	0.03	0.01	0.12	0.48	0.22	0.38
C23:0	0.01^{b}	0.03^{b}	0.17^{b}	0.92^{a}	0.22	0.02
C20:4n6	0.22	0.28	1.51	2.48	0.77	0.12
C22:2	0.19^{b}	0.10^{b}	1.58^{ab}	2.63^{a}	0.67	0.03
C24:0	0.04	0.09	1.18	0.37	0.41	0.19
C20:5	0.28	0.41	0.83	1.28	0.48	0.42
C24:1	0.93^{b}	0.48^{b}	5.25^{a}	5.47^{a}	1.44	0.03
C22:6	0.63^{b}	0.62^{b}	3.07^{a}	4.41^{a}	0.79	<0.01

Table 3. Fatty Acids Profile of Broiler Breast Meats (continued)

Fatty acids (g/100 g)	Dietary treatments				SE	P value
	CONT	BACI	FERM	FERB		
Total SFA	11.5	9.65	7.49	13.6	2.11	0.23
Total MUFA	58.2^{ab}	62.6^{a}	53.7^{bc}	46.8^{c}	2.47	<0.01
Total PUFA	30.1^{b}	27.7^{b}	38.8^{a}	39.6^{a}	1.87	<0.01

^{a,b,c}Means with different letters within the same row reflect significant difference

CONT: maize-soybean-based feed with no additive, BACI: maize-soybean-based feed supplemented with 0.1% zinc bacitracin, FERM: feed containing 20% of FCPMO, FERB: feed containing 20% of FCPMO and 0.1% *B. subtilis*, SE: standard error

Total SFA= C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0
Total MUFA= C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1 + C21:0 + C22:1 + C24:1
Total PUFA= C18:2n6 + C18:2 + C18:3n3 + C18:3n6 + C20:2 + C20:3n3 + C20:3n6 + C20:4n6 + C22:2 + C20:5 + C22:6
SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

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CONFLICT OF INTERESTS

The authors had no conflict of interest.

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